of water and the aglycon filtered off; yield 0.2 g., 50%; m. p. 235° after one recrystallization from ethanol and water.

The aqueous filtrate was concentrated to dryness. The sirup was taken up in 1 ml. of methanol. Upon standing in the cold for a week, crystals formed; yield 64 mg., m. p. 184–188°, after one recrystallization from methanol m. p. 188°; no depression when mixed with an authentic sample of methyl α-D-mannoside; $[\alpha]^{28}D$ +86.7° (*c*, 1, methanol). Reported for methyl α-D-mannoside, m. p. 193–194°; $[\alpha]^{20}D$ +87.5° (*c*, 1, methanol).

Effect of Varying Concentrations of Barium Methoxide on the Methanolysis.—A solution of glucoside tetraacetate of VIII was made up to contain 3.33 g. per 100 ml. of methanol. To 10-ml. aliquots, 0.02, 0.04, 0.08, 0.16 and 0.32 ml. of 0.573 *N* barium methoxide was added. The rotation was followed until the change in ten minutes was less than 0.02°. The results are plotted in Fig. 3.

Summary

1. A series of glycosides of 3-phenyl-4-hydroxycoumarin (VIII) has been prepared by condensing the enol silver salt of the aglycon with the respective acetylglycosyl bromides.

2. The glycosides all undergo methanolysis when treated with catalytic amounts of barium methoxide in dry methanol. The mechanism appears to be the same in each case. A Walden inversion accompanies the cleavage. From the β-D-glycosides of VIII are formed methyl α-D-glycosides, and from the α-D-arabinoside of VIII methyl β-D-arabinoside is obtained.

3. An electronic mechanism, in which the electrophilic nature of VIII promotes electronic shifts which labilize the sugar–oxygen bond, is offered as a rationalization of the products of the alkaline cleavage.

MADISON, WISCONSIN RECEIVED JUNE 2, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

Alkaline Methanolysis of Theobromine β-D-Glucoside Tetraacetate[1]

BY CLINTON E. BALLOU AND KARL PAUL LINK

The observations of Huebner, *et al.*,[2] and Spero, *et al.*,[3] concerning the cleavage products formed during the attempted deacetylation of 3-phenyl-4-hydroxycoumarin D-glycoside tetraacetates, have stimulated an investigation of various other alkali labile glycosides. This paper is on the alkaline methanolysis of theobromine D-glucoside tetraacetate (I).

Fischer[4] first described the difficulty of deacetylating certain purine glucoside tetraacetates without cleaving the glucosidic linkage. From theobromine D-glucoside tetraacetate (I) in aqueous alkali, Fischer obtained theobromine and glucose (II). Due to the sensitivity of this glucoside to aqueous acid and alkali, he postulated the glucosidic linkage as being to position 2 or 6 of the enol form of the purine residue.

In our studies of the cleavage of alkali sensitive glycosides the reactions are run in dry methanol[5] in the presence of catalytic amounts of barium methoxide. Under these conditions cleavage involves a methanolysis of the glycosidic linkage, and in those cases reported[2,3] a methyl glycoside and the free aglycon are formed. The cleavage is accompanied by a Walden inversion, and the products formed suggest a mechanism for the cleavage.

According to the mechanism advanced by Isbell,[6] the cleavage anion may add to either the sugar residue or the aglycon, depending upon the shift of electrons between these two parts of the molecule. During the cleavage of the glycosidic linkage under the influence of basic catalysts, a cationoid center is derived from either the sugar residue or the aglycon. If the cation comes from the sugar residue it may be attacked by an anion such as hydroxyl or methoxyl, to give the free sugar or a methyl glycoside. This sugar cation may also be attacked by an anion present within the sugar molecule with the formation of an inner anhydride. If the cationoid center derives from the aglycon it likewise may be attacked by an anion present, again hydroxyl or methoxyl, to give the free aglycon or a methoxyl derivative, or it may coördinate with an adjacent carbon atom to form a carbon–carbon double bond, a proton being expelled during the reaction.

The cleavage of 3-phenyl-4-hydroxycoumarin β-D-glucoside tetraacetate[2] with the formation of methyl α-D-glucoside represents a case in which the oxygen–sugar bond is split. This is indicated because the anion, in this reaction the methoxyl group, becomes attached to the sugar residue, and the configuration of the anomeric carbon atom changes from the β to the α form. Peat[7] has thoroughly reviewed this type of inversion as encountered in alkaline scission of anhydro sugar rings.

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. This paper was presented before the Division of Sugar Chemistry and Technology at the 116th Meeting of the American Chemical Society, Atlantic City, September, 1949.

(2) Huebner, Karjala, Sullivan and Link, THIS JOURNAL, **66**, 906 (1944).

(3) Spero, Ballou and Link, *ibid.*, **71**, 3740 (1949).

(4) Fischer and Helferich, *Ber.*, **47**, 210 (1914).

(5) The dry methanol was obtained by refluxing commercial (99.9%) methanol with magnesium turnings (10 g./liter) until the magnesium had completely reacted. The methanol was then distilled and the center fraction was collected in small bottles and stored for use.

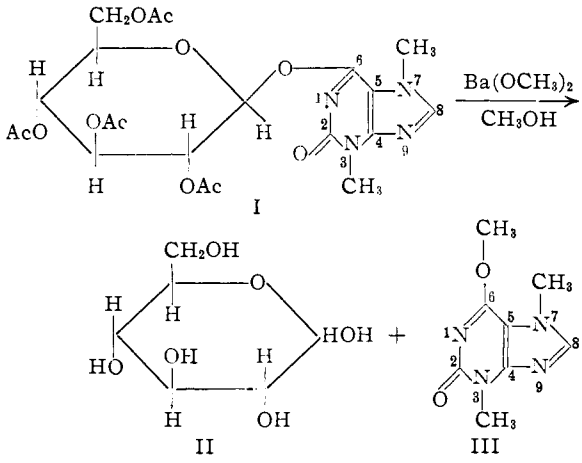
(6) Isbell, *Ann. Rev. Biochem.*, **XII**, 215 (1943).

(7) Peat, *Adv. in Carbohydrate Chem.*, Vol. 2, 45 (1946).

He states, "If the carbonium cation derives from an asymmetric atom then the configuration change becomes manifest in an inversion of optical rotation (Walden inversion)." In the cleavage of the glycosides of 3-phenyl-4-hydroxycoumarin, the cationoid center derives from the anomeric carbon atom, and is attacked by the methoxyl anion. The Walden inversion is manifested by the formation of a methyl α-D-glycoside from the original β-D-glycoside.

Possibly representative of the cleavage of the oxygen–aglycon linkage is the reaction reported by Kuhn and Löw[8] for the alkaline hydrolysis of picrocrocin. In this reaction an unsaturated carbon–carbon linkage arises in the aglucon, indicating cleavage of the oxygen–aglucon bond with a simultaneous expulsion of a proton from an adjacent carbon atom. Evidence was offered to indicate that the mechanism does not follow hydrolysis of the glucoside linkage, with subsequent dehydration of the aglucon to form the unsaturated product.

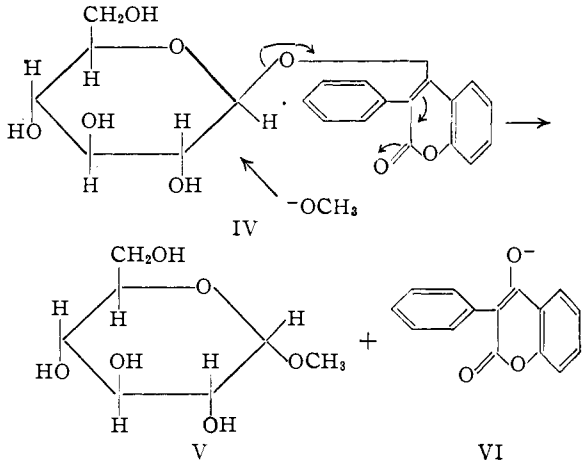
A clear-cut illustration of this second type of glycosidic cleavage is reported herewith. Theobromine D-glucoside tetraacetate (I) was cleaved in absolute methanol containing catalytic amounts of barium methoxide to form glucose (II) and a methoxy-oxy-3,7-dimethyl purine (III) in a yield of 95%. This reaction may be formulated as



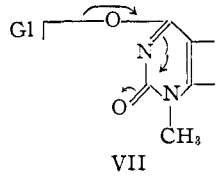
The fact that the aglucon was recovered as a methoxyl derivative indicates that the point of attack of the methoxyl anion was between the glucosidic oxygen and the aglucon. Thus the cationoid center must derive from the aglucon. This is in contrast to the cleavage of the glycosides of 3-phenyl-4-hydroxycoumarin in which the methoxyl anion attacks the anomeric carbon atom of the sugar residue.

An electronic rationalization of this reaction is not embraced by the concepts outlined by Isbell.[6] The expected electron shifts in the aglucon would tend to labilize the sugar–oxygen bond, not the aglucon–oxygen bond. It is possible that the inherent pyridinium nature of the pyrimidine portion of the purine nucleus may be involved.[9]

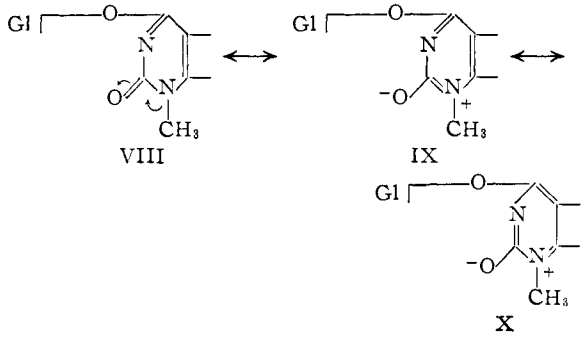
In the structure IV, the indicated "discontinuous" polarizations permit the methoxyl anion to attack the glucose portion to give V and VI.



Representing the glucoside of theobromine (I) as shown in VII, the effect of the carbonyl group would be to permit the same type of attack (weaken the glucosyl oxygen bond).



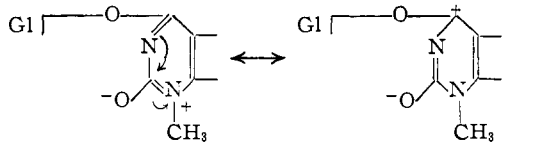
However, the products of methanolysis indicate that this is not so. As shown in VIII, the polarization of the nitrogen in position 3 could establish the continuous system IX, of which X is a resonance hybrid.



These forms are favored due to higher resonance, and would be further stabilized by the barium ions in solution. IX and X may give rise to XI as indicated

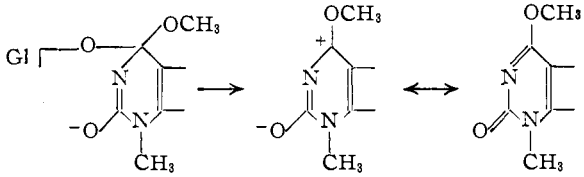
(8) Kuhn and Löw, *Ber.*, **74**, 219 (1941).

(9) We are deeply indebted to Prof. S. M. McElvain of the Chemistry Department of the University of Wisconsin for his generous suggestions concerning the electronic interpretations presented in this paper, and to Dr. H. S. Isbell of the National Bureau of Standards for reading the manuscript prior to submission for publication, and for the helpful ideas he gave.



IX XI

and upon attack by a methoxyl anion, there would be formed the intermediate XII. In methanol solution the decomposition of XII to give XIV and the glucose anion would be favored.



XII XIII XIV

This interpretation implies a fundamental difference in the activating effect upon the glycosidic linkage between the discontinuous system as represented by IV, and the continuous system formulated in IX and X. The latter permit the consideration of an aromatic type structure in which a positive center could become located on the carbon atom of the aglucon involved in the glucosidic linkage, and make this atom susceptible to attack by the methoxyl anion. Obviously the corresponding aromatic oxonium form of the 3-phenyl-4-hydroxycoumarin glycosides is not as effective a contributing structure in the alkaline cleavage of those compounds as is the ammonium cation of IX.

Whether the imidazole ring of the purine nucleus contributes to the labilizing effect of the aglucon is not known. The xyloside of 1-methyluracil is labile to alkali,[10] and it is possible that this same type of methanolysis could be duplicated with this compound.

The exact position of the methoxyl group in the theobromine residue (III) was not established. It could be either on position 2 or 6 as shown, since enolization would be possible at either carbon atom. It seems quite likely that the methoxyl group will occupy the position at which the sugar molecule was attached. The data on the ultraviolet absorption spectra indicate this (Table I).

TABLE I

ULTRAVIOLET ABSORPTION DATA OF PURINES IN METHANOL

Compound	Wave length of maxima, mμ	Mol. ext. coeff. of maxima	Wave length of minima, mμ
Methoxy-oxy-3,7-dimethylpurine (III)	245	0.36×10^4	235
	295	$.85 \times 10^4$	260
Theobromine β-D-glucoside tetraacetate (I)	245	$.33 \times 10^4$	235
	295	$.67 \times 10^4$	260
Theobromine	275	$.91 \times 10^4$	245
Caffeine	275	$.87 \times 10^4$	245

(10) Levene and Sobotka, *J. Biol. Chem.*, **65**, 469 (1925).

It is seen that the maxima and minima of I and III are identical. These are significantly different from the characteristics shown by theobromine and caffeine. By a similar study of the appropriate purine bases it should be possible to determine whether the correct linkage in the glucoside (I) is to position 2 or 6. This method has been most successfully employed by Gulland and Holiday[11] in establishing linkages in the ribosylpurines.

The methoxy-oxy-3,7-dimethylpurine (III) formed in this reaction was characterized by carbon and hydrogen analysis, by a methoxyl determination, by conversion to caffeine via the well known imidoester rearrangement,[12] and by hydrolysis to theobromine. The formation of glucose is indicated by the final rotation of the methanolysis reaction mixture, and by its reducing power.

Hydroxycaffeine D-glucoside tetraacetate, also prepared according to Fischer,[4] was treated in the same manner with dry methanol and barium methoxide. The expected 8-methoxycaffeine was not isolated, and the only substance found was the aglucon hydroxycaffeine. The structure of this glucoside is not strictly comparable to that of the theobromine glucoside, since the glycosidic linkage is to position 8 in the imidazole ring of the purine nucleus. However, it is still labile to alkali, and the methanolysis should produce definitive products. It is possible that these conditions simply effect deacetylation of the glucoside, and the process of working up the reaction, which introduces moisture, hydrolyzes the deacetylated glucoside.

Experimental

Preparation of Theobromine D-Glucoside Tetraacetate (I).—I was made from the silver salt of theobromine and tetraacetyl-D-glucosyl bromide as described by Fischer,[4] but extreme care was necessary in the preparation of the silver salt of theobromine before the reaction could be completed successfully. Thirty grams of theobromine was dissolved in 2 liters of boiling water containing an excess of ammonia, and an ammoniacal solution of silver nitrate (1 equivalent plus 2% excess) was added with stirring. The salt precipitated immediately.

To remove the last traces of ammonia, the precipitated salt was washed several times with water, and then resuspended in about a liter of water. This suspension was concentrated by boiling at atmospheric pressure until the volume was about 200 ml. This step was repeated. The salt was filtered off, washed with absolute ethanol, and dried in an oven at 130°. If this purification of the theobromine salt was not carried out, the glucoside formed in the coupling reaction was cleaved during the subsequent recrystallizations. An acetone–Skelly "B" mixture was found to be a more desirable solvent for recrystallization of the glucoside than ethyl acetate as directed by Fischer.

Methanolysis of Theobromine D-Glucoside Tetraacetate (I).—Three grams of I was dissolved in 240.4 g. of dry methanol.[5] The optical rotation of this solution was −0.37° (25°, 1 dcm.). To this solution was added 1.0 ml. of 0.387 *N* barium methoxide, and the rotation was observed until it stopped changing twelve hours later at the value +0.23° (25°, 1 dcm.). Complete conversion of the glucoside to free glucose would give a value of +0.23 (25°, 1 dcm.). To this solution was

(11) Gulland and Holiday, *Nature*, **132**, 782 (1933).

(12) Hibbert and Johnson, THIS JOURNAL, **52**, 2001 (1930).

added 4.0 ml. of 0.096 *N* sulfuric acid to precipitate the barium ions. The barium sulfate was removed by filtration through an asbestos mat. The filtrate was concentrated to dryness *in vacuo* at 50°. The white crystalline product obtained was dissolved in the minimum amount of hot absolute ethanol. The solution was filtered, and upon cooling, fine white needles separated. They were filtered off and dried in a desiccator over phosphorus pentoxide, yield 1.0 g., m. p. 244–245° when heated rapidly. Otherwise, it begins to sinter at 230° and undergoes rearrangement to caffeine. The substance is very soluble in cold water and hot alcohol. Theobromine is quite insoluble in both.

Anal. Calcd. for $C_7H_8ON_4(OCH_3)$: C, 49.5; H, 5.15; OCH_3, 15.95. Found: C, 49.7; H, 5.38; OCH_3, 15.83.

The ethanolic filtrate was diluted with water to a known volume and an aliquot was taken for the determination of the reducing power by the method of Shaffer and Somogyi.[13] Ninety per cent. of the glucose of the original glucoside was accounted for. The reducing value, the rotation of the reaction given above, and a 95% recovery of the aglucon as the methoxy derivative III establishes the formation of glucose.

Rearrangement of the Methoxy-oxy-3,7-dimethylpurine (III) to Theobromine.—Lactim ethers readily undergo rearrangement to the isomeric lactam ($N{=}C{-}OCH_3 \rightarrow CH_3{-}N{-}C{=}O$) upon heating.[12] This rearrangement was employed to establish the structure of the purine residue III. About 0.2 g. of III was heated in a sealed tube at 290–300° in a lead-bath for one hour. After cooling, the tube was opened and the contents ground up in a mortar. The mixture was heated in a crucible, and the substance that sublimed was collected on the under surface of a watch glass over the crucible. The sublimate was recrystallized from absolute ethanol. It formed fine white needles, m. p. 227–231°, yield 0.15 g. One more recrystallization from the same solvent gave m. p. 230–232°. The substance showed no depression of the melting point when mixed with an authentic sample of caffeine which melted at 232–233°. A methoxyl determination was negative.

(13) Shaffer and Somogyi, *J. Biol. Chem.* **100**, 695 (1933).

The substance formed a mercuric chloride salt, m. p. 246°; reported for caffeine-mercuric chloride salt, m. p. 246°.

Conversion of III to Theobromine.—Ethers of the enolic forms of purines and pyrimidines (lactim ethers) are easily hydrolyzed by dilute acid. A small amount of III was dissolved in water and 0.1 volume of concentrated hydrochloric acid was added. The solution was boiled for five minutes. Upon cooling, it was neutralized with dilute sodium hydroxide and a white crystalline powder separated. This was filtered off and dried. The substance begins to sublime at 300° and melts at 350–355° with decomposition. An authentic sample of theobromine showed this same behavior. The material showed the characteristic solubility of theobromine, and formed an insoluble silver salt when ammoniacal silver nitrate was added to a solution of the substance in dilute ammonia water.

Anal. Calcd. for $C_7H_8O_2N_4$: C, 46.6; H, 4.45. Found: C, 46.8; H, 4.74.

Summary

1. Two purine glucosides, theobromine D-glucoside tetraacetate and hydroxycaffeine D-glucoside tetraacetate were treated with barium methoxide in dry methanol in an attempt to cleave the glucosidic linkage by methanolysis.

2. Methanolysis of theobromine D-glucoside tetraacetate occurred and the products of the cleavage were glucose and a methoxyl derivative of the aglucon. This indicates that the cleavage occurs between the glucosidic oxygen and the aglucon. The cleavage of the glucoside of hydroxycaffeine did not result in products which were indicative of the mechanism of the splitting.

3. An electronic interpretation of this reaction is offered in an attempt to rationalize the results with respect to the present concepts of the various mechanisms involved in the alkaline cleavage of the glycosidic linkage.

MADISON, WISCONSIN RECEIVED JUNE 2, 1949

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORY]

The Reaction of Amides with Isocyanates. II. N-Substituted Amides

BY PAUL F. WILEY

In previous studies[1,2,3] of the reaction of isocyanates with amides in which the amide nitrogen was not substituted the reaction has been shown to occur according to the equation (R′ = H).

$$RCONHR' + R''NCO \longrightarrow RCONCONHR''$$
$$\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\quad R'$$

R = alkyl or aryl
R″ = alkyl or aryl

Recent investigators[4,5,6,7] who have had occasion to use the reaction of nitrogen-substituted amides with isocyanates have generally made no mention of any divergence from the above equation. However, Kühn[1,2] has reported unknown products from formanilide, acetanilide, acetnaphthalide and benznaphthalide and phenyl isocyanate as well as the formation of N,N′-diphenylbenzamidine from benzanilide. A further investigation along the lines of Kühn's[1,2] earlier work has been made in the present study.

In these experiments phenyl isocyanate was the only isocyanate used, and in addition to the amides shown in Table I, N-*n*-butylacetamide, N-isobutylundecylenamide and N-ethylbenzamide were used. The reactions were run using either boiling toluene or xylene as reaction media or no solvent at temperatures varying from 120 to 220°. Table I lists identified products obtained and

(1) Kühn, *Ber.*, **17**, 2880 (1884).
(2) Kühn, *ibid.*, **18**, 1476 (1885).
(3) Wiley, THIS JOURNAL, **71**, 1310 (1949).
(4) French and Wirtel, *ibid.*, **48**, 1736 (1926).
(5) Lüdke, *Z. physiol. Chem.*, **150**, 215 (1925).
(6) Berchet, U. S. Patent 2,333,914 (November 9, 1943).
(7) Foster, U. S. Patent 2,333,922 (November 9, 1943).