bath maintained at 25.00 ± 0.02°. Equilibrium was acertained by repeated analysis after varying periods of time, and by approach from supersaturation. Alcohol-rich mixtures reached equilibrium more slowly than those which were water-rich. The yellow color of the chromate was visible even in absolute alcohol.

Analyses were made by evaporating weighed samples of the saturated solutions to dryness, and were reproducible to about ±3 in the last reported digit. Densities were determined with calibrated pipets and with a pycnometer, and are estimated correct to one part per thousand.

Solvent Vol. % C_2H_5OH	Saturated soln. Wt. % K_2CrO_4	Density	Solvent Vol. % C_2H_5OH	Saturated soln. Wt. % K_2CrO_4	Density
0	39.67	1.387	60.0	1.238	0.905
10.00	30.69	1.263	75.0	0.160	.863
20.00	21.70	1.152	85.0	.035	.834
30.00	13.50	1.062	95.0	.021	.801
40.00	7.19	0.992	100	.005	.786
50.00	3.214	.942			

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Two Liquid Phases in the Lithium–Ethylamine System at 0°[1]

BY WILLIAM H. HOWLAND AND LEO F. EPSTEIN

RECEIVED SEPTEMBER 25, 1953

Lithium has been reported to be "extremely soluble" in ethylamine.[2] Investigation of this system at 0° has shown that at equilibrium there are two liquid phases present, similar to the behavior of sodium–ammonia solutions[3] at low temperatures, where the system has a critical solution temperature in the neighborhood of −45°.

Eastman "anhydrous" ethylamine was dried by refluxing over lithium until the appearance of the deep blue color, which is observed with solutions of the alkali metals in ammonia and lower primary amines. This anhydrous ethylamine was distilled in a dry helium atmosphere into a glass tube (containing an excess of clean lithium) maintained at 0° with an ice-bath. Almost immediately the deep blue color appeared in the distillate which was vigorously agitated by means of a magnetic stirrer. After the resulting lithium–ethylamine system was equilibrated at 0°, it was observed that two liquid layers separated when the stirring was stopped. The upper layer was deep blue in color and somewhat smaller in volume than the colorless lower layer. Due to slight convection currents, streamers of the blue colored phase slowly circulated through the colorless and slightly denser phase.

In this study great care was observed to keep air and moisture out of the system. Unlubricated ground glass joints sealed with mercury were used throughout, and inert gas atmospheres were employed to eliminate contact of air with the purified ethylamine and lithium.

The apparatus used in this exploratory study was not adapted to the sampling of a two-phase system, so that no information was obtained on the composition of the two layers. Further work on this system has been set aside and therefore, the above observations are being reported at this time.

Seiler has stated[4] that a trace of ammonia is needed to catalyze the formation of a lithium–ethylamine solution and that the blue color appeared only after ten hours of stirring. No phase separation was observed in her work. The difference between these earlier observations and those reported here is considered to be entirely due to the relative cleanliness of the two systems with respect to air and moisture.

From this investigation the following preliminary conclusions can be drawn.

1. Ethylamine and lithium form two liquid phases at 0°, one blue, the other colorless.
2. The densities of the two phases are nearly equal, the colorless phase being the denser.
3. No catalyst is needed to initiate the solution of lithium in ethylamine, providing that the solvent is adequately dried and air is kept out of the system. With these precautions, the lithium dissolves rapidly.

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(1) The Knolls Atomic Power Laboratory is operated by the General Electric Company for the Atomic Energy Commission. The work reported here was carried out under contract No. W-31-109 Eng-52.

(2) G. N. Lewis and F. G. Keyes, THIS JOURNAL, **35**, 340 (1913).

(3) C. A. Kraus and W. W. Lucasse, *ibid.*, **44**, 1949 (1922).

(4) E. F. Seiler, *Astrophysical J.*, **52**, 129 (1920).

The Catalytic Hydrogenolysis of Proteins and Related Model Compounds[1]

BY H. A. LILLEVIK[2] AND W. M. SANDSTROM

RECEIVED APRIL 2, 1953

Introduction

Adkins[3] has shown that the carbon-to-nitrogen linkage in amides is cleaved at high temperatures and pressure by hydrogen in the presence of catalysts such as copper chromite or Raney nickel. Our purpose was to apply this reaction to proteins and to compare the results with those obtained upon some related model compounds. Zein and acetyl zein were examined together with nylon, polyacrylamide, butyl hippurate and acetamide. Since *n*-butyl alcohol employed as solvent could enter into the reaction, studies were included to differentiate the products of butanolysis from those due to hydrogenolysis. The products were examined to determine the extent of the two reactions upon the primary and secondary (peptide) amide linkages.

A survey of the literature reveals that no attempt has been made to apply Adkins' reduction to proteins, their derivatives or split products. Milder reducing agents such as cyanide or sodium–alcohol have been used; however, these are essentially without effect upon the peptide or amide linkages. Alcoholysis, particularly with methanol or ethanol under pressure, results in partial cleavage of proteins with loss of ammonia and volatile amines. Many of these reactions are considered in a survey article.[4]

(1) From part of a thesis submitted by Hans A. Lillevik to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Published with the approval of the Director of the Minnesota Agricultural Experiment Station as Journal Series No. 2730.

(2) Kedzie Chemical Laboratory, Department of Chemistry, Michigan State College, East Lansing, Mich.

(3) H. Adkins, "Reactions of Hydrogen with Organic Compounds over Copper–Chromium Oxide and Nickel Catalysts," University of Wisconsin Press, Madison, Wisconsin, 1937, pp. 112–119.

(4) R. M. Herriot, "Advances in Protein Chemistry," Vol. III, The Academic Press, Inc., New York, N. Y., 1947, p. 169.

Experimental

Materials.—Zein was selected as a protein because it could be dispersed in anhydrous *n*-butyl alcohol at the temperatures used for hydrogenolysis. Anhydrous solvent was necessary since the copper chromite catalyst is poisoned in aqueous medium. Zein I was prepared by the method of Evans, Foster and Crosston[5]; Zein II was a commercial sample isolated by peptization of starch-free corn gluten with ethanol–water. Acetyl zein was prepared by the procedure of Veatch[6] to the stage where the product is isolated by pouring the reaction mixture into cold water. This procedure invariably yielded a sticky mass, but if the reaction mixture was slowly poured into ice-cold water swirling in a Waring blendor, a granular precipitate could be readily separated. Three precipitations yielded a pale brown product free from acetic acid and inorganic ions.

Polyhexamethyleneadipamide ("Nylon 66") of molecular weight above 7,000 was chosen as a high polymer containing the repeating –CONH– groups, and *n*-butyl hippurate as a compound possessing one such linkage. As corresponding compounds containing the primary amide linkage, polyacrylamide and acetamide were used. Raw Nylon[7] fiber was extracted with acetone and ground to a fine powder in a semi-micro Wiley mill. Polyacrylamide had been prepared by the method of Jones, Zomlefer and Hawkins.[8]

It was necessary to esterify hippuric acid for use with copper chromite. The *n*-butyl ester was prepared by an adaptation of the method of Gurin and Segal.[9] The *n*-butyl hippurate was purified by distillation at 175–180° and 3 mm. Upon standing for several weeks in the cold room, large prismatic crystals were obtained; m.p. 33–35°; Kjeldahl nitrogen found was 6.2%. Acetamide was purified by redistillation of a commercial sample; its final m.p. was 82°.

The copper chromite catalyst was prepared by the directions of Lazier and Arnold[10] and the Raney nickel was made by Mozingo's method.[11]

It was desirable to conduct the reactions in liquid phase at 250° and for this purpose *n*-butyl alcohol was found suitable since its critical temperature is around 287°. Furthermore, traces of butanol, unlike lower alcohols, did not interfere in the nitrous acid method of determining nitrogen. The solvent was dried and redistilled through a fractionating column before use.

Procedure.—The high pressure reaction vessel employed was of the type described by Adkins[3] and part of a complete assembly made by the American Instrument Company. All compounds were dried *in vacuo* over phosphorus pentoxide to constant weight and prepared in 6% (w./v.) dispersions with *n*-butyl alcohol. The ratio of catalyst to reactant was 3 to 2. The mixture (100 ml.) was subjected to the action of hydrogen in the rocking bomb for 10 hr. and maximum pressure of about 238 atmospheres. The temperature of hydrogenolysis was maintained at 250° with the copper chromite catalyst and at 185° with the Raney nickel catalyst. The reaction taking place in the presence of catalyst is referred to as hydrogenolysis. To determine the extent of butanolysis, identical conditions were employed except that catalyst was omitted. During hydrogenolysis treatment at 250°, pressures decreased in terms of moles of hydrogen absorbed per 6-g. sample as follows: zein I, 0.059; zein II, 0.043; acetyl zein, 0.005; nylon, 0.018; polyacrylamide, 0.018; butyl hippurate, 0.012; and acetamide 0.042. This, as a result of drop in pressure at constant temperature, was taken to indicate uptake of hydrogen. By contrast, no pressure change occurred during conditions of butanolysis except in the case of acetamide where an increase was noted.[12] The resulting nitrogen products in the gaseous and liquid phases were examined. A solid product was obtained only from nylon treated under conditions of butanolysis; it was found to be unreacted material.

Analytical Methods.—Basic nitrogen in the gaseous phase was determined by bleeding it from the cold bomb into standard acid. The products in the liquid phase were analyzed for the following forms of nitrogen: total (Kjeldahl), amino (Van Slyke, nitrous acid method), peptide (increase in amino nitrogen after hydrolysis with 20% HCl for 24 hr.), free ammonia (Nessler) and/or volatile bases. The volatile basic nitrogen remaining in solution after hydrogenolysis and butanolysis was determined titrimetrically upon the distillate of a sample made alkaline with CaO and warmed to 45° under 16 mm. The data obtained after butanolysis include both free amino and peptide nitrogen present; the volatile bases were determined before and after 24 hr. of hydrolysis with 20% HCl. Certain of these data for the model compounds are given in Table I.

TABLE I

THE FORMS OF NITROGEN[a] IN THE LIQUID PHASE AFTER REACTION AT 250°

Model compounds	Hydrogenolysis: Peptide, %	Hydrogenolysis: Volatile bases,[b] %	Butanolysis: Primary amino, %	Butanolysis: Peptide, %	Butanolysis: Volatile bases, % Before hydrolysis	Butanolysis: Volatile bases, % Liberated by hydrolysis
Nylon	1.7	89.8	9.8	71.0	8.0[c]	1.4[c]
Polyacrylamide	0.0	84.9	...	...	37.5	31.8
n-Butyl hippurate	0.0	89.6	0.0	100.0	0.0	0.0
Acetamide	0.0	99.5	...	...	59.6	30.8

[a] As % of total N. [b] Identified as tri-*n*-butylamine. [c] Negative Nessler test; others positive.

The three proteins before treatment were subjected to semi-micro analysis for Van Slyke nitrogen distribution and similarly after hydrogenolysis and butanolysis. It is recognized that the conventional terms applied to protein hydrolysates may not apply to their hydrogenolysates or alcoholysates. Thus the "ammonia" nitrogen may include volatile amines; in fact such fractions from hydrogenolysis treatment contained no ammonia as confirmed by the Nessler test. The data are presented in Table II.

TABLE II

THE NITROGEN DISTRIBUTION (IN PER CENT. OF THE TOTAL NITROGEN) OF THE PROTEINS AND THEIR PRODUCTS AFTER HYDROGENOLYSIS AND BUTANOLYSIS

Materials and treatment	Ammonia N, %	Humin N: Insol., %	Humin N: Sol., %	P.T.A. bases, %	Filtrate N: Am., %	Filtrate N: Non-am., %	Total N recovd., %
Zein I							
Untreated	19.4	0.1	0.0	6.3	65.9	7.5	99.2
After hydrogenolysis[a]	89.4[b]	2.6	3.0	..	..	..	95.0
After butanolysis[c]	21.3	5.9	6.6	13.6	39.2	11.5	98.1
Zein II							
Untreated	21.7	0.7	1.3	6.2	62.8	7.8	100.5
After hydrogenolysis[d]	25.9[b]	0.1	3.6	17.0	42.5	7.3	96.4
After butanolysis[c]	16.7	1.0	2.0	5.5	59.8	14.2	99.2
Acetyl zein							
Untreated	17.5	0.5	2.6	7.2	65.5	6.8	100.1
After hydrogenolysis[a]	50.0[b]	0.3	4.6	15.6	13.8	15.6	99.9
After butanolysis[c]	25.1	4.0	5.2	12.5	38.2	15.0	100.0

[a] At 250° with copper chromite catalyst. [b] Identified as tri-*n*-butylamine. [c] At 250°. [d] At 185° with Raney nickel catalyst. [e] At 185°.

Results

Under conditions of hydrogenolysis, using either catalyst, no gaseous basic nitrogen was found. However, after butanolysis for 10 hr. at 250° certain samples contained basic nitrogen: zein II, acetyl zein, polyacrylamide and acetamide showed 1.2, 2.4, 4.9 and 9.7% of their total nitrogen, respectively, in this phase.

(5) C. D. Evans, R. J. Foster and C. B. Crosston, *Ind. Eng. Chem.*, **37**, 175 (1945). We thank Dr. C. W. Ofelt for a sample.

(6) C. Veatch, U. S. Patent 2,236,768; *C. A.*, **35**, 4524 (1941).

(7) A sample was kindly made available by E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.

(8) G. D. Jones, J. Zomlefer and K. Hawkins, *J. Org. Chem.*, **9**, 500 (1944). We thank Dr. Giffin Jones for a sample.

(9) S. Gurin and C. F. Segal, THIS JOURNAL, **58**, 2107 (1936).

(10) W. A. Lazier and H. R. Arnold, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., p. 142.

(11) R. Mozingo, *Org. Syntheses*, **21**, 15 (1941).

(12) Subsequent analysis showed 69.3% conversion to ammonia.

From the reaction mixture it was found that the volatile amine fraction accounted for 85% or more of the total nitrogen of the proteins and model compounds subjected to hydrogenolysis with copper chromite at 250°. The exception was the case of acetyl zein in which the linkages appear to be partly protected. Less was produced from zein II upon Raney nickel hydrogenolysis at 185°. In every instance of hydrogenolysis the volatile amine fraction gave a negative Nessler test for ammonia; instead the reagent produced a white, chalky precipitate. All of this base in each case was identified as tri-*n*-butylamine which was isolated and purified as the picrate. All samples melted from 103–105° with no depression when mixed with authentic sample. The nitrogen content by modified micro-Kjeldahl was 12.9%, the calculated value is 13.5%. However, a value of 12.9% was also obtained on an authentic sample of tri-*n*-butylamine picrate. No primary amine nitrogen was found; such would be expected since the conditions of hydrogenolysis favor alkylation, as has been shown by Adkins.[3] During hydrogenolysis the amide and peptide linkages of the compounds appear to be attacked to the same extent with conversion of their nitrogen to the fully alkylated base.

During butanolysis, primary amino nitrogen was liberated and ammonia appeared in all products except from nylon and butyl hippurate. No alkylated nitrogen product was detected. Peptide nitrogen is markedly more resistant to butanolysis than hydrogenolysis; this is particularly evident with butyl hippurate and nylon. Furthermore, the peptide linkages are more resistant to hydrogenolysis than the primary amide bonds of acetamide and polyacrylamide.

Hydrogenolysis produced marked changes in the forms of nitrogen as revealed by the Van Slyke nitrogen analyses summarized in Table II. Most striking is the enormous increase in the "ammonia" fraction which was all identified as tri-*n*-butylamine. After butanolysis the "phosphotungstic acid bases" and the "non-amino" nitrogen in the filtrate are increased at the expense of the "soluble amino" nitrogen. Similar but less drastic changes are produced upon zein II by hydrogenolysis with Raney nickel at 185°; as may also be seen with acetyl zein.

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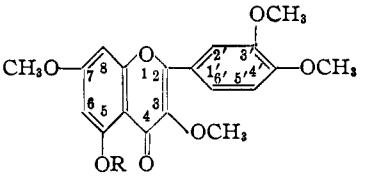
A Novel Acetylation of Quercetin 3,3′,4′,7-Tetramethyl Ether (5-Hydroxy-3,3′,4′,7-tetramethoxyflavone)

BY J. H. LOOKER AND F. C. ERNEST

RECEIVED AUGUST 3, 1953

In an investigation of the methanesulfonation of quercetin and its derivatives,[1] we have observed that quercetin 3,3′,4′,7-tetramethyl ether (I) cannot be methanesulfonated by the action of methanesulfonyl chloride in pyridine. Several modifications of the usual procedure were then employed, one of which involved the action on I of a mixture of methanesulfonyl chloride and acetic acid in pyridine at room temperature. Instead of the methanesulfonate there was obtained a colorless, sulfur-free product, identified as 5-acetoxy-3,3′,4′,7-tetramethoxyflavone (II).



I, R = H
II, R = $COCH_3$

The difficulty encountered in attempting to methanesulfonate I is not surprising, since the 5-hydroxyl of I is known to be also difficult to methylate,[2,3] and acetylation has been reported only when the vigorous conditions of boiling acetic anhydride and anhydrous sodium acetate were used.[4] Our acetylation procedure accordingly seems remarkable, employing as it does very mild conditions.

Experimental

Quercetin 3,3′,4′,7-Tetramethyl Ether (I).—Quercetin was methylated by the method of Waliaschko,[5] with retention of the product, m.p. 155–157°, considered by Waliaschko to be a trimethyl ether, but subsequently shown[3] to be I, thus confirming Herzig's contention.[2]

5-Acetoxy-3,3′,4′,7-tetramethoxyflavone.—To 0.1 g. of quercetin 3,3′,4′,7-tetramethyl ether, dissolved in 11 ml. of reagent pyridine, was added first 0.7 ml. of glacial acetic acid, then 0.6 g. of methanesulfonyl chloride. Heat was evolved, and crystalline material (water-soluble) separated in approx. 30 seconds. The pyridine solution was decanted from solid material after 40 minutes, and the pyridine decantate permitted to stand at room temperature an additional 42 hours. The crude acetate was isolated by pouring the reaction mixture into 90 ml. of water, and permitting the aqueous suspension to stand for three hours. The crude, crystalline solid deposited was collected by filtration, washed with water and air-dried for several days; m.p. 165–170°. After sodium fusion, the crude product gave negative tests for sulfur both with lead acetate and sodium nitroprusside. The crude acetate was recrystallized twice from absolute ethanol to give colorless, silken needles, m.p. 169–171° (lit. m.p. 167–169°,[4] 171–172°[6]), no depression upon admixture with authentic 5-acetoxy-3,3′,4′,7-tetramethoxyflavone (m.p. 169–170.5°), prepared by the method of Herzig.[4]

(1) J. H. Looker and A. L. Krieger, unpublished observations.

(2) J. Herzig, *Sitzungsber.*, **121**, 333 (1912).

(3) Methylation of the 5-hydroxyl of I has been achieved, however, by employment of dimethyl sulfate and solid potassium hydroxide [A. S. Gomm and M. Nierenstein, THIS JOURNAL, **53**, 4408 (1931)].

(4) J. Herzig, *Monatsh.*, **5**, 86 (1884).

(5) N. Waliaschko, *Ber.*, **42**, 727 (1909).

(6) N. Krassowski, *Chem. Centr.*, **80**, [I] 772 (1909).

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Chlorotetranitronaphthalenes

BY WALTER E. MATHEWSON

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Although two isomeric bromotetranitronaphthalenes were described seventy years ago, no mention has been found in the literature of chlorotetranitronaphthalenes.

The two chlorotetranitronaphthalenes described here were prepared mainly for trial as colorimetric