difficulty soluble in water. In contrast the barium salt of the unoxidized starting material is extraordinarily soluble. The methoxyl content of the unoxidized barium salt was 10.4% with a barium content of 12.5%. The barium salt of the oxidized material has a methoxyl content of 6.4% with barium content of 17.2%. This indicates the cleavage from the anion of a methoxyl-containing fragment during periodate oxidation. From the oxidized material an oxime has been prepared containing 2.7% nitrogen.

Complete accounts of these experiments and other studies now in progress on the periodate oxidation of lignin sulfonic acids and other lignins will be given in future communications.

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RECEIVED JUNE 10, 1946

ON THE PEPTIDE NATURE OF VITAMIN Bc CONJUGATE FROM YEAST

Sirs:

Evidence has been presented to support the view that vitamin Bc conjugate consists of vitamin Bc linked to an ultraviolet-transparent nitrogeneous moiety.[1] The non-vitamin Bc portion of the molecule has been found to consist of six molecules of 1(+)-glutamic acid in peptide linkage.

Following hydrolysis (18% hydrochloric acid for sixteen hours at 100°) 59.6% of the total N reacted as α-amino acid N,[2] which was accounted for as glutamic acid nitrogen by microbiological assay.[3,4] From 298 mg. of conjugate methyl ester 220 mg. of 1(+)-glutamic acid hydrochloride was isolated. $[\alpha]^{24}D$ +25.4° (5.1% solution in 1 *N* hydrochloric acid; C, 32.75; H, 5.6; N, 7.8, 7.6. Calcd.: C, 32.7; H, 5.5; N, 7.6. Under comparable hydrolytic conditions 20.1% of the total nitrogen of vitamin Bc reacted as α-amino acid nitrogen and as glutamic acid nitrogen by microbiological assay.

(1) Pfiffner, Calkins, O'Dell, Bloom, Brown, Campbell and Bird, *Science*, **102**, 228 (1945).

(2) Van Slyke, *J. Biol. Chem.*, **16**, 121 (1913).

(3) Hier, Graham, Freides and Klein, *ibid.*, **161**, 705 (1945).

(4) We wish to thank Dr. O. D. Bird for conducting the microbiological determinations.

The ratio of the $E^{1\%}_{1\,cm.}$ values of vitamin Bc to the conjugate was determined as 2.72:1. With the molecular formula of vitamin Bc established as $C_{19}H_{19}O_6N_7$ (mol. wt. 441.4)[5] the above ratio suggests a minimum molecular weight for the conjugate of 1200 (found by diffusion 1400).[6] A molecule consisting of one molecule of the vitamin in peptide linkage with a peptide chain consisting of six 1(+)-glutamic acid residues would have the molecular formula $C_{49}H_{61}O_{24}N_{13}$ (1216.1). This formulation is in agreement with elementary analytical findings some of which have been reported.[1] That the conjugate is not a mixture of peptides with an average of 7 glutamic acid residues is evidenced by its homogeneous behavior on electrophoresis.[6] Following the suggested nomenclature of Angier, *et al.*,[7] vitamin Bc conjugate may be designated pteroylhexaglutamylglutamic acid.

The conjugate is essentially inactive microbiologically, whereas the fermentation *L. casei factor* reported by Angier, *et al.*,[7] to yield 3 moles of glutamic acid has high microbiological activity.

Demonstration of the peptide nature of the conjugate identifies the conjugase enzymes[8] which split the microbiologically active compound from the conjugate as peptidases. Since conjugases do not liberate vitamin Bc from the conjugate methyl ester[1] they can be further classified as carboxypeptidases.

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(5) Our analytical data allowed a choice between $C_{19}H_{19}O_6N_7$ and $C_{21}H_{21}O_6N_5$ as the probable molecular formula while our degradation results ruled out the latter formulation. Angier, *et al.* (*Science*, **103**, 667 (1946)) demonstrated by degradation and synthesis the structure of the liver *L. casei factor* to be N-[4-{[(2-amino-4-hydroxy-6-pteridyl)-methyl]amino}-benzoyl]-glutamic acid and suggested the name pteroylglutamic acid. A sample of the synthetic compound was generously supplied us by the Lederle Laboratories, and we found it to be identical with the compound which we isolated from liver and yeast and tentatively called vitamin Bc (*Science*, **97**, 404 (1943)).

(6) We wish to thank Dr. J. M. Vandenbelt for the ultraviolet absorption, diffusion and electrophoresis determinations.

(7) Angier, *et al.*, *Science*, **103**, 667 (1946).

(8) Bird, Binkley, Bloom, Emmett and Pfiffner, *J. Biol. Chem.*, **157**, 413 (1945).