at 135–140° for two hours produced 17.3 g. (80.8%) of (VI), a yellow liquid, at 183–185° (8 mm.).

(2) Twenty-nine grams of N,N′-dibutyl-ethylenediamine dithiocarbamate heated at 130–135° for about two hours gave 21.5 g. (85%) of (VI) at 183–184° (8 mm.).

1,3-Dibutyl-trimethylenethiourea (VII).[11]—(1) Starting with 21.4 g. (0.1 mole) of N-formyl-(III) and 4.0 g. of sulfur and heating at 145–150° for two hours and at 175° for one-half hour, 9.2 g. of unconverted N-formyl-(III) was recovered, and 5.8 g. (25%) of (VII) was obtained as a yellow liquid, b. p. 177–178° (3 mm.).

(2) A stirred solution of 37.2 g. (0.2 mole) of (III) in 50 cc. of methanol was treated with a solution of 15.2 g. (0.2 mole) of carbon disulfide in 40 cc. of methanol in the course of fifteen minutes. The solvent was evaporated from the resulting solution and the remaining thick liquid, the dithiocarbamate, was heated at 150–155° until the evolution of gas ceased (about two hours). The reaction mixture then was distilled, yielding 12.4 g. of recovered (III), b. p. 107° (7 mm.), and 14.6 g. (31%) of (VII), b. p. 177–178° (3 mm.).

1,3-Diphenyl-ethylenethiourea.—This product was prepared in 71% yield by heating N-formyl-(V) with sulfur, but in this case little reaction was observed below 195°. (V) did not form a dithiocarbamate on treatment with carbon disulfide and water under reflux at atmospheric pressure for twenty hours.

(11) N-Monosubstituted-trimethylenethioureas have been made by pyrolysis of the dithiocarbamates of the corresponding N-substituted-trimethylenediamines; Goldenring, *Ber.*, **23**, 1171 (1890); Fränkel, *ibid.*, **30**, 2501 (1897).

Acknowledgments.—The author is indebted to Mrs. J. D. Nevins and Mrs. R. C. Schropp of the Monsanto Analytical Laboratory for the analyses reported.

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RECEIVED JANUARY 17, 1946

COMMUNICATIONS TO THE EDITOR

CRYSTALLINE VITAMIN A METHYL ETHER

Sir:

In recent years much interest has been shown in the synthesis of vitamin A ethers. However, since no data are available concerning the biological activity of these ethers, we have undertaken the preparation of vitamin A methyl ether from the natural vitamin.

The methyl ether was prepared by the action of dimethyl sulfate on the lithium derivative of the vitamin, which was formed by the reaction of *n*-butyl lithium[1] and crystalline vitamin A alcohol.[2] It was purified by chromatography on activated alumina[3] and was obtained as an orange oil, which crystallized from methanol after several months at −70°, m. p. 31–33°. After three recrystallizations from methanol and two from a 65–70° hydrocarbon fraction [Purified Skelly Solve B],[4] vitamin A methyl ether was obtained as light yellow crystals melting at 33–34°. *Anal.* Calcd. for $C_{21}H_{32}O$: C, 83.95; H, 10.74; OCH_3, 10.34. Found: C, 83.76; H, 11.07; OCH_3, 9.94.

The spectrophotometric curve for crystalline vitamin A methyl ether is identical in all respects with that of vitamin A alcohol, both having absorption maxima at 326 mμ on the Beckman spectrophotometer. The extinction coefficient ($E^{1\%}_{1\,cm.}$) in isopropanol at 326 mμ is 1660. This corresponds to an equivalent extinction coefficient of 1742 for vitamin A alcohol.

Vitamin A methyl ether possesses a biological potency greater than 3,000,000 U. S. P. XII units per gram and is of the same order of activity as crystalline vitamin A alcohol.

The experimental details and complete biological data will appear in a forthcoming paper.

(1) Gilman, Langham and Moore, THIS JOURNAL, **62**, 2327 (1940).
(2) Distillation Products, Inc., Rochester, N. Y.
(3) Aluminum Ore Co., East St. Louis, Illinois.
(4) Purified by treatment with concentrated sulfuric acid and distillation.

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RECEIVED JUNE 17, 1946

AMINOMETHYLATION OF THIOPHENE

Sir:

During the course of formylation studies with thiophene it was noted that in the presence of ammonium chloride and formaldehyde thiophene appeared to undergo a reaction to give water-soluble amine hydrochlorides. From the reaction mixture was isolated 2-thenylamine (2-aminomethylthiophene) (I), b. 58° (5 mm.), $n^{20}D$ 1.5589; secondary di-(2-thenyl)-amine, b. p. 150–152° (10 mm.), $n^{22}D$ 1.5914; and a third amine (III). Amine III is polymeric in nature and is believed to contain methylol groups. The hydroxyl number of III produced by the reaction of one mole of thiophene with four moles of 37% formaldehyde and one mole of ammonium chloride at the reflux was 475, indicating that methylol groups may be substituted around the thiophene in all remaining positions. Other analysis obtained on the product were as follows: 20.6% sulfur and 7.3% nitrogen. With the use of aqueous 37% formaldehyde in excess III is obtained exclusively and molecular weights of 600–750 are the usual order. The use of trioxymethylene with a few per cent. by weight acetic acid (to promote depolymerization at lower temperatures) gave

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

(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-