ylamino-4,6-dichloro-1,3,5-triazine instead of the desired monochlorotriazine. In the course of numerous attempts to prepare 1,3,5-triazine from known triazine derivatives[3] we also studied this reaction some time ago.

An excess of lithium aluminum hydride in ethereal solution at room temperature converts cyanuric chloride almost completely into a mixture of inorganic compounds insoluble in ether, consisting mainly of lithium chloride, aluminum chloride and lithium aluminum cyanide, $LiAl(CN)_4$. The ether contains only traces of organic material. During the reaction hydrogen is evolved. Since these experiments have been carried out, we have discovered that 1,3,5-triazine is identical with the long known compound erroneously described as the dimer of hydrocyanic acid,[1] and we feel therefore now able to give a possible explanation of the path of this reaction

$$3LiAlH_4 + 4C_3N_3Cl_3 \longrightarrow 3LiCl + 3AlCl_3 + 4C_3H_3N_3 \quad (A)$$

The so-formed triazine is then catalytically depolymerized by the aluminum chloride[4]

$$4C_3H_3N_3 \longrightarrow 12HCN \quad (B)$$

Hydrocyanic acid reacts with lithium aluminum hydride according to Wittig and Bille[5]

$$3LiAlH_4 + 12HCN \longrightarrow 3LiAl(CN)_4 + 12H_2 \quad (C)$$

As it is impossible to isolate either triazine or hydrocyanic acid even when working with the stoichiometrical amount of the reducing agent, reactions B and C must occur with a higher rate than A. However, adding an insufficient amount of lithium aluminum hydride to a large excess of cyanuric chloride in ethereal solution at $-10°$ enabled us to separate by fractional sublimation from the unreacted starting material 2,4-dichloro-1,3,5-triazine, in small yield, obviously the first intermediate in the reduction of cyanuric chloride according to A. From the less volatile parts of the reaction mixture we obtained as a further by-product 2-dimethylamino-4,-6-dichloro-1,3,5-triazine indicating that part of the triazine formed according to equation A undergoes further hydrogenolysis to dimethylamine which then reacts with unchanged cyanuric chloride.[2] Even under these conditions the main reaction product is a mixture of ether-insoluble salts as mentioned above.

2,4-Dichloro-1,3,5-triazine is a volatile crystalline compound, m.p. 50–52°, with a pungent odor similar to cyanuric chloride but much more irritating. It is identical with the product obtained by mixed polymerization of hydrocyanuric acid and cyanogen chloride[6] following the general method described some time ago.[7] Water hydrolyzes it at least as easily as cyanuric chloride, and concentrated aqueous ammonia converts it into 2,4-diamino-1,3,5-triazine (formoguanamine).

(3) C. Grundmann, H. Ulrich and A. Kreutzberger, *Chem. Ber.*, **86**, 181 (1953).

(4) L. E. Hinkel, E. E. Ayling and J. H. Beynon, *J. Chem. Soc.*, 674 (1935).

(5) G. Wittig and H. Bille, *Z. Naturforsch.*, **6B**, 226 (1951).

(6) D. W. Kaiser and F. C. Schaefer (to American Cyanamid Co.), U. S. Patent 2,653,934 (September 29, 1953).

(7) C. Grundmann, G. Weisse and S. Seide, *Ann.*, **577**, 77 (1952).

Experimental

To a well-stirred solution of 9.2 g. of cyanuric chloride in 200 ml. of dry ether cooled by an ice–salt-bath to $-10°$ was added 1.9 g. of lithium aluminum hydride in small portions during 15 minutes in an atmosphere of nitrogen with exclusion of moisture. During the reaction the temperature rose to $-6°$. After 30 minutes the cooling bath was removed and the reaction mixture allowed to warm to room temperature during one hour. The white precipitate was removed by filtration and washed with ether (7.8 g.). The ethereal filtrate yielded on evaporation a crystalline residue soaked with oil (3.5 g.) which was dried on a porous clay plate and then sublimed at 10 mm. (bath temperature 80 to 130°). The first portion of the sublimate (0.5 g.) yielded after resublimation at 80° (10 mm.) 0.2 g. of 2,4-dichloro-1,3,5-triazine, colorless prisms, m.p. 50–52°. The analysis indicates that the material is not completely free of cyanuric chloride.

Anal. Calcd. for $C_3HN_3Cl_2$: N, 28.02; Cl, 47.22. Found: N, 26.77; Cl, 48.25

The residue from the sublimation which was essentially free of cyanuric chloride was extracted with ether; from the extract 1.6 g. of crude 2-dimethylamino-4,6-dichloro-1,3,5-triazine was isolated, m.p. after repeated recrystallization from petroleum ether 122–124°. It showed no depression in m.p. when mixed with an authentic sample.[8] Finely powdered 2,4-dichloro-1,3,5-triazine (0.15 g.) was suspended in 10 ml. of 28% aqueous ammonia, kept overnight at room temperature and then held for one hour at 80°. After evaporation of the water in an open dish on the steam-bath, the residue was recrystallized from 5 cc. of boiling water with the addition of a few drops of 40% sodium hydroxide. 2,4-Diamino-1,3,5-triazine (0.1 g.) was obtained in thin long needles melting at 316° identified by mixed melting point with an authentic sample.

(8) W. M. Pearlman and C. K. Banks, THIS JOURNAL, **70**, 3726 (1948).

OHIO STATE UNIVERSITY RESEARCH FOUNDATION
COLUMBUS 10, OHIO, AND
II. CHEMISCHES INSTITUT, HUMBOLDT UNIVERSITAET
BERLIN, GERMANY

Reaction of Azide Ion with N-Methyl-N-nitroso-N-nitroguanidine and N-Nitro-S-methylisothiourea

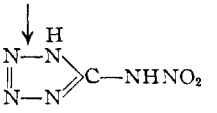
BY RONALD A. HENRY AND ROBERT H. BOSCHAN

RECEIVED NOVEMBER 4, 1953

McKay and Wright[1] demonstrated that primary alkyl or aryl amines and N-methyl-N-nitroso-N′-nitroguanidine react with the elimination of the methylnitrosamino group and the formation of substituted nitroguanidines. More recently, Fishbein and Gallaghan[2] found that these same nucleophilic reagents would eliminate methyl mercaptan from N-nitro-S-methylisothiourea with the formation of similar nitroguanidine derivatives. These reactions have now been extended to another nucleophilic reagent, namely, the azide ion. Thus, sodium azide and either N-methyl-N-nitroso-N′-nitroguanidine or N-nitro-S-methylisothiourea react to form 5-nitroaminotetrazole, which is conveniently isolated as its sparingly soluble guanidinium salt in 50 to 60% yield. The intermediate nitroguanylazide, which results from the elimina-

$$RC(NH)NHNO_2 + N_3^- \longrightarrow R^- + N_3C(NH)NHNO_2$$

R = $CH_3N(NO)$ or CH_3S



(1) A. F. McKay and G. F. Wright, THIS JOURNAL, **69**, 3028 (1947); A. F. McKay, *ibid.*, **71**, 1968 (1949).

(2) L. Fishbein and J. A. Gallaghan, *ibid.*, **76**, 1877 (1954).

tion step, immediately cyclizes under the reaction conditions to the isomeric tetrazole.

5-Nitroaminotetrazole has been prepared previously by diazotizing nitroaminoguanidine in buffered acetic acid solution,[3] or by cyclizing nitroguanylazide in the presence of inorganic or organic bases[3,4] or by nitrating 5-aminotetrazole.[5]

Experimental

Sodium Azide and N-Methyl-N-nitroso-N'-nitroguanidine.—A slurry, consisting of 0.75 g. (0.115 mole) of sodium azide, 1.5 g. (0.0102 mole) of methylnitrosonitroguanidine and 15 ml. of water, was allowed to stand at room temperature; a slow evolution of gas began immediately. Complete solution was obtained after 16 hours. The solution was then heated on the steam-bath for 30 minutes, treated with 1.3 g. of guanidine nitrate, adjusted to *p*H 3.5, diluted with 10 ml. of water and cooled to 5°. The crystalline product was removed by filtration, washed with three 5-ml. portions of cold water and dried. The yield was 1.1 g. (58.2%); m.p. 217–218° dec. One recrystallization from 25 ml. of water raised the melting point to 222–223° dec.; a mixture melting point with an authentic sample of guanidinium 5-nitroaminotetrazole[3] was not depressed. X-Ray powder patterns[6] were also identical. The calculated equivalent weight is 189.15; found, 188.7.

Sodium Azide and N-Nitro-S-methylisothiourea.—When a slurry of sodium azide (1.1 g., 0.0169 mole), N-nitro-S-methylisothiourea (1.95 g., 0.0144 mole) and 20 ml. of water was refluxed on the steam-bath for 12 hours, methyl mercaptan was evolved slowly (very little reaction occurred during 16 hours at room temperature). The product, which was isolated in the same manner as described in the previous experiment, weighed 1.7 g (62.2%) and melted at 220° dec. A mixture melting point with a known sample of guanidinium 5-nitroaminotetrazole was not depressed; X-ray powder patterns were also identical.

(3) E. Lieber, E. Sherman, R. A. Henry and J. Cohen, THIS JOURNAL, **73**, 2327 (1951).
(4) E. Lieber, C. C. Herrick and E. Sherman, *ibid.*, **74**, 2684 (1952).
(5) R. M. Herbst and J. A. Garrison, *J. Org. Chem.*, **18**, 941 (1953).
(6) L. A. Burkardt and D. W. Moore, *Anal. Chem.*, **24**, 1579 (1952).

ORGANIC CHEMISTRY BRANCH
CHEMISTRY DIVISION
U. S. NAVAL ORDNANCE TEST STATION
CHINA LAKE, CALIFORNIA

Enzymatic Hydrolysis Studies on Certain Flavonoid Glycosides

BY CHARLES W. NYSTROM, BYRON L. WILLIAMS AND SIMON H. WENDER

RECEIVED DECEMBER 4, 1953

Definite experimental evidence sufficient to resolve the question as to whether the glucosyl unit is attached alpha or beta onto quercetin (3,3′,4′,-5,7-pentahydroxyflavone) had not been previously recorded, to our knowledge, for isoquercitrin (quercetin-3-glucoside) and quercimeritrin (quercetin-7-glucoside). The present study was, therefore, undertaken to aid in the establishment or confirmation of the alpha or beta union of glucose to the aglucone in these two quercetin glucosides as well as in a quercetin glucoside of unknown structure from apricots,[1] and in hesperetin-7-glucoside and naringenin-7-glucoside.

Using an α-glucosidase prepared by utilizing paper electrophoresis, and also emulsin in buffers of *p*H 5, with controls in every experiment, and following any hydrolysis by means of paper chromatography on the resulting flavonoid aglucone, each of the above-mentioned glucosides was found to be hydrolyzed by the emulsin preparation, but not by the α-glucosidase, as seen in Table I.

TABLE I

Flavonoid glucoside	Hydrolysis catalyzed by Emulsin	Hydrolysis catalyzed by α-Glucosidase
Hesperetin-7-glucoside	Yes	No
Isoquercitrin	Yes	No
Naringenin-7-glucoside	Yes	No
Quercimeritrin	Yes	No
Unknown apricot[1] quercetin glucoside	Yes	No

A relatively simple method for obtaining directly from takadiastase an α-glucosidase free from β-glucosidase activity in a micro amount for further paper chromatographic enzyme studies has been described below. The α-glucosidase was obtained by filter paper electrophoresis over a 40-hr. period, using Whatman 3 MM. paper saturated with citrate buffer of *p*H 3.

Experimental

Preparation of the α-Glucosidase.—Forty milligrams of takadiastase (Parke, Davis and Co.) was dissolved in 0.125 *M* citrate buffer, *p*H 3, and spotted as a band at the center of a Whatman 3 MM. paper strip, 46 × 19 cm. The paper strip, saturated with the citrate buffer of *p*H 3, was placed between two 38 × 20 cm. glass plates. The ends of the paper strips were allowed to dip into troughs containing the citrate buffer. The system was allowed to equilibrate for 45 minutes, and then 110 v. direct current was applied for 40 hr. The paper was then removed from between the glass plates, and 2-cm. wide strips were cut parallel to the point of application of the enzyme solution (origin) on toward the side of the paper which had been attached to the positive pole. Each 2-cm. wide strip was eluted with citrate buffer until 0.5 ml. of eluant from each was obtained. Each eluant was divided into two portions. To one portion was added an equal volume of 0.5 *M* citrate buffer of *p*H 7, containing maltose (16 mg./ml.). To the second portion was added an equal volume of the *p*H 7 citrate buffer containing cellobiose (16 mg./ml.). The solutions were mixed by shaking and allowed to incubate at room temperature for 6 hr. Aliquots of the solutions were spotted on Whatman No. 1 paper and developed for 12 hr. with a butanol–pyridine–water system (2:1:1.5 by volume, upper layer removed and one more part of pyridine added). The glucose, if present, could then be located by spraying with an aniline–hydrogen oxalate solution and identification indicated by comparing the spots developed with those of glucose, maltose and cellobiose.[2]

A fraction, no. 1, eluted from the strip cut from the filter paper extending 0 to 2 cm. from the origin, apparently contained substances interfering with clearcut analysis of results, and was eventually discarded. A fraction, no. 2, eluted from the strip cut from the paper extending 2 to 4 cm. from the origin, hydrolyzed both maltose and cellobiose. A fraction, no. 3, eluted from the strip cut from the paper extending 4 to 6 cm. from the origin, hydrolyzed maltose, but not cellobiose. Additional fractions eluted from 2 cm. strips cut from the paper in the region 6 to 12 cm. from the origin also were capable of catalyzing hydrolysis of maltose, but not of cellobiose. There was a marked decrease, however, in the activity. Many repetitions of this experiment gave essentially the same results. Similar experiments were run on the β-glucoside salicin and on methyl α-D-glucoside. The fraction no. 2 hydrolyzed both of these glucosides; whereas fractions no. 3 catalyzed hydrolysis of methyl α-D-glucoside but not of salicin. Therefore, the fraction no. 3, eluted from the strip cut from the paper extending 4 to 6 cm. from the origin on the positive charged side was selected as the enzyme solution to be used for studies on the flavonoid glucosides.

(1) B. L. Williams and S. H. Wender, *Arch. Biochem. Biophys.*, **43**, 319 (1953).

(2) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).