5. Alkaline hydrolysis of diazomethane methylated sugar humic acid at 60° leads to a decomposition product having the empirical formula $(C_{33}H_{31}O_{11})_x$ or $[C_{32}H_{28}O_{10}(OCH_3)]_x$.

6. The evidence obtained indicates clearly that the two types of humic acid have markedly different structures.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, TEACHERS COLLEGE, COLUMBIA UNIVERSITY]

# Studies of Crystalline Vitamin $B_1$. III. Cleavage of Vitamin with Sulfite

BY ROBERT R. WILLIAMS, ROBERT E. WATERMAN, JOHN C. KERESZTESY AND EDWIN R. BUCHMAN

When in the fall of 1933 supplies of crystalline vitamin $B_1$ became available[1] to us for study, careful consideration was given to the choice of cleavage reactions adapted to reveal facts about its constitution. Such care was considered necessary not only for the sake of economy of vitamin but also because the lability of the substance suggested that drastic treatments would probably cause splitting at several points in the molecule. This indication appeared to be confirmed by the experience of others, notably Van Veen,[2] that a miscellany of substances is produced by the more usual oxidative and hydrolytic reagents.

A review of our experience extending over many years in connection with isolation of the vitamin offered a promising suggestion. Several years ago an attempt to utilize sulfurous acid as a preservative against bacterial decay of rice polish extracts had led to a very prompt and complete loss of the antineuritic activity at room temperature. In a preliminary experiment, a few milligrams of the crystalline vitamin dissolved in two portions of sodium sulfite solution were allowed to stand at room temperature under toluene for thirty-six hours after adjustment to *p*H 1 and *p*H 4, respectively. The solutions were then made alkaline with baryta to remove sulfite ion. The neutralized filtrate of the solution which had been adjusted to *p*H 1 had lost about half its activity as shown by injection of polyneuritic rats; that at *p*H 4 was quite devoid of activity.

We have since had considerable experience with this reaction of the vitamin and find that it proceeds readily at about *p*H 5, under which conditions it is complete at room temperature in twenty-four to forty-eight hours; at steam-bath temperature it is complete in an hour or less according to a single experiment. The use of saturated aqueous sulfurous acid gives only very slow destruction of activity. After three months at room temperature, 50% yields of cleavage products were obtained. Under favorable conditions, it proceeds quantitatively to form crystalline products according to the equation

$$C_{12}H_{16}N_4OS + H_2SO_3 \longrightarrow \underset{\text{I}}{C_6H_9N_3SO_3} + \underset{\text{II}}{C_6H_9NOS}$$

With sulfite at *p*H 5.0, the yield of each cleavage product has reached 97% of the calculated and the products are well defined. The constancy of these results from a quantitative standpoint and the corresponding degree of destruction of physiological activity which occurs simultaneously afford strong assurance of the essential purity of the crystalline vitamin. The reaction has been carried out thirteen times. A detailed description of the isolation of the cleavage products is given in the experimental part.

I is an acidic substance, sparingly soluble in water and insoluble in other solvents; II is a basic substance soluble in water but extractable by means of chloroform from an alkaline aqueous solution. It has been dealt with principally as the hydrochloride which can readily be obtained in a crystalline condition. Results of investigation of the constitution of both the acidic and basic cleavage products will be reported in papers now in preparation.

## Experimental

1.000 gram of vitamin was dissolved in 15 cc. of sodium sulfite solution containing sufficient excess sulfurous acid to bring the *p*H to 4.8–5.0. The total sulfite content was 2.6 *N*. After standing overnight at room temperature the liquid had deposited copious amounts of the sparingly soluble acidic cleavage product in crystalline form. After standing for several days, the crystalline product was collected, washed and dried; weight 535.8 mg.

The mother liquor and washings were brought to *p*H 10 with strong sodium hydroxide and the alkaline solution extracted seven times with 50 cc. of chloroform each time.

(1) R. R. Williams, R. E. Waterman and J. C. Keresztesy, THIS JOURNAL, **56**, 1187 (1934).

(2) A. S. Van Veen, *Rec. trav. chim.*, **51**, 279 (1932).

The combined chloroform extracts were extracted with dilute hydrochloric acid, the acid aqueous extract was evaporated *in vacuo* and the residue was extracted with absolute alcohol. The alcoholic solution on evaporation left a residue of 518.8 mg. of the crystalline hydrochloride of the basic cleavage product, the purity of which was demonstrated by analysis; yield 97.4%. The recrystallization of this material is effected by dissolving in a minimum amount of absolute alcohol, adding an excess of dioxane and allowing to stand.

From the alkaline liquor after chloroform extraction, a further quantity of 25.3 mg. of acidic cleavage product was obtained by neutralizing and concentrating to a small volume; total yield 561 mg. or 93.1% of the theoretical. Fine white needles are obtained on recrystallization from hot water.

TABLE I

ANALYSES

| | | C | H | N | S | Cl |
|---|---|---|---|---|---|---|
| I. Dried for anal. in vac. over $P_2O_5$ at 100° | | | | | | |
| Calcd. for $C_6H_9N_3SO_3$ | | 35.44 | 4.46 | 20.68 | 15.78 | ... |
| Found | Not recryst. | 35.34 | 4.03 | ... | 15.88 | ... |
| | Water recryst. | 35.36 | 4.60 | 20.53[a] | 16.01 | ... |
| II. Dried for anal. in vac. over $P_2O_5$ | | | | | | |
| Calcd. for $C_6H_9NSO \cdot HCl$ | | 40.09 | 5.61 | 7.80 | 17.85 | 19.74 |
| Found | | 39.80[b] | 5.89[b] | 7.81[c,d] | 17.85[c] | 19.48[c] |

[a] Kjeldahl. [b] Not recryst.; dried at 55°. [c] Recryst. from alcohol–dioxane and dried at room temp. [d] Dumas.

In another experiment 203 mg. of vitamin hydrochloride was allowed to react at *p*H 5.0 in a sealed tube at room temperature with a solution containing by analysis 231.9 mg. of sodium bisulfite. After standing for forty-eight hours the solution was made alkaline with baryta and the precipitate so formed was suspended in water, decomposed with hydrochloric acid and excess sulfite determined iodimetrically. The sulfite consumed was 74.5 mg. or 116%. Blank experiments accounted for the excess above one mole equivalent as due to losses of sulfur dioxide by atmospheric oxidation and volatilization. The reaction products were isolated as indicated above.

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## Summary

Vitamin $B_1$ is split quantitatively at room temperature by sulfite at *p*H 5 into two products having the compositions $C_6H_9N_3SO_3$ (I) and $C_6H_9NSO$ (II), respectively.

I is a sparingly soluble acidic product.

II is a chloroform soluble base which has been isolated in the form of a crystalline hydrochloride.

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[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

# The Configuration and the Mechanism of Hydrolysis of the Maltose Derivatives with Orthoester Structure

BY EUGENE PACSU

Several years ago Freudenberg and Ivers[1] prepared a new acetochloromaltose from octaacetylmaltose using an ether solution of dry hydrogen chloride. From the methyl alcoholic suspension of this halogeno derivative, on treatment with pyridine, Freudenberg and co-workers[2] obtained a new heptaacetylmethylmaltoside. Both of these maltose derivatives were later recognized by Freudenberg,[3] as possessing "orthoester" structures. It has been suggested by Haworth[4] that formation of such orthoester derivatives may occur "whenever, as in the β-mannose, -lyxose, and -rhamnose series, adjacent hydroxyl groups congregate in clusters on the same side of the six-atom ring." Since no congestion of hydroxyl groups is prevalent in maltose, it appears that two adjacent hydroxyl groups on the same side of the plane are sufficient for the formation of orthoester derivatives. If this be true, then Freudenberg's maltose derivatives must have α-configurations, since β-maltose is so constituted that each hydroxyl group occupies a *trans* position with respect to its neighbor. Hydrolysis experiments with very dilute hydrochloric acid on the "γ"-monoacetylmethylmaltoside[2,3] (I) confirm this. Two consecutive reactions were found to take place, when the hydrogen-ion concentration of the solution corresponded to *p*H 4 (Table I).

The first reaction, strongly catalyzed by hydrogen ions, was so rapid that it was completed in less than two minutes. During this time the original specific rotation of $[\alpha]_D^{23}$ 103.7° increased

(1) Freudenberg and Ivers, *Ber.*, **55**, 929 (1922).

(2) Freudenberg, v. Hochstetter and Engels, *ibid.*, **58**, 666 (1925).

(3) Freudenberg, *Naturwiss.*, **18**, 393 (1930); Freudenberg and Scholz, *Ber.*, **63**, 1969 (1930).

(4) Haworth, "Rapports sur les Hydrates de Carbone," Xième Conférence de l'Union Internationale de Chimie, Liége, 1930.