of hydrocyanic acid" with a tertiary amine,[1,2] by thermal decomposition of formamidine hydrochloride or trimerization of the free base,[3] or by trimerization of ethyl formimidate.[4] Other methods have been summarized by Smolin and Rapoport.[5] We now wish to describe a new and convenient method for the preparation of small quantities of *s*-triazine by the dehydration of formamide.

Although *s*-triazine is formed in the thermal decomposition of thioformamide,[6] Grundmann and Kreutzberger have shown that thermal dehydration of formamide itself leads only to the monomer, hydrocyanic acid.[2] We have now found that when formamide is mixed with a suitable dehydrating agent such as calcium carbide (or calcium nitride, calcium hydride, lithium carbide, etc.) and the mixture is heated, *s*-triazine is readily formed.

Several anhydrous transition metal halides, such as nickelous bromide, cobaltous chloride, and ferric chloride, when dissolved in the formamide in concentrations up to 10%, serve to improve the yield. Ferric chloride is especially useful since it also inhibits formation of black, tarry HCN polymer. A side reaction which reduces the efficiency of the preparation regardless of the order of addition is the base-catalyzed decomposition of formamide to carbon monoxide and ammonia. About 30% of the starting material is lost in this way. Unwanted by-products which tend to distill from the reaction mixture with the triazine and to cause decomposition should be removed from the product before collection. This can be accomplished by passing the triazine vapor through a short column of Linde molecular sieve.

EXPERIMENTAL

Preparation of s-triazine. The apparatus consisted of a three-neck flask fitted with an addition buret, a nitrogen inlet, and a curved adapter outlet (packed with a 3-in. column of Linde molecular sieve Type 4A) leading to a chilled receiver. To 10.0 g. of pulverized calcium carbide maintained at 100° was added dropwise 10 ml. of a filtered solution of formamide containing approximately 10% anhydrous ferric chloride. The product sublimed immediately from the red-brown residue which formed. The reaction flask was subsequently heated to *ca.* 150° to obtain more product. A total of 1.17 g. of *s*-triazine was collected (19% yield based on the formamide added). The product was identified by melting point (82–83° uncorrected), infrared,[7] and mass spectroscopic analysis.

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AMERICAN CYANAMID CO.
STAMFORD, CONN.

(1) L. E. Hinkel and R. T. Dunn, *J. Chem. Soc.*, 1834 (1930).
(2) C. Grundmann and A. Kreutzberger, *J. Am. Chem. Soc.*, **76**, 5646 (1954).
(3) F. C. Schaefer, I. Hechenbleikner, G. A. Peters, and V. P. Wystrach, *J. Am. Chem. Soc.*, **81**, 1466 (1959).
(4) F. C. Schaefer and G. A. Peters, *J. Org. Chem.*, **26**, 2778 (1961).
(5) E. M. Smolin and L. Rapoport, *s-Triazines and Derivatives*, Interscience Publishers, Inc., New York, 1959, pp. 9–10.
(6) R. Willstätter and T. Wirth, *Ber.*, **42**, 1908 (1909).
(7) J. E. Lancaster, R. F. Stamm, and N. B. Colthup, *Spectrochim. Acta*, **17**, 155 (1961).

Synthesis and Properties of 4-Methyl-2-oxo-1,2-benzopyran-7-yl β-D-Galactoside (Galactoside of 4-Methylumbelliferone)

R. STRACHAN, J. WOOD, AND RALPH HIRSCHMANN

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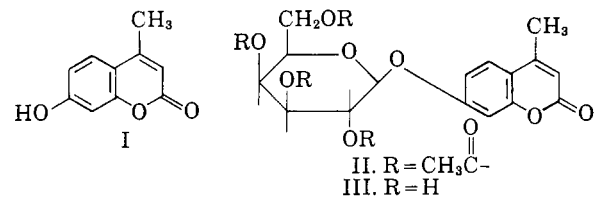
Mead, Smith, and Williams[1] observed that while 4-methylumbelliferone (I) fluoresced strongly in ultraviolet light at a *p*H 9–10, its conjugates showed little or no fluorescence. These workers were, therefore, able to show that the glucuronide of I, isolated from urine, served as a highly sensitive substrate for the determination of glucuronidase levels. Robinson[2] extended the fluorimetric method to the glucoside of I and Marsh and Levy[3] described the conversion of the glucoside to the glucuronide. Finally Leaback and Walker[4] described methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucoside. We wish to report the synthesis of the galactoside (III).[4a] Fluorometric analysis showed the product to be substantially free of I.



EXPERIMENTAL[5]

Methylumbelliferyl tetra-O-acetyl-β-D-galactopyranoside (II). A mixture of 11.5 g. (0.065 mole) of I (Eastman Kodak), m.p. 188–189°, and of 19.0 g. (0.0462 mole)[6] of 2,3,4,6-tetra-*O*-acetyl-α-D-galactosyl bromide[7] was suspended in

(1) J. A. R. Mead, J. N. Smith, and R. T. Williams, *Biochem. J.*, **60**, XV (1955); *Biochem. J.*, **61**, 569 (1955).
(2) D. Robinson, *Biochem. J.*, **63**, 39 (1956).
(3) C. A. March and G. A. Levy, *Nature*, **178**, 589 (1956).
(4) D. H. Leaback and P. G. Walker, *Biochem. J.*, **78**, 151 (1961).
(4a) The utilization of this substrate for the estimation of β-D-galactosidase levels in cell cultures will be described by A. A. Tytell and A. I. Shepartz (**Federation Proc. 21**, 1962).
(5) Analyses were carried out by Mr. R. Boos and his associates and the infrared spectra were determined by Messrs. R. Walker and N. Allan. The rotations were measured by Dr. D. Williams.
(6) The molar ratios employed are those of R. D. Robinson.[2]
(7) R. G. Hansen, W. J. Rutter, and P. Krichevsky, *Biochemical Preparations*, **4**, 1 (1955).

140 ml. of acetone and treated with a solution of 3.6 g. of sodium hydroxide in 90 ml. of water. The mixture was stirred for about 18 hr. at room temperature. The acetone was removed *in vacuo*, and the bulk of the remaining water was distilled azeotropically with benzene. The residue was dissolved in ethanol and stirred with 3 g. of decolorizing charcoal.

The filtrate was concentrated until an oily product separated. The supernatant was removed by decantation and afforded, after the addition of water, 2.0 g. of methylumbelliferone characterized by its melting point and its infrared spectrum. The oil was triturated with ether to afford 8.05 g. of II, m.p. 140–142°. One recrystallization from 75 ml. of ethanol gave 7.40 g. melting at 141–143°. An analytical specimen, 6.67 g., m.p. 143–145.5°; λ_{max} (CH_3OH) 314 mμ (log ϵ 4.11); 286 mμ (3.94), 245 mμ (3.39), was obtained from the same solvent. The infrared spectrum in chloroform showed maxima at 5.7–5.8 μ (acetate and lactone), 8–8.05 μ (acetate), 6.15, 6.35, 6.62 μ (olefinic). The compound exhibited little rotation in chloroform at 589 mμ, $[\alpha]^{25}$ − 6.5° (*c*, 1.54) but at 365 mμ $[\alpha]^{25}$ − 73°.

Anal. Calcd. for $C_{24}H_{26}O_{12}$: C, 56.91; H, 5.17. Found: C, 56.54; H, 5.17.

Methylumbelliferyl-β-D-galactopyranoside (III). The acetyl groups were removed by ester exchange, using only a catalytic amount of base. A solution of 5 g. II (0.00987 mole), m.p. 143–145.5°, in 160 ml. of spectral grade methanol was stirred with 7 ml. of 1.05*N* methanolic sodium methoxide (0.007 mole) for 5 min. The product, which had separated, was removed by filtration and afforded 3.34 g., m.p. 254–256.5°. The product was recrystallized from ethanol–water and then suspended in 50 ml. of acetone and stirred at room temperature for 16 hr. to remove any methylumbelliferone. The product, 2.48 g., m.p. 227.5–230°, $[\alpha]_D^{24}$ − 37° (*c*, 1.23% in dimethylformamide), λ_{max} (methanol) 315 mμ (log ϵ 4.13), sh 290 mμ (3.92), λ_{max} 245 mμ (3.75); λ_{max} (Nujol) 2.99 μ, 3.15–3.20 μ (OH), 5.85–5.91 μ (lactone), 6.18 μ (aromatic), was submitted for analysis.

Anal. Calcd. for $C_{16}H_{18}O_8$: C, 56.80; H, 5.36. Found: C, 57.00; H, 5.31.

There was no selective absorption in the 8-μ region (acetate) in the infrared spectrum.[8]

Fluorometric studies. Fluorescence and excitation spectra for I and III were determined with an Aminco-Keirs spectrophosphorimeter modified for fluorescence measurements. For each of the compounds the position of the excitation and fluorescence maxima was the same at *p*H 7.0 as at *p*H 10.3 (0.15*M* glycine buffer). Compound I gave maximum fluorescence at 445 mμ when excited at 365 mμ, and the fluorescence at *p*H 10.3 was approximately 100 times as intense as at *p*H 7. The much weaker fluorescence of the galactoside was maximal at 368 mμ when excited at 331 mμ, but the fluorescence of III was only 1.15 times as intense at the higher *p*H as at neutrality. For the free phenol fluorescence at 445 mμ was found to be a linear function of concentration at least up to $c = 2.2 \times 10^{-4}$ mg./ml. We found that as little as 0.0007% of I in III caused a significantly greater increase in fluorescence at 445 mμ than at 368 mμ with a shift in *p*H from 7 to 10.3, whereas, for our sample of III, m.p. 227.5–230°, the increase in fluorescence in alkaline solution at 445 mμ was no greater than at 368 mμ. We may conclude, therefore, that the product contained less than 0.0007% of I as a contaminant and should therefore, be entirely satisfactory for the determination of galactosidase levels.

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RAHWAY, N. J.

(8) D. H. Leaback [*Biochem. J.*, **78**, 22P (1961)] reported the preparation of III but neither m.p. nor other characteristic constants were given in this preliminary announcement.

Further Observations Concerning the Periodic Acid Oxidation of Hydroxylamine Derivatives[1]

THOMAS F. EMERY[2] AND J. B. NEILANDS

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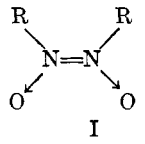
In a previous communication[3] we reported the periodic acid oxidation of certain secondary hydroxamic acids and hydroxylamine derivatives of the general structure R′N(OH)R. The oxidation of either free or acylated *N*-alkylhydroxylamine (R′ = H or acyl, R = alkyl) gave the corresponding *cis*-nitrosoalkane dimer (I. R = alkyl). Primary



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hydroxamic acids (R′ = acyl, R = H) and free hydroxylamine (R′ = R = H) were also attacked, but the precise nature of the oxidation product remained obscure.

In this report we describe the products formed by periodate oxidation, under similar conditions, of free hydroxylamine and of benzohydroxamic acid. In addition, the application of the reaction for the detection and determination of natural and synthetic "active acyl" compounds will be illustrated.

The action of periodate on hydroxylamine gave nitrous oxide as the principal product. Although intermediates have not been specifically investigated, it may be relevant that both nitroxyl dimer (I. R = H) and hyponitrous acid are known to decompose to nitrous oxide.[4]

The oxidation of potassium benzohydroxamate would be expected[3] to yield benzoic acid and nitrous oxide. Instead, *N,O*-dibenzoylhydroxylamine was found.

As the molar absorption coefficient of compound I (R = CH_3) is almost 10,000,[5] the formation of this substance via periodic acid oxidation should provide a sensitive test for the detection of synthetic and natural *N*-alkylhydroxylamines. The values shown in Table I substantiate this statement.

(1) This work was derived in part from the doctoral dissertation of Thomas F. Emery, University of California, Berkeley, and was supported by a grant from the Office of Naval Research.

(2) Present address: Department of Biochemistry, Yale University, New Haven, Conn.

(3) T. Emery and J. B. Neilands, *J. Am. Chem. Soc.*, **82**, 4903 (1960).

(4) E. Abel, O. Orlicek, and J. Proisl, *Monatsch. Chem.*, **72**, 1 (1938); F. Raschig, *Sulfur and Nitrogen Studies*, Leipzig-Berlin (1924).

(5) B. G. Gowenlock and J. Trotman, *J. Chem. Soc.*, 4190 (1955); 1670 (1956).