

in the geographic location of the two collection points. The latitude of West Fargo is about 47° north and the winters are quite severe. Fort Worth is situated close to the thirty-third parallel and the winter months are comparatively mild.

Conclusions

Calculated on the desiccated fat-free basis, the yearly average for hog thyroid glands from North Dakota is 0.32% iodine, while that of Texas glands is 0.60% iodine.

The seasonal variation in the iodine content is most pronounced in the northern glands.

The higher iodine level of the Texas glands and the greater seasonal fluctuations of the North Dakota thyroids are attributed to the geographic location of the two states.

CHICAGO, ILLINOIS

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF BRISTOL]

THE ACETYLATION OF ORTHO-HYDROXY ALDEHYDES

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Herzig and Wenzel¹ found that when phloroglucin aldehyde is heated with acetic anhydride and anhydrous sodium acetate, no trace of the corresponding coumarin was produced, and that the condensation product consisted mainly of 2,4,6-triacetoxybenzylidenediacetate (I), which melted at 155–156°. Later, Knoevenagel² showed that benzylidenediacetate formation is general to all aromatic aldehydes, being favored by rise of temperature. On the other hand, Pratt and Robinson³ apparently succeeded in preparing for the first time triacetylphloroglucin aldehyde (II) by the interaction of phloroglucin aldehyde with acetic anhydride and anhydrous potassium carbonate. They found that during the reaction considerable heat was evolved and that the resulting product melted at 151°, a melting point subsequently raised to 156–157° by Robertson and Robinson.⁴ The agreement between the melting points found by Herzig and Wenzel and Robertson and Robinson, respectively, for the two substances I and II seemed to us very striking, and since Robinson's triacetylphloroglucin aldehyde is an important starting material in the syntheses of a large number of the anthocyanidins,⁵ we thought it desirable to

¹ Herzig and Wenzel, *Monatsh.*, **24**, 864 (1903).

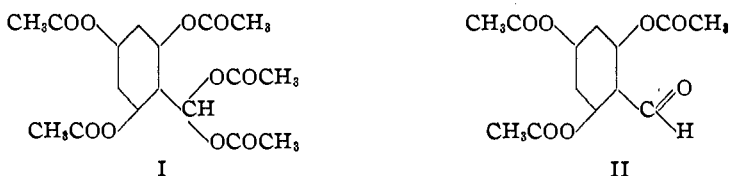
² Knoevenagel, *Ann.*, **402**, 115 (1913).

³ Pratt and Robinson, *J. Chem. Soc.*, **127**, 1184 (1925).

⁴ Robertson and Robinson, *ibid.*, 1713 (1927).

⁵ See Pratt and Robinson, *J. Chem. Soc.*, **127**, 1182 (1925); Malkin and Robinson, *ibid.*, **127**, 1190 (1925); Gatewood and Robinson, *ibid.*, 1959 (1926); Nolan and Robinson *ibid.*, 1968 (1926); Robertson and Robinson, *ibid.*, 1710 (1927); Malkin and Nierenstein, *Ber.*, **61**, 791 (1928).

re-investigate this material. Our results leave no doubt whatever that the so-called triacetylphloroglucin aldehyde (II) of Robinson and his co-workers is in fact the penta-acetate (I) of Herzig and Wenzel. This incidentally also offers an explanation for the analytical difficulties commented on by Pratt and Robinson, who found that their product did not give correct values for triacetylphloroglucin aldehyde (II) and assigned to it the empirical formula $C_{13}H_{12}O_7 + \frac{1}{2}H_2O$.⁶



In the present communication, a method for the preparation of triacetylphloroglucin aldehyde (II) is described, and this method has also been extended to the preparation of diacetyl- β -resorcylic aldehyde and acetyl-salicylic aldehyde. All these aldehydes are smoothly acetylated in ethereal solution with acetic anhydride and anhydrous potassium carbonate in which manner all rise of temperature is controlled. That evolution of heat should be avoided is evident from the fact that if salicylic aldehyde is warmed for a few minutes with acetic anhydride and anhydrous potassium carbonate, 2-acetoxybenzylidenediacetate only is obtained. Cooling in ice does not, however, sufficiently retard the formation of benzylidenediacetate and leads, for example, in the case of phloroglucin aldehyde, to a quantitative yield of the penta-acetate (I) and not the triacetate (II).

The ease with which the formation of benzylidenediacetate takes place clearly accounts in our opinion for the low yields which are obtained when coumarin is prepared by the Perkin synthesis,⁷ a fact well known to industry.⁸

The failure of Herzig and Wenzel to obtain 5;7-dihydroxycoumarin by the Perkin reaction is thus in harmony with the facile benzylidenediacetate formation of phloroglucin aldehyde, although Gattermann⁹ states that this coumarin is formed by heating phloroglucin aldehyde, anhydrous sodium acetate and acetic anhydride in a sealed tube for five hours at 160–175°. Gattermann, however, draws attention to the fact that the deacetylated product shows no fluorescence in concentrated sulfuric acid, the character-

⁶ The analytical data of Pratt and Robinson refer to the product melting at 151°, which was obviously impure penta-acetate (I). No analyses are recorded by Robertson and Robinson for their pure product (m. p. 156–157°).

⁷ According to Simonis, "Die Cumarine," Stuttgart, 1916, p. 59, the yield of 37.5% recorded by Gnehm, *Ber.*, 14, 262 (1881), is the highest yield so far given in the literature for coumarin.

⁸ See May, *Perfumery Essential Oil Record*, 16, 47 (1925).

⁹ Gattermann, *Ann.*, 357, 345 (1907).

istic property of 7-hydroxycoumarins. Gattermann's synthesis, therefore, still requires confirmation.

The application of the true *o*-acetoxy aldehydes in the syntheses of flavylum compounds is in progress, and is reserved for subsequent publications.

Experimental

Phloroglucin aldehyde and β -resorcylic aldehyde were prepared by Adams and Levine's modification of the Gattermann reaction¹⁰ and only those who have worked with the original Gattermann method can appreciate the advance for which Adams' modification stands. In our experience, however, the comparatively large excess of zinc cyanide as used by Adams and Levine is unnecessary and cleaner products are obtained when only a very slight excess over the theoretical is employed.

Acetyl-salicylic Aldehyde.—To a solution of 10 g. of salicylic aldehyde in 50 cc. of dry ether are added 15 g. of anhydrous potassium carbonate and 15 cc. of acetic anhydride, and the reaction mixture vigorously shaken for thirty minutes. The solution is then filtered on the pump and the residual solid washed several times with dry ether. The residue left on evaporation of the ether is treated with water and the solid collected. The product crystallizes from ligroin (b. p. 60–80°) in needles which melt at 38°, in agreement with the melting point given in the literature.

2-Acetoxybenzylidenediacetate.—Ten grams of salicylic aldehyde dissolved in 30 cc. of acetic anhydride is warmed for a few minutes with 10 g. of anhydrous potassium carbonate. The oil formed on pouring into water and standing for some time is separated from the aqueous layer and dissolved in a little alcohol. On standing, thick prisms separate which on crystallization from alcohol melt at 107°. This product is identical with the "2-hydroxybenzylidenediacetate" (m. p. 101°) of Perkin,¹¹ as already pointed out by Barbier¹² and Knoevenagel,¹³ who give its melting point as 104–105° and 103°, respectively.

Diacetyl- β -resorcylic Aldehyde.—Three grams of β -resorcylic aldehyde, dissolved in 100 cc. of dry ether, was reacted on with 6 g. of anhydrous potassium carbonate and 10 cc. of acetic anhydride in the manner already described for the preparation of acetyl-salicylic aldehyde. The crude product (3.5 g.) separated from absolute alcohol in colorless needles which melted at 69°. The alcoholic solution gives no coloration with ferric chloride.

Anal. Subs., 4.580, 5.101, 4.891 mg.: CO₂, 9.960, 11.150, 10.660 mg.; H₂O, 1.81, 2.12, 1.96 mg. Calcd. for C₁₁H₁₀O₅: C, 59.46; H, 4.50. Found: C, 59.31, 59.63, 59.45; H, 4.42, 4.65, 4.48.

Triacetylphloroglucin Aldehyde (II).—For the preparation of phloroglucin aldehyde in small quantities, the following slight modification of the original method of Adams and Levine was found to give excellent results. Into a solution of 2 g. of anhydrous phloroglucin in 25 cc. of dry ether containing, 1.2 g. of zinc cyanide, a good stream of dry hydrogen chloride is passed until the oil formed solidifies. After standing for two to three hours, the solid is collected, washed with ether, and the imide salt dissolved in 40 cc. of water and hydrolyzed on a water-bath. The solid (2 g.) obtained on cooling is collected and dissolved in ether, filtered from traces of a red-colored by-product, and the ether evaporated. Phloroglucin aldehyde thus obtained crystallizes

¹⁰ Adams and Levine, *THIS JOURNAL*, **45**, 2373 (1923).

¹¹ Perkin, *Ann.*, **146**, 371 (1868).

¹² Barbier, *Compt. rend.*, **90**, 37 (1881).

¹³ Knoevenagel, *Ann.*, **402**, 124 (1914).

in long cream-colored needles from water containing a trace of sulfur dioxide. As already recorded by Gattermann, the product has no definite melting point.

In the acetylation, 2.3 g. of phoroglucin aldehyde in 100 cc. of dry ether containing 7.5 g. of anhydrous potassium carbonate and 15 cc. of acetic anhydride are worked up in the manner already described. The crude solid (3 g.) crystallizes from alcohol in thick colorless prisms which melt at 101°. The alcoholic solution gives no coloration with ferric chloride.

Anal. Subs., 5.100, 4.869 mg.: CO₂, 10.420, 9.930 mg.; H₂O, 1.98, 1.85 mg. Subs., 5.333, 5.930 mg.: 5.68, 6.37 cc. of *N*/100 NaOH. Calcd. for C₁₃H₁₂O₇: C, 55.71, H, 4.29; CH₃CO, 46.07. Found: C, 55.74, 55.64; H, 4.34, 4.25; CH₃CO, 45.82, 46.21.

The 2,4,6-triacetoxybenzylidenediacetate (I), obtained by the method of Pratt and Robinson, melted at 157°.

Anal. Subs., 4.979, 5.075 mg.: CO₂, 9.730, 9.930 mg.; H₂O, 2.06, 2.09 mg. Subs., 6.769, 6.181 mg.: 8.57, 7.81 cc. *N*/100 NaOH. Calcd. for C₁₈H₁₂O₇ (triacetylphloroglucin aldehyde): C, 55.71; H, 4.29; CH₃CO, 46.07. Calcd. for C₁₇H₁₈O₁₀ (2,4,6-triacetoxybenzylidenediacetate): C, 53.39; H, 4.71; CH₃CO, 56.27. Found: C, 53.30, 53.36; H, 4.63, 4.61; CH₃CO, 54.47, 54.36.

For the micro-acetyl estimations we are indebted to Dr. Arnulf Soltys and to Dr. M. Zacherl, which were carried out by them in Professor Pregl's laboratory, according to the Pregl-Soltys method.¹⁴

Summary

A general method for the acetylation of *o*-hydroxy-aldehydes is described. The acetylation is carried out with acetic anhydride and anhydrous potassium carbonate in the presence of ether, in which manner benzylidenediacetate formation is avoided.

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A METHOD FOR THE DETERMINATION OF PEROXIDASE ACTIVITY¹

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A mixture of α -naphthol and *p*-phenylenediamine has frequently been used for both the qualitative and quantitative estimation of oxidase activity.² This depends on the oxidation of the reagent by atmospheric oxygen to form an indophenol, oxidase acting as the catalyst. In the estimation of peroxidase, hydrogen peroxide takes the place of oxygen in the reaction. The α -naphthol-*p*-phenylenediamine mixture has, however, usually been regarded as too sensitive for peroxidase, and other compounds

¹⁴ Pregl, "Die Quantitative Organische Mikroanalyse," Berlin, Dritte Auflage, 1930, p. 216.

¹ Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 13.

² H. M. Vernon, *J. Physiol.*, **42**, 402-432 (1911).