of absolute ether. After all the hydride had dissolved, a solution of 50 g. of N-methyl camphorimide in 500 ml. of absolute ether was added dropwise with rapid stirring. The rate of addition was adjusted so that the mixture refluxed gently. During the addition a fine suspension of the complex precipitated. After the addition was completed, the stirring was continued for several hours and the mixture allowed to stand overnight. The flask was cooled in an ice bath and, with vigorous stirring, the reaction mixture was decomposed by the dropwise addition of water. The addition of water was regulated so that reflux was just maintained and then 10 cc. in excess was added at the end. After decomposition, the mixture was stirred an additional hour and filtered with suction. The inorganic precipitate was well pressed and washed with three portions of ether. After drying over sodium sulfate, the ether was stripped and the residue distilled under reduced pressure. There was obtained 36 g. of material boiling at 99–102° at 38 mm. (68° at 10 mm.) n_D^{25} 1.4776.

Anal. Calcd. for $C_{11}H_{21}N$: C, 78.98; H, 12.65; N, 8.37. Found: C, 79.39; H, 12.52; N, 8.45.

The methiodide was prepared by heating with a slight excess of methyl iodide in a bomb tube at 100° for 8 hr. using methanol as a solvent. After recrystallization from methanol-ether the product melted over 300°.

Anal. Calcd. for $C_{12}H_{24}NI$: I, 41.04. Found: I, 40.65.

The hydrochloride was prepared in the usual way by means of alcoholic hydrogen chloride and melted at 226–227° after recrystallization from methanol-ether.

Anal. Calcd. for $C_{11}H_{22}NCl$: Cl, 17.42. Found: Cl, 17.54.

Dialkylaminoalkyl camphorimides. Into a flask fitted with a reflux condenser was placed 0.4 mole of camphoric anhydride. With cooling and intermittent shaking, 0.41 mole of the appropriate dialkylaminoalkylamine was added slowly. After the reaction had subsided, the reaction mixture was heated until a clear homogeneous melt was obtained and then maintained by means of an oil bath at 180° for 2 hr. The resulting crude product was fractionated in vacuum and the pure imide obtained as a colorless oil. (See Table I.)

The hydrochlorides of the above imides were prepared in the usual way by means of alcoholic hydrogen chloride and recrystallized from methanol or isopropanol-ether mixtures.

The methiodides of the above imides were prepared by reaction with methyl iodide in absolute alcohol in the usual way and recrystallized from methanol or isopropanol-ether.

N-Dialkylaminoalkyl-1,8,8-trimethyl-3-azabicyclo-[3.2.1]*-octanes.* These were prepared in a manner analogous to that of the N-methyl camphidine base above. (See Table III.)

The dihydrochlorides and monomethiodides of the N-substituted camphidine bases above were prepared in the usual manner and recrystallized from methanol-ether.

The dimethiodides of these bases were obtained by heating the base in a bomb tube at 100° for 8 hr. with an excess of methyl iodide and were recrystallized from methanol.

α,ω-Bis(1,8,8-trimethyl-3-azabicyclo[3.2.1]-3-octyl)alkane dimethonium salts. (See Table V.)

To 0.06 mole of N-methyl camphidine dissolved in 20 ml. of isopropanol in a bomb tube was added 0.03 mole of the α,ω-dihalogenated alkane. The mixture was allowed to stand at room temperature for 1 hr. and then heated to 100° and maintained at this temperature for an additional 8 hr. The crude product was filtered, washed with alcohol-ether mixture, recrystallized from a mixture of methanol and ethanol, and dried.

WASHINGTON 7, D. C.

[CONTRIBUTION FROM U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE, PUBLIC HEALTH SERVICE, THE ROBERT A. TAFT SANITARY ENGINEERING CENTER]

Structure of Dactylin

G. E. INGLETT[1]

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Dactylin is shown to be isorhamnetin 3,4′-diglucoside, I.

A flavonoid glycoside, dactylin, was found to occur in pollens of timothy and orchard grass in 1931 by Moore and Moore.[2] This flavonoid glycoside has become of particular importance recently since it was shown by Johnson *et al.*[3] to possess allergenic activity. Whether this activity is associated with the pure dactylin or with impurities in the pigment remains to be established. However, the present studies are concerned only with the structure of dactylin.

Heyl[4] in 1919 isolated a quercetin glucoside as the least soluble flavonoid from ragweed pollen and isorhamnetin by hydrolysis of the more soluble fractions. Since this report, several investigators have reported the presence of flavonoids in pollens.[5–7] Kuhn and Löw[8] have isolated and characterized a flavonoid glycoside of *Crocus Sir John Bright* to be isorhamnetin 3,4′-diglucoside.

Dactylin was obtained as a light yellow solid from a 1955 crop of defatted timothy pollen by alcoholic extraction. It gave only one spot on chromatograms developed in three different solvent systems. Methoxyl content was 3.60%, and the dactylin aglycone, obtained by 2*N* sulfuric acid hydrolysis of dactylin, revealed 6.93% methoxyl content. Infrared spectra, ultraviolet spectra, and melting point data revealed the aglycone to be impure isorhamnetin. Acetylation of dactylin aglycone

(1) Present address, Box 345, Corn Products Refining Co., Argo, Ill.

(2) M. B. Moore and E. E. Moore, *J. Am. Chem. Soc.*, **53**, 2744 (1931).

(3) M. C. Johnson, S. F. Hampton, A. W. Schiele, S. Frankel, *J. Allergy*, **25**, 82 (1954).

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(6) M. S. El Ridi, L. A. Strait, and M. H. A. Wafa, *Arch. Biochem. and Biophys.*, **39**, 317 (1952).

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(8) R. Kuhn and I. Löw, *Ber.*, **77**, 196 (1944).

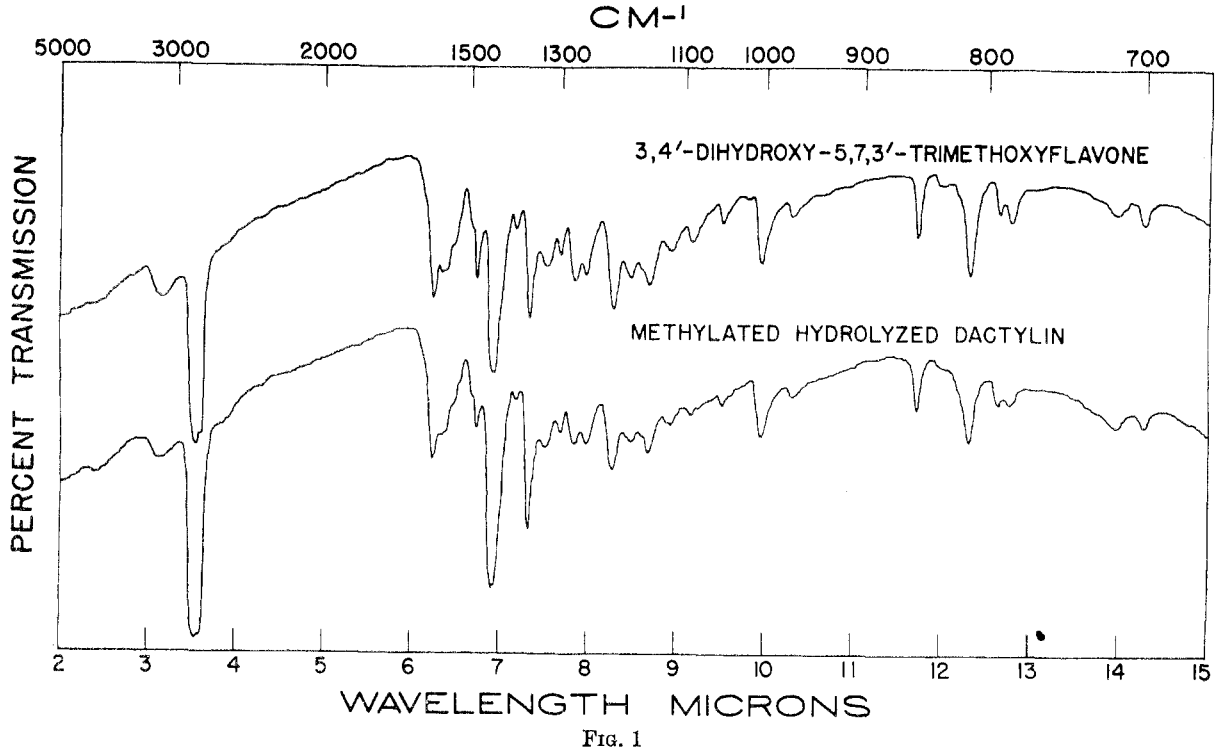
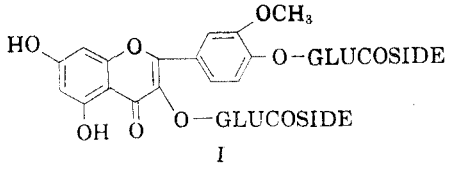


FIG. 1

gave 3,5,7,4′-tetraacetoxy-3′-methoxyflavone that gave an infrared spectrum identical in every respect with a known sample. Methylation of the aglycone with diazomethane gave 3,7,3′,4′-tetramethoxy-5-hydroxyflavone that gave an infrared spectrum that was also identical in every respect with a known sample.

When dactylin was methylated and hydrolyzed 3,4′-dihydroxy-5,7,3′-trimethoxyflavone was obtained that was identified by mixed melting point and identical infrared spectrum with a known sample (Fig. 1). The structure of dactylin is, therefore, shown to be isorhamnetin 3,4′-diglucoside (I). It is interesting to note that this compound (I) has the same structure as the one obtained by Kuhn and Löw[8] from the *Crocus Sir John Bright.*



I

EXPERIMENTAL

Dactylin, isolation and properties. A 50 g. sample of timothy pollen (*Phleum pratense*)[9] from a 1955 crop was extracted continuously in a Soxhlet with chloroform for 3 hr. The thimble containing the defatted pollen was dried in the air, and afterward it was extracted continuously with 95% ethanol for 24 hr. The yellow alcoholic solution was evaporated to an oily residue on a steam bath. To the residue 25 ml. of acetone was added, the solution was heated to a boil and decanted, and this process repeated twice more. The remaining gummy residue was dissolved in 10–15 ml. of water on heating to a boil. The solution was filtered and cooled. On storing at 2° for several weeks, crystallization occurred. The crystalline material was collected on a filter and dried, 0.384 g. It was recrystallized from water to give a light yellow solid, 150 mg., m. p. 187–190°. For a 1:1 mixture of $C_{27}H_{30}O_{17}\cdot H_2O$ with $C_{28}H_{32}O_{17}$ the following calculations are given.

Anal. Calcd. for $C_{27}H_{30}O_{17}\cdot H_2O$ with $C_{28}H_{32}O_{17}$: C, 51.38; H, 5.02; OCH_3, 2.41. Found: C, 50.61; H, 5.73; OCH_3, 3.60.

Moore and Moore did not report methoxyl determination of their dactylin samples from orchard grass (*Dactylis glomerata* L.) or timothy pollens, but their carbon and hydrogen analyses agreed with our sample as well as the other general properties of the flavonoid glycoside. Kuhn's[8] flavonoid glycoside, isorhamnetin-3,4′-diglucoside, isolated from the pollen of *Crocus Sir John Bright* had practically the same empirical formula.

Paper chromatography of timothy pollen extracts revealed dactylin to be the major flavonoid glycoside with R_F values of 0.48 and 0.24 in *n*-butyl alcohol–acetic acid–water (8–2–5) at 26° and *m*-cresol–acetic acid–water (48–2–50) at 20°, respectively. Recrystallized dactylin from these extracts gave R_F values of 0.48, 0.42, 0.36, and 0.24 in *n*-butyl alcohol–acetic acid–water (8–2–5) at 26°, *n*-butyl alcohol–acetic acid–water (4–1–5) at 20°, *n*-butyl alcohol–acetic acid–water (4–1–5) at 26°, and *m*-cresol–acetic acid–water (48–2–50) at 20°, respectively. Dactylin was detected on the papergrams by spraying the dried paper strips with 5% aqueous aluminum chloride and observing the yellow fluorescence spot under ultraviolet light in a dark room. In a similar manner, on spraying the strip with aqueous ammonia, a yellow spot can be observed in the visible and fluorescence in the ultraviolet. Ammoniacal silver nitrate was not reduced by dactylin. In a similar manner, vanillin, 2,4-dihydroxybenzaldehyde, 3-hydroxyflavone, 2,4-dihydroxyacetophenone, 2,4-dihydroxybenzoic acid, hesperidin and arbutin, when spotted on paper and sprayed with ammoniacal silver nitrate, did not show any appreciable reaction with this reagent. However, catechol, 2,6-dimethoxyphenol, rutin, quercetin, and isoquercitrin reduced this reagent

(9) Purchased from the Greer Drug Co., Inc., Lenoir, N. C.

immediately, and reduction with isorhamnetin, phloroglucinol, syringic acid, and resorcinol occurred after several minutes.

The extract of orchard grass revealed the presence of dactylin and another component having R_F values of 0.66 and 0.45 in *n*-butyl alcohol–acetic acid–water (8–2–5) at 26° and *m*-cresol–acetic acid–water (48–2–50) at 20°, respectively. Isoquercitrin from the extract of giant ragweed pollen has the same R_F values in these two solvent systems. Identical R_F values to the ones reported by Bates-Smith[10] for isoquercitrin, quercitrin, quercetin, and rutin were obtained in the two solvent systems used at 20°.

An ultraviolet spectrum of dactylin in water gave λ_{max} 252, 262 and 340 mμ (ϵ = 19600, 20500, 14100, respectively). On adding three drops of 0.1*N* sodium hydroxide to the cell, λ_{max} 273 and 364 mμ (ϵ = 25600 and 11500, respectively) peaks were observed.

Dactylin aglycone (isorhamnetin). A 110-mg. sample of dactylin was hydrolyzed on mixing with 20 ml. of 2*N* hydrochloric acid and refluxed for 20 min. A yellow crystalline solid separated and after cooling was collected on a filter. After drying, 47.7 mg. (43.4% of dactylin) of dactylin aglycone was obtained, m.p. 302–304° (dec.)[11], no sublimation noted. Quercetin obtained from the hydrolysis of rutin gave a melting point of 305–308° with sublimation that appears to be a distinguishing feature. For a 1:1 mixture of $C_{15}H_{10}O_7$ with $C_{16}H_{12}O_7$ the following calculations are given.

Anal. Calcd. for $C_{15}H_{10}O_7$ with $C_{16}H_{12}O_7$: C, 60.18; H, 3.59; OCH_3, 5.01. Found: C, 60.41; H, 4.07; OCH_3, 6.93.

Paper chromatography of the dactylin hydrolysis product gave tailing in the solvent systems used for studying dactylin. The material was detected by spraying the dried strips with 5% aluminum chloride solution and observing fluorescence under ultraviolet light. Quercetin and isorhamnetin give identical behavior on papergrams, but no accurate R_F values could be obtained because of excessive tailing.

Paper chromatography of the dactylin hydrolysis filtrate revealed glucose to be the only sugar present. R_F values of 0.21, 0.26, and 0.62 were observed for dactylin sugar that corresponded to the R_F values of glucose in ethyl acetate–pyridine–water (2–1–2), *n*-butyl alcohol–acetic acid–water (4–1–5), and *n*-butyl alcohol–acetic acid–water (8–2–5) solvent systems at 26°. The sugar was detected using anisidine hydrochloride spray. Moore and Moore identified glucosazone from the hydrolysis of their material after reaction with phenylhydrazine, but this osazone could have been derived from fructose or mannose as well as glucose. A quantitative determination of dactylin sugar using the method of Morris[12] on the filtrate gave 58 mg. (53% of dactylin).

The ultraviolet spectrum of dactylin aglycone in 95% ethanol gave λ_{max} 256 (ϵ = 10200) and λ_{max} 373 (ϵ = 21000). On adding a few drops of 0.1*N* sodium hydroxide to the cell, a spectrum was obtained with λ_{max} 248 (ϵ = 7020) and λ_{max} 324 (ϵ = 16400). Similar ultraviolet spectra for isorhamnetin are reported by Kuhn[8] and Tappi and Menziani[7] for isorhamnetin.

Isolation of dactylin aglycone. An alcoholic extract of 50 g. of timothy pollen as described above was evaporated to a small volume. The resulting oily material was suspended in the *n*-butyl alcohol layer of *n*-butyl alcohol–acetic acid–water (4–1–5) mixture and passed over a cellulose column. The cellulose column was prepared from Whatman cellulose powder (Standard Grade) that had previously been washed with the *n*-butyl alcohol mixture and dried. The column (2 cm. × 28 cm.) was composed of approximately 36 layers. A rapid moving, brown colored layer was the first material to pass from the column in 75 ml. of collected effluent. A light yellow band remained on the column and was removed in the following 75 ml. of solvent. The yellow colored fraction was concentrated to near dryness on a steam bath, and the resulting oil was hydrolyzed in 20 min. with 2*N* sulfuric acid. The resulting solid that separated was collected on a filter and washed with 95% ethanol. On drying, 65 mg. sample of yellow dactylin aglycone was obtained.

Acetylated dactylin aglycone (3,5,7,4′-tetraacetoxy-3′-methoxyflavone). In a centrifuge tube, 2.5 ml. of acetic anhydride, 28 mg. of dactylin aglycone and two drops of pyridine were refluxed for 4 hr. The acetic anhydride, acetic acid, and pyridine were removed under a jet of air. Crystallization occurred when a few drops of absolute ethanol were added to the residue. The solid was recrystallized from absolute ethanol to give a white solid, m.p. 199–201°. A second recrystallization from absolute ethanol–acetone gave a solid with m.p. 204–205°.

A sample of 3,5,7,4′-tetraacetoxy-3′-methoxyflavone was prepared by the acetylation of isorhamnetin in the same manner as described to give a melting point of 209–210°. On admixture of 10% acetylated dactylin aglycone with 3,5,7,4′-tetraacetoxy-3′-methoxyflavone, no depression of melting point was observed. Infrared spectra of the two samples were identical in every respect.

Methylated Dactylin Aglycone (3,7,3′,4′-Tetramethoxy-5-hydroxyflavone). A 65 mg. sample of dactylin aglycone was suspended in 5 ml. of methanol, and approximately 700 mg. of diazomethane from nitrosomethylurea in 25 ml. of ether (dried over potassium hydroxide pellets) was added with shaking. The solution became a brick red color, but after standing stoppered overnight, the solution was light amber. Two drops of glacial acetic acid were added to decompose excess diazomethane. The solution was filtered and evaporated to a syrup. Absolute ethanol was added, and, on allowing to evaporate slowly in the cold to a small volume, yellow crystals separated from the solution. The supernatant was removed by decantation and the crystals were dried under a flow of nitrogen to give 18.3 mg. of yellow solid. A second 15.5 mg. crop of crystals was obtained from the mother liquor. The two crops were combined, m.p. 145–146°. The compound was recrystallized from absolute alcohol to give a white solid, m.p. 146–147°.

Anal. Calcd. for $C_{19}H_{18}O_7$: C, 63.67; H, 5.07; OCH_3, 34.66. Found: C, 64.47; H, 5.35; OCH_3, 36.12.

An infrared spectrum of this compound was found to be identical in every respect with 3,7,3′,4′-tetramethoxy-5-hydroxyflavone.

3,7,3′,4′-Tetramethoxy-5-hydroxyflavone. A 178 mg. sample of doubly recrystallized quercetin (from hydrolyzed rutin) was allowed to react with diazomethane by the usual technique. The 41.6 mg. sample of 3,7,3′,4′-tetramethoxy-5-hydroxyflavone was recrystallized from absolute ethanol to give yellow colored needles, m.p. 159.5°, 25.3 mg.

From the mother liquors a second form[13] of 3,7,3′,4′-tetramethoxy-5-hydroxyflavone was obtained as white pellets. The solid was collected on a filter and washed with absolute ethanol, 30.2 mg., m.p. 145–147°. It was recrystallized from absolute alcohol and the white pellets collected on a filter, m.p. 146–148°.

Anal. Calcd. for $C_{19}H_{18}O_7$: C, 63.67; H, 5.07; OCH_3, 34.66. Found: C, 64.74; H, 5.12; OCH_3, 35.63.

Methylated hydrolyzed dactylin (3,4′-dihydroxy-5,7,3′-trimethoxyflavone). A 93.8 mg. sample of recrystallized dactylin was allowed to react with diazomethane by the usual technique. The material obtained of this reaction was hydrolyzed for 10 min. with 2*N* sulfuric acid to give a bright red colored solution that turned yellow on allowing to stand in a hot water bath. Yellow crystals separated and were collected on a filter and dried, 55 mg. The solid was recrystallized from absolute ethanol by allowing the filtered alcoholic

(10) E. C. Bates-Smith, *Partition Chromatography*, Biochemical Society Symposia No. 3, p. 62, 1950.

(11) Fisher-Johns hot stage was used for taking these melting points. The other melting points were obtained in a capillary and are corrected.

(12) D. L. Morris, *Science*, **107**, 254 (1948).

(13) A. S. Gomm and M. Nierenstein, *J. Am. Chem. Soc.*, **53**, 4408 (1931).

solution to cool overnight in a refrigerator. The crystalline material was collected on a filter and dried, 23.8 mg., m.p. 205–7°. The material was again recrystallized to give a slightly yellow colored solid, m.p. 205–7°.

Anal. Calcd. for $C_{18}H_{16}O_7$: C, 62.77; H, 4.69. Found: C, 61.94; H, 4.81.

On admixture of methylated hydrolyzed dactylin with 3,4′-dihydroxy-5,7,3′-trimethoxyflavone, no depression of melting point was observed. Infrared spectra of the two samples were identical in every respect as shown in Fig. 1.

Acknowledgment. The author wishes to thank Dr. Richard Kuhn, who graciously supplied samples that allowed a rapid solution to this problem.

CINCINNATI, OHIO

[CONTRIBUTION FROM KAY-FRIES CHEMICALS, INC.]

Preparation of Cytosine

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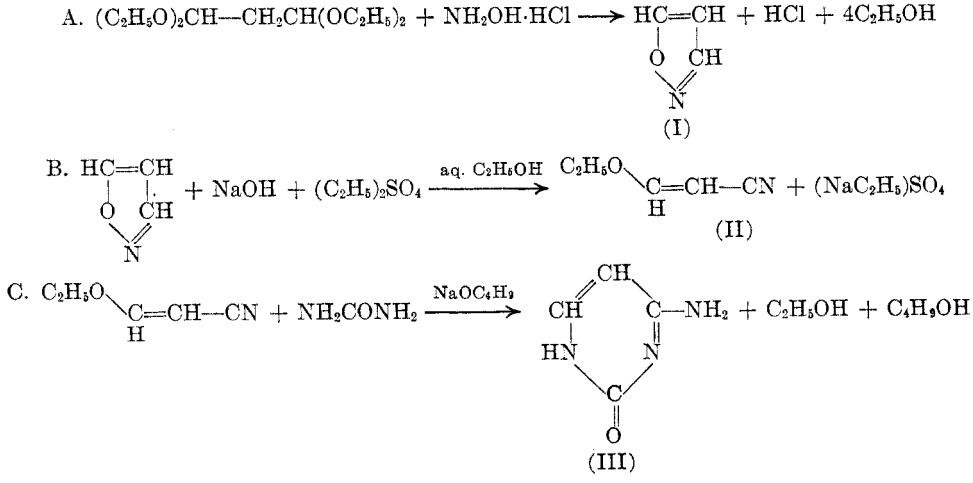
A new method for the preparation of cytosine is described. Isoxazole is prepared by the reaction of malonaldehyde tetraethyl acetal with hydroxylamine hydrochloride. β-ethoxyacrylonitrile is prepared by reaction of isoxazole with diethyl sulfate in alkaline solution. Cytosine is prepared by condensing β-ethoxyacrylonitrile with urea in a sodium alcoholate solution.

In the development of certain work in this laboratory, cytosine was desired. It therefore became necessary to prepare cytosine in sufficient quantities so that further reactions of it could be studied. The preparation of cytosine from uracil, thiouracel, dithiouracil, and cyanoacetal has been reported.[1–4] The procedures and results obtained by the above methods did not seem suitable for our purposes, since yields are invariably low and the processing of the necessary intermediates is involved and time consuming. Consequently, an alternative method of preparation of cytosine was developed.

This method employed the following sequence of reactions:



Isoxazole (I) was obtained in 70% yield from 1,1,3,3-tetraethoxypropane (malonaldehyde acetal) and hydroxylamine hydrochloride. The reaction of osoxazole with diethyl sulfate and sodium hydroxide to form β-ethoxyacrylonitrile (II) proceeded to give a yield of 85–90% of a mixture of β-ethoxyacrylonitrile and cyanacetaldehyde acetal. The condensation of β-ethoxyacrylonitrile with urea in a refluxing sodium butylate solution resulted in a 43% yield of cytosine (III). All the steps were characterized by the absence of by-products except in the case of β-ethoxyacrylonitrile which invariably contained varying amounts of cyanoacetaldehyde acetal. The β-ethoxyacrylonitrile was obtained substantially pure by dealcoholating a mixture containing the β-ethoxyacrylonitrile and cyanoacetaldehyde acetal with heat at atmospheric pressure.

EXPERIMENTAL

Isoxazole (I). Two hundred twenty grams (1.0 mole) of malonaldehyde tetraethyl acetal prepared by the method of

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(2) D. J. Brown, *J. Soc. Chem. Ind. (London)*, 69, 353 (1950).

(3) G. Hitchings and P. Russell, *J. Biol. Chem.*, 177, 357 (1949).

(4) A. Bendich, H. Getler, and G. Brown, *J. Biol. Chem.*, 177, 565 (1949).