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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RECEIVED JUNE 14, 1946

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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RECEIVED JUNE 19, 1946

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[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-