The Emulsin Preparation.—Tests similar to the above, but using an emulsin preparation (Nutritional Biochemical Corp., Cleveland, Ohio) were performed on cellobiose, salicin, methyl α-D-glucoside and maltose. The emulsin behaved as it was supposed to do, catalyzing the hydrolysis of the two β-glucosides, but not of the α-glucosides.

Enzymatic Hydrolysis of Isoquercitrin.—A preparation of α-glucosidase was obtained as described above and its *p*H was adjusted to approximately 5 by the addition of an equal volume of 0.5 *M* citrate buffer of *p*H 7. A few crystals of authentic isoquercitrin were then added to the solution and the mixture agitated to dissolve the flavonoid glucoside. The mixture was allowed to incubate at room temperature for 6 hr.

For the emulsin studies, a few crystals of the isoquercitrin were added to 0.5 ml. of a citrate buffer of *p*H 5 and brought nto solution by shaking and heating. To the mixture was added approximately 3 mg. of the solid emulsin, and the mixture shaken and allowed to incubate at room temperature for 6 hr. Individual controls on each of the citrate buffers plus isoquercitrin, but containing no enzyme, were run at the same time.

The presence or absence of the aglycone quercetin and/or the original, unhydrolyzed isoquercitrin was ascertained at the end of the incubation period by paper chromatography, using a 15% acetic acid–water system. No hydrolysis of the isoquercitrin could be detected with the controls and with the α-glucosidase preparation, but practically complete hydrolysis of the isoquercitrin was observed with the emulsin under the experimental conditions used.

A sample of isoquercitrin prepared by the partial hydrolysis of rutin[3] and chromatographically identical with isoquercitrin from grapes likewise was hydrolyzed by emulsin but not by the α-glucosidase.

Enzymatic Hydrolysis of Other Flavonoid Glucosides.—By the method described above for isoquercitrin, the flavonoid glucosides hesperetin-7-glucoside, naringenin-7-glucoside, quercimeritrin and the unknown quercetin glucoside from apricots were investigated. The first two of these were prepared as described by Fox, *et al.*,[3] and the quercimeritrin was obtained through the courtesy of T. R. Seshadri, Delhi, India. All four of these were hydrolyzed with emulsin, but not with the α-glucosidase.

Hesperidin, from which the hesperetin-7-glucoside had been prepared, has been shown by Zemplén and co-workers[4] to be a β-rhamnoglucoside. Apparently the partial hydrolysis of the hesperidin with formic acid to obtain the hesperetin-7-glucoside did not alter the glucosyl attachment to the aglycone in this particular case. If this were also to apply to the partial hydrolysis of rutin (quercetin-3-rhamnoglucoside)[5] and to naringin (naringenin-7-rhamnoglucoside), then from the results reported here these two rhamnoglucosides could possibly be considered to have the glucosyl unit attached beta onto the aglycone. Experimentally, rutin is not hydrolyzed with emulsin or the α-glucosidase.

Acknowledgment.—This research was supported in part by the Office of Naval Research.

(3) D. N. Fox, W. L. Savage and S. H. Wender, THIS JOURNAL, **75**, 2504 (1953).

(4) G. Zemplén, A. K. Tettamanti and S. Faragó, *Ber.*, **71B**, 2511 (1938).

(5) G. Zemplén and A. Gerecs, *ibid.*, **68B**, 1318 (1935).

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Products of the Oxidative Degradation of Thiophene by Nitric Acid[1a]

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The action of nitric acid on thiophene results in the oxidative decomposition of the thiophene molecule with, apparently, a quantitative conversion of the sulfur to sulfuric acid.[2] In the present research, an attempt was made to discover something of the nature of the intermediate products and by-products of this interesting reaction.

It has been observed that 3,4-dibromothiophene and tetrabromothiophene both give dibromomaleic acid when subjected to treatment with cold concentrated nitric acid.[3] In a similar manner, 3-methyl-2,4,5-tribromothiophene gave rise to bromocitraconic acid, $HOOCC(Br)=C(CH_3)COOH$, and the 2-methyl-3,4,5-tribromo isomer was converted to dibromoacetylacrylic acid, $CH_3CO—CBr=CBr—COOH$.[3] The action of ozone on a cold suspension of thiophene in water gave oxalic acid, carbon dioxide and sulfuric acid.[4] With oxygen in the presence of light, formic acid was formed in addition to oxalic acid.[5] On the other hand, succinic acid (dithallous succinate) is said to be formed by bubbling air through a suspension of thiophene in aqueous thallous hydroxide.[6] The action of potassium chlorate on tetraphenylthiophene (thionessal) produced dibenzoylstilbene, $C_6H_5CO—C(C_6H_5)=C(C_6H_5)—COC_6H_5$, in good yield.[7] The products of the oxidation of thiophene and its bromo derivatives by hydrogen peroxide,[8] hypochlorous acid[9] or calcium hypochlorite[10] were not identified.

Experimental

For the purpose of isolating and identifying the reaction products, 30 ml. of a 5% solution of thiophene in cyclohexane was refluxed with 300 ml. of 8 *N* nitric acid. The reaction mixture went through the following color changes: colorless, turbid, yellow, orange, clear yellow. The reaction was terminated a few minutes after attaining the clear yellow stage. The solution was allowed to cool, and the cyclohexane layer was removed by means of a separatory funnel. The cyclohexane phase was then extracted with distilled water and the washings added to the yellow aqueous solution.

The Disappearance of Thiophene.—A small portion of the yellow aqueous solution was tested with isatin in concentrated sulfuric acid, but no color was obtained. This indicates that all the thiophene in the aqueous phase had already been consumed.

The Presence of Sulfate.—Another small portion of the solution was diluted with distilled water and tested with barium chloride solution. The resulting copious precipitate of barium sulfate bore evidence to the fact that the reaction had proceeded at least to the point where sulfuric acid was being formed.

Isolation of 2-Nitrothiophene.—A 150-ml. portion of the acidic yellow solution was neutralized with solid sodium hydroxide, whereupon the color changed to dark reddish-orange. A little concentrated hydrochloric acid was added until the solution became bright yellow again and the slightly acidic solution was steam distilled. Yellow-white crystals formed within the condenser. On recrystallizing from ethyl alcohol, long white crystals of 2-nitrothiophene were obtained; m.p. 45–46°. A mixed melting point with 2-nitrothiophene, obtained by nitration of thiophene in acetic acid solution,[11] showed no depression. It was confirmed

(1) (a) Taken from a portion of the Doctoral Dissertation of Leonard S. Levitt, Temple Univ., 1953. (b) Chemistry Department, Stevens Institute of Technology, Hoboken, N. J.

(2) L. S. Levitt and E. Howard, Jr., *Anal. Chem.*, **25**, 196 (1953).

(3) A. Angeli and G. Ciamician, *Ber.*, **24**, 74, 1347 (1891).

(4) M. Freri, *Gazz. chim. ital.*, **63**, 281 (1933).

(5) E. Sernagiotto, *Atti Accad. Lincei*, **28**, I, 432 (1919).

(6) G. T. Morgan and W. Ledbury, *J. Chem. Soc.*, **121**, 2882 (1922).

(7) J. Dorn, *Ann.*, **153**, 352 (1870).

(8) M. Lanfry, *Compt. rend.*, **153**, 73 (1911).

(9) E. G. Ardagh and W. H. Bowman, *J. Soc. Chem. Ind.*, **54**, 267 (1935).

(10) E. G. Ardagh, W. H. Bowman and H. S. Weatherburn, *ibid.*, **58**, 249 (1939).

(11) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, New York, N. Y., 1943, p. 466.

that this compound gives a negative test with isatin in sulfuric acid.[12]

Identification of 2,5-Dinitrothiophene.—The remainder of the original acidic solution (145 ml.) was twice extracted with 100-ml. portions of diethyl ether, resulting in the removal of practically all of the yellow coloring of the solution. The combined ether extracts were evaporated down, and there remained a yellow-orange semi-solid residue. Since it was suspected that this substance might be, at least in part, 2,5-dinitrothiophene, a test[11,13] for this compound was performed. A small amount of the substance was dissolved in alcohol, and concentrated sodium hydroxide solution was then added. Formation of a deep pink color which disappeared on addition of an excess of sodium hydroxide indicated the presence of 2,5-dinitrothiophene. It was discovered that a much more sensitive test for this compound (and perhaps for other dinitro compounds) may be performed by dissolving the slightest trace in a few ml. of acetone and adding a pellet of solid sodium hydroxide. After a few minutes an intensely deep blue color[14] develops, which, likewise, disappears after more of the sodium hydroxide has dissolved. Repetition of these tests with 2-nitrothiophene revealed that no noticeable color was produced, thus confirming the specificity of the tests.

Isolation of Maleic Acid.—The nearly colorless aqueous solution remaining from the previous extraction was extracted five more times with 50-ml. portions of ether. The combined extracts were evaporated to dryness and a very small quantity of a yellow-white powder remained. This residue was very acidic and gave no test for sulfur or nitrogen in an elemental analysis. On attempting to determine its melting point, it was found to char at about 140°. An anilide was made by heating the sodium salt of the compound with aniline hydrochloride at 200° for four hours.[15] The tan colored anilide was recrystallized from alcohol; m.p. 188–190°. Since maleic acid melts at 130° and decomposes at 135° and its anilide melts at 187°, it is probable that the compound isolated here was maleic acid.

Isolation of Oxalic Acid.—The aqueous solution remaining after the many ether extractions was evaporated to 15 ml. and cooled with ice, whereupon a white crystalline acidic material precipitated. It was recrystallized from water; m.p. 101° (the melting point of oxalic acid dihydrate). The identity of this compound was confirmed by its immediate decolorization of hot dilute permanganate solution, and by preparation of the diethyl ester.

Discussion

The products formed by the action of 8 *N* nitric acid on thiophene in cyclohexane are 2-nitrothiophene, 2,5-dinitrothiophene, maleic acid, oxalic acid and sulfuric acid. These are all substances which one might logically expect to appear in a reaction of this type, particularly in the light of evidence obtained previously in the oxidation of derivatives of thiophene.[3–7]

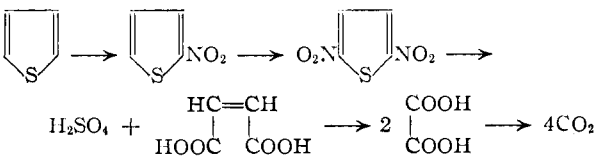
It is apparent that the mononitro derivative must be formed prior to the dinitro compound, just as maleic acid must precede oxalic acid. Whether or not the nitrothiophenes are necessarily precursors of the two acids is more a matter for speculation. Some evidence, however, might be brought to bear on this point.

The yellow intermediates (presumably nitro compounds) are formed shortly after the start of the reaction and, since no appreciable concentration of thiophene derivatives can remain at the end of the reaction because of the conversion of the sulfur to sulfate,[2] it would seem that the nitrothiophenes are among the first of the reaction products and probably precede the stage of oxidative ring cleavage which gives rise to the four- and two-carbon dicarboxylic acids. The concentration of the nitro compounds must be built up to a maximum when the deepest orange color of the reaction mixture has been attained, and then, with little or none of the original thiophene remaining as a source of additional nitrothiophenes, the predominant reaction must become that of oxidative ring opening. The maleic acid thus formed would then be split oxidatively at the double bond to give oxalic acid, which must, in its turn, be oxidized to carbon dioxide, since it cannot long survive the conditions of the reaction.[2]

As an alternative, it would appear possible that direct cleavage of the thiophene ring could occur simultaneously with the competing reaction of nitration. In such an event, those molecules of thiophene which were first nitrated would have to be cleaved in a subsequent reaction.

Conclusive evidence which would seem to preclude the possibility of this second alternative is furnished by a kinetic run made on the thiophene–nitric acid reaction in homogeneous solution of water and dioxane at 65°. Within the first 45 minutes of the kinetic run, all the thiophene had been consumed,[16] but not until after 75 minutes had any sulfate been detected. This shows unequivocally that under the conditions of the kinetic run (which, except for the lower temperature and the presence of dioxane, were essentially the same as in the isolation studies) all the thiophene is converted to an intermediate compound before any of it is completely decomposed to sulfuric acid.

In the light of the evidence obtained during this investigation, it appears almost certain that the oxidative decomposition of thiophene by nitric acid follows the sequence



It cannot be said with certainty how many steps are involved in the conversion of 2,5-dinitrothiophene to maleic acid. It seems probable that the actual cleavage must be preceded by oxidation at the sulfur atom. This might take place by removal of one or more electrons from the sulfur atom in one or more distinct steps, the most likely electron extractors being NO_2^+, NO^+ or HO^+. This could occur, presumably, with or without the formation of a short-lived sulfoxide or sulfone, which would appear to be likely products of such oxidative attack. All attempts to prepare a thiophene sulfoxide or sulfone by direct oxidation have failed.[17] Such reactions lead invariably to decomposition of the thiophene molecule, probably because of the decreased

(12) V. Grignard, G. Dupont and R. Locquin, "Traité de Chimie Organique," Vol. XVIII, Masson and Cie., Paris, 1945, p. 571.

(13) V. Meyer and H. Stadler, *Ber.*, **17**, 2778 (1884).

(14) This blue solution exhibits a sharp absorption maximum at 590 mμ. at which wave length it obeys Beer's law, thus providing a spectrophotometric method for the determination of 2,5-dinitrothiophene.

(15) S. M. McElvain, "The Characterization of Organic Compounds," The Macmillan Co., New York, N. Y., 1947, p. 184.

(16) The samples were tested with isatin in concentrated sulfuric acid. It should be noted that neither 2-nitrothiophene nor any 2,5-disubstituted thiophene forms a colored product with isatin.

(17) R. C. Elderfield, "Heterocyclic Compounds," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1950, p. 214.

availability of the sulfur atom's two lone electron pairs for resonance stabilization of the aromatic system.

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Derivatives of 5-Amino-4-methylpyrimidine

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We have been interested in synthetic routes which lead to aminopyrimidines easily converted to aminovinylpyrimidines.[3] A number of workers,[4-6] particularly in the last few years, have reported the synthesisis of 5-amino-4-methylpyrimidine (I) and we wish to report some new experimental procedures for the synthesis of this compound and several new derivatives.

2,4-Dichloro-6-methyl-5-nitropyrimidine was reduced with excess Raney nickel to give 5-amino-2,4-dichloro-6-methylpyrimidine (II) in 66% yield. Reaction of II with hydrogen with palladium-on-charcoal catalyst and magnesium oxide gave I in 31% yield and 5-amino-2-chloro-4-methylpyrimidine in 34.7% yield. The latter compound was easily dechlorinated to give I in 78% yield with the above catalyst.

We investigated a second route for the preparation of I, namely, the chlorination of 5-amino-6-methyluracil to give II; however, this method is inferior due to low yields and formation of a by-product, an aminochloro-N-methylanilinomethylpyrimidine, since dimethylaniline is used along with phosphorus oxychloride in the reaction.

Reductive alkylation[7] of I with a large excess of freshly distilled acetaldehyde with palladium on charcoal gave a 78% yield of 5-N-ethylamino-4-methylpyrimidine; with Raney nickel the reaction was unsuccessful.

An attempt was made to prepare 5-bromo-4-methylpyrimidine by the catalytic dechlorination of 5-bromo-2,4-dichloro-6-methylpyrimidine. 5-Bromo-6-methyluracil was chlorinated with phosphorus oxychloride in 84.8% yield. The compound was catalytically reduced with palladium-on-charcoal and magnesium oxide until two moles of hydrogen was absorbed. However, only a 42.8% yield of 4-methylpyrimidine was isolated from the reaction mixture. Very recently Whittaker[8] attempted to prepare 5-bromopyrimidine through the same synthetic route with the reaction following a similar course as that described for the methyl derivative above.

(1) Public Health Research Fellow, 1951–1953.

(2) This note comprises portions of theses presented by Irving C. Kogon in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Graduate School of the Polytechnic Institute of Brooklyn and by W. J. Einstman in partial fulfillment of the requirements for the degree of Master of Science in the Graduate School of the Polytechnic Institute of Brooklyn.

(3) (a) C. G. Overberger and I. C. Kogon, THIS JOURNAL, **76**, 1065 (1954); (b) **76**, 1879 (1954).

(4) J. R. Marshall and J. Walker, *J. Chem. Soc.*, 1004 (1951).

(5) K. Yanai, *J. Pharm. Soc. Japan*, **62**, 315 (1951); *C. A.*, **45**, 5150 (1951).

(6) S. Gabriel and J. Colman, *Ber.*, **34**, 1234 (1901).

(7) W. S. Emerson, "Organic Reactions," Edited by R. Adams, Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1948, Chap. 3, p. 174.

(8) N. Whittaker, *J. Chem. Soc.*, 1646 (1953).

Experimental[9]

5-Amino-2,4-dichloro-6-methylpyrimidine.—2,4-Dichloro-6-methyl-5-nitropyrimidine was prepared by the chlorination of 6-methyl-5-nitrouracil following the procedure of Marshall and Walker.[4] The nitro group was then reduced as follows: a mixture of 10.3 g. (0.05 mole) of 2,4-dichloro-6-methyl-5-nitropyrimidine, 35 ml. of absolute alcohol and $^1/_2$ teaspoon of Raney nickel catalyst was hydrogenated for three hours until the theoretical amount of hydrogen was absorbed. The catalyst was removed by filtration and washed with ethanol. After the removal of the solvent a purple residue remained which was sublimed at 110° (3 mm.) to give 5.9 g. (66%), m.p. 108–110°, of a light yellow crystalline material. Two sublimations raised the melting point to 113–114°, (m.p. 115–116.5° prepared by reduction of the nitro compound with palladium-on-charcoal in ether).[5]

Preparation of this compound from 5-amino-6-methyluracil and phosphorus oxychloride gave low yields of 5-amino-2,4-dichloro-6-methylpyrimidine under a wide variety of experimental conditions with and without small additions of dimethylaniline.

5-Amino-4-methylpyrimidine. (A).—When 5-amino-2,4-dichloro-6-methylpyrimidine was hydrogenated over 10% palladium-on-charcoal in the presence of excess magnesium oxide a 31% yield of 5-amino-4-methylpyrimidine was obtained. References 4, 5 and 6 all prepared this compound using different reducing agents. The mother liquor was distilled off on a steam-bath and the crystalline residue was sublimed at 83° (0.01 mm.). A slightly yellowish, crystalline compound, 5-amino-2-chloro-4-methylpyrimidine, (34.7%), m.p. 91–92°, was collected (m.p. 92°, no yield reported),[5] (m.p. 87°, no yield reported),[4] (m.p. 93.5°).[5]

(B).—A mixture of 2.4 g. (0.016 mole) of 5-amino-2-chloro-4-methylpyrimidine, 0.1 g. of 10% palladium-on-charcoal catalyst, 10 ml. of absolute ethanol, 20 ml. of distilled water and 4 g. (0.1 mole) of magnesium oxide was hydrogenated for 1 hour until no more hydrogen was absorbed. The catalyst was removed by filtration and the filtrate extracted continuously with chloroform for 48 hours and the solution dried over anhydrous sodium sulfate. After the removal of the drying agent and solvent, a solid residue remained, 1.8 g. (78%), m.p. 149–150°. A mixed melting point with an authentic sample, m.p. 149–151°, gave no depression, m.p. 149–150°.

5-N-Ethylamino-4-methylpyrimidine.—A mixture of 5.2 g. (0.048 mole) of 5-amino-4-methylpyrimidine, 5.3 g. (0.120 mole) of acetaldehyde, 1 g. of 10% palladium-on-charcoal and 20 ml. of absolute ethanol was hydrogenated for 5 hours at 50°. After the theoretical quantity of hydrogen was absorbed, the mixture was filtered and the catalyst washed well with ethanol. After the removal of the solvent, the residue was distilled to give a yellow liquid, 5.6 g. (86.1%), b.p. 124–126° (7 mm.). On redistillation a pale yellow liquid, 5.1 g. (78.4%), b.p. 123–125° (7 mm.), was obtained which solidified on standing, m.p. 55–56°.

Anal.[10] Calcd. for $C_7H_{11}N_3$: C, 61.21; H, 8.09; N, 30.63. Found: C, 60.95; H, 8.32; N, 30.43.

The picrate was prepared by adding excess ethereal picric acid to an ethereal solution of the free base. The yellow crystalline precipitate was recrystallized from ethanol and had a m.p. 138–139°.

Anal. Calcd. for $C_{13}H_{14}N_6O_7$: N, 22.9. Found: N, 22.8.

5-Bromo-2,4-dichloro-6-methylpyrimidine.—5-Bromo-6-methyluracil was prepared by the procedure described by Behrend[11] (97.6%), m.p. 238° with dec. (90%, m.p. 230°, with slow dec.).[11] A mixture of 14 g. (0.0683 mole) of 5-bromo-6-methyluracil and 31.8 g. (0.2 mole) of phosphorus oxychloride (b.p. 105–107°) was refluxed for 3 hours. After cooling, the dark brown mixture was carefully poured with rapid stirring into 300 g. of an ice–water mixture. A green crystalline precipitate appeared which was removed by filtration and air dried for several hours. The compound was

(9) All melting points are corrected.

(10) Analyses by Drs. Weiler and Strauss, Oxford, England; Dr. K. Ritter, Zurich, Switzerland, Dr. F. Schwarzkopf, Long Island City, N. Y.

(11) R. Behrend, *Ann.*, **229**, 17 (1885).