products that were insoluble in all common solvents and no molecular weights have been determined.

The yields of I, II and III are 40, 20 and 40%, respectively, based on the thiophene reacted, when two moles of thiophene was treated with one mole of formaldehyde and three moles of ammonium chloride. The excess reactants were recoverable. Attempts to improve the yields of I and II are being made.

Hexamethylenetetramine was found to react with thiophene in the presence of concentrated hydrochloric acid to give 7% of I, 25% of II and 68% of III on a weight per cent. basis.

Superficially, at least, this reaction appears to be similar to the Mannich reaction with ketones. It differs in that free amine bases and formaldehyde appear not to react and that primary and secondary amine hydrochlorides do not react as rapidly as ammonium chloride.

A preliminary study of the reaction with thiophene derivatives indicates wide applicability. Full details of the reaction with such derivatives as 2- and 3-methylthiophene, 2-chlorothiophene, and 2-*t*-butylthiophene will be reported in a later communication.

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MICRO-ANALYSIS OF MIXTURES (AMINO ACIDS) IN THE FORM OF ISOTOPIC DERIVATIVES

Sir:

A mixture is treated with a reagent containing a stable or radioactive isotope to form quantitatively a stable derivative of the desired constituent. An overwhelming excess, W, of the unlabelled derivative (the carrier) is added and purified to constant concentration, C_c. If C_r is the isotopic concentration of pure isotopic derivative prepared with the same reagent, the amount of derivative present is $W(C_c/C_r)$.

The method has much higher sensitivity than the familiar isotope dilution technique,[1] being theoretically operable at the level of trade substances. Furthermore, the use of racemic carriers avoids errors due to partial racemization. One isotopic reagent suffices for the analysis of many compounds.

As the labelled reagent we used *p*-iodophenyl sulfonyl chloride (PIPSYLchloride), prepared from radioactive iodide ion and *p*-diazobenzenesulfonic acid, followed by treatment with phosphorus pentachloride. A 5–10-fold excess reacts quantitatively with amino acids (glycine, alanine, isoleucine) as indicated by the disappearance of amino nitrogen.

β-Lactoglobulin was analyzed for glycine as follows: 0.3 ml. of an acid hydrolysate (1.13 mg. protein), 20 mg. of PIPSYLchloride, and excess sodium carbonate were shaken in a Folin tube at 90° for ten minutes. The walls were washed down, 5 mg. of labelled reagent added and the procedure repeated. One ml. of ammonia was added. The mixture, together with an acetone solution of some solid reaction products, was added to 200 mg. of normal PIPSYLglycine in ammonia, acidified, extracted with *n*-butanol, and iodobenzenesulfonate ion removed by passing the butanol over Duolite C3 (ion exchange resin). Ligroin was added and the carrier extracted into alkali and purified by repeated precipitation by acid, solution in ammonia, and treatment with activated charcoal, the amount at any stage being estimated spectrophotometrically at 2500 Å. and its radioactivity measured in solution with a Geiger counter. Values obtained at stages of purification corresponding to carrier recoveries of about 12.5, 10 and 7.5 were 1.59, 1.52 and 1.54% glycine for one analysis and 1.52, 1.52 and 1.50% for another. Rittenberg and Foster reported 1.5%.[1]

Less than one-hundredth per cent. of *d*(−)alanine was found in the β-lactoglobulin hydrolysate using PIPSYL *d*(−)alanine carrier. Seven and four-tenths per cent. of alanine was found when racemic carrier was employed. Chibnall reported 6.7%[2]; Brand, 6.2%.[3] Four and seven-tenths per cent. alanine was found in insulin. Chibnall reported 4.6%.[2] When 113 micrograms of alanine was added to a β-lactoglobulin hydrolysate containing 105 micrograms, 215 micrograms was found.

In two analyses, <0.2 and <0.5% isoleucine were found in human hemoglobin, confirming the low values previously reported.[4,5] The isotope concentration of the carrier diminished so slowly that only a few tenths per cent. of the carrier remained when the values were calculated.

The systematic application of this method to protein analysis is in progress.

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(1) D. Rittenberg and G. L. Foster, *J. Biol. Chem.*, **133**, 737 (1940).

(2) A. C. Chibnall, *J. Int. Leather Trades Chem.*, **30**, 1 (1946).

(3) E. Brand, *et al.*, THIS JOURNAL, **67**, 1524 (1945).

(4) E. Brand and J. Grantham, *ibid.*, **68**, 721 (1946).

(5) A. Albanese, *J. Biol. Chem.*, **157**, 613 (1946).

(6) Aided by a grant from the John and Mary Markle Foundation

STREPTOMYCES ANTIBIOTICS. IX. DIHYDROSTREPTOMYCIN

Sir:

Streptomycin has been catalytically hydrogenated to dihydrostreptomycin which is active against *B. subtilis in vitro* and *S. schottmülleri in vivo*.

Streptomycin trihydrochloride was hydrogenated in aqueous solution with a platinum cata-

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 *p*H 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*.

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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.