lyst at atmospheric pressure. About one molar equivalent of hydrogen was absorbed. The product obtained by drying the filtered solution from the frozen state was a white granular solid which showed $(\alpha)^{25}D$ $-88.7°$ (c, 1.0 in water), and had an activity of about 750 units/mg. as compared with 800 units/mg. for streptomycin.

Dihydrostreptomycin trihydrochloride was converted to the trihelianthate as described for streptomycin.[1] A sample of the trihelianthate after recrystallization three times melted at 215–225° (dec.), activity about 400 units/mg. *Anal.* Calcd. for $C_{21}H_{41}N_7O_{12}(C_{14}H_{15}N_3O_3S)_3$:[2] C, 50.46; H, 5.79; N, 14.94. Found: C, 50.14; H, 5.83; N, 15.08.

Conversion of dihydrostreptomycin trihelianthate to the trihydrochloride as described for streptomycin[1] gave a white powder, m. p. 185–190° (dec.), $(\alpha)^{25}D$ $-89.5°$ (c, 0.98 in water), activity about 750 units/mg. Potentiometric titration of this sample gave an equivalent weight of 690; calcd. mol. wt. 693; pK_A 7.75. *Anal.* Calcd. for $C_{21}H_{41}N_7O_{12}\cdot 3HCl$: C, 36.40; H, 6.40; N, 14.15. Found: C, 36.50; H, 6.21; N, 13.91.

The presence of a free or potential carbonyl group in streptomycin was demonstrated by the formation of an oxime and semicarbazone.[3] Streptomycin was inactivated[3] by hydroxylamine in aqueous pyridine solution at pH 4. Dihydrostreptomycin is not inactivated by hydroxylamine under these conditions, which is evidence that the carbonyl group in the streptobiosamine moiety is the functional group which was reduced. Acid hydrolysis of dihydrostreptomycin yields streptidine; hence, the reduction involves only the streptobiosamine moiety.

Dihydrostreptomycin is not inactivated by cysteine under conditions[4] which cause the inactivation of streptomycin: thus, it appears that a mechanism involving the reaction of the carbonyl group with the amino and/or mercapto groups of cysteine is involved in the activation.

Dihydrostreptomycin is not degraded to maltol[5] when treated with alkali.

Tests by Dr. H. Robinson and Mr. O. Graessle of the Merck Institute for Therapeutic Research have shown that single doses of 85 units of dihydrostreptomycin trihydrochloride as contrasted with 45 units of streptomycin trihydrochloride–calcium chloride double salt were required to protect 50% of the mice against one lethal dose of *Salmonella schottmülleri*.

RESEARCH LABORATORIES
MERCK AND CO., INC.
RAHWAY, N. J.

ROBERT L. PECK
CHARLES E. HOFFHINE, JR.
KARL FOLKERS

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(1) Kuehl, Peck, Hoffhine, Graber and Folkers, THIS JOURNAL, **68**, in press (1946).

(2) Results to be published on streptomycin degradation products (Kuehl, Flynn, Brink and Folkers) are in best agreement with the formula $C_{21}H_{39}N_7O_{12}$ for streptomycin.

(3) Brink, Kuehl and Folkers, *Science*, **102**, 506 (1945).

(4) Denkewalter, Cook and Tishler, *ibid.*, **102**, 12 (1945).

(5) Schenck and Spielman, THIS JOURNAL, **67**, 2276 (1945).

OXIDATION OF LIGNIN SULFONIC ACIDS BY PERIODIC ACID

Sir:

It doubtless has occurred to many investigators that the degradation of cellulose in wood by periodic acid oxidation might provide a mild and facile method of obtaining lignin, provided that ligneous substances are not attacked by the reagent. Wald, Ritchie and Purves reported[1] the isolation of lignin by the action of periodic acid. We are moved therefore to make a preliminary report on our study of periodic acid oxidation of lignin sulfonic acids and other isolated lignins in progress in this Laboratory for more than a year. Periodic acid has been found to oxidize lignin sulfonic acids, including samples scrupulously freed of carbohydrate material originating from the wood pulping process. The purification processes employed to remove carbohydrates are: (a) fermentation with yeast; (b) diffusion of fermented sulfite waste liquor in Northup-Anson type sintered glass diffusion cells for sixty-two days; (c) preparation of barium ligno-sulfonate soluble in 40% acetone–water solution, insoluble in 70% acetone–water solution; (d) dialysis of fermented sulfite waste liquor against running water for one hundred and sixty-eight hours[2]; (e) purification by a quinoline extraction method, precipitation from quinoline solution of quinolinium ligno-sulfonates by the addition of ether and re-solution of the quinolinium salts in aqueous ammonium hydroxide.

Table I lists the various preparations and the equivalent weights of lignin sulfonic acids per mole of periodate reduced.

TABLE I

Preparation	% Methoxyl content of ammonium salt	Equiv. wt./mole of periodate reduced
a	8.5	200
b	10.1	304
c	9.7	347
d	13.0	525
e	11.9	525

It is apparent that preparations of low methoxyl content contain extraneous periodate-reducing substances, probably carbohydrates; but as refinement improves, the approach of the methoxyl content to a limiting value is accompanied by a similar constancy in the periodate equivalent weight. We believe samples (d) and (e) to be substantially free of carbohydrate material. The equivalent weights of 525 for preparations (d) and (e) correspond approximately to a ratio of two methoxyl groups for each linkage oxidizable by periodic acid.

Periodic acid oxidation of lignin sulfonic acids prepared by method (e) yields a barium salt,

(1) W. H. Wald, P. F. Ritchie and C. B. Purves, abstract No. 8, p. 4C, Division of Cellulose Chemistry, Abstracts of Papers 109th Meeting, American Chemical Society, Atlantic City, New Jersey, April 8, 1946.

(2) This material and its analysis were kindly supplied by Dr. Quentin Peniston of this Laboratory.

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.

PULP MILLS RESEARCH PROJECT
UNIVERSITY OF WASHINGTON DERROL PENNINGTON
SEATTLE 5, WASHINGTON D. M. RITTER

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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.

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.

J. J. PFIFFNER
RESEARCH LABORATORIES D. G. CALKINS
PARKE, DAVIS AND COMPANY E. S. BLOOM
DETROIT, MICHIGAN B. L. O'DELL

RECEIVED JUNE 20, 1946

(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.

(5) Our analytical data allowed a choice between $C_{19}H_{19}O_6N_7$ and $C_{21}H_{20}O_6N_8$ 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).