of the sulfonic acid as the sodium salt and its conversion to the sulfonyl chloride,[6] but these operations require equipment not available in the ordinary laboratory. The nitration of the sulfonyl chloride according to the literature[4a] furnishes the 4-nitro and 5-nitro derivative in an 8:3 ratio. In our work the ratio of 4-nitro to 5-nitro was closer to 9:1.

2,4-Dinitrothiophene. Crude 2,4-dinitrothiophene obtained by the nitration of 2-nitrothiophene[4a] was purified by three crystallizations from ethanol and one from petroleum ether (b.p. 40–60°). Solutions were prepared and handled in the same way as with 2-nitrothiophene. About 2 g. of the dinitrothiophene will dissolve in 10 ml. of ethanol or 250 ml. of petroleum ether at 40°. The recovery from ethanol is about 15%, from petroleum ether about 60%. The pure dinitrothiophene melts at 49.8–50.2°, corr., in a Hershberg apparatus.

2,4-Dinitrothiophene from 2-Iodo-3,5-dinitrothiophene. A solution of 4.5 g. of sodium iodide in 15 ml. of acetone was added to 3.0 g. of 2-iodo-3,5-dinitrothiophene dissolved in 15 ml. of acetone and 5 ml. of glacial acetic acid. After two weeks the dark brown reaction mixture was poured into a solution of 5.0 g. of sodium bisulfite in 140 ml. of water and the dark oily precipitate was stirred until it became granular. The yellow-brown solid was dissolved in 25 ml. of hot ethanol and the solution, after it had been decolorized with Norit and filtered, was diluted with 40 ml. of water to yield 1.2 g. (66%) of 2,4-dinitrothiophene, m.p. 51–52°, whose identity was confirmed by a mixed melting point. In another experiment the solid was digested with ligroin; the extract on evaporation furnished 2,4-dinitrothiophene. The residue from the ligroin extraction when crystallized from ethanol gave unreacted 2-iodo-3,5-dinitrothiophene.

QUEENS COLLEGE
FLUSHING 67, N. Y.

(6) See reference 2a. p. 513.

An Improved Micro Synthesis of Thiamine-S^{35} [1]

M. JACQUELINE VERRETT AND LEOPOLD R CERECEDO

Received March 14, 1957

In connection with a study of the metabolism of thiamine which is in progress in this laboratory, it became necessary to prepare thiamine-S^{35} of a high specific activity. Williams and Ronzio[2] have synthesized labeled thiamine from thiourea-S^{35} and thiourea-2-C^{14}. We attempted to duplicate this synthesis, and obtained very low yields when less than 300 mg. of thiourea-S^{35} was used for the initial step of the synthesis. We therefore revised the steps of the synthesis and, starting with 50–100 mg. of carrier-free thiourea-S^{35}, have obtained high yields.

EXPERIMENTAL

2-Amino-5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} hydrochloride. Thiourea-S^{35} (100 mg.)[3] was coupled with 330 mg. of γ-aceto-γ-chloropropanol (1:1.5 molar ratio, respectively) according to the method of Todd *et al.*[4] The substances were mixed in a 5-ml. beaker on the steam bath, and the mixture stirred continuously until the thiourea-S^{35} went into solution, and then 5 min. longer. The solution was removed from the steam bath, and stirred until the mass solidified. The solid mass was broken up, transferred to a centrifuge tube, extracted with anhydrous ether, and the ether was discarded. The residue was dissolved in the minimum amount of hot anhydrous ethanol (1–1.5 ml.). After the solution was cooled, absolute ether was added dropwise until crystallization began. A fivefold excess of ether was then added and the crystallization allowed to go to completion while standing for about 12 hr. in the refrigerator. The precipitate was filtered, rinsed with anhydrous ether, and dried in a vacuum desiccator to yield 220 mg. (86%) of 2-amino-5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} hydrochloride, m.p. 148–150°.

It was found that when 75–100 mg. of thiourea were coupled with γ-aceto-γ-chloropropanol in a molar ratio of 1:1.5, respectively, an average yield of 82% of the 2-aminothiazole was obtained. When very small amounts (less than 75 mg.) of thiourea were used, increasing this ratio of reactants to 1:2 resulted in an average yield of 78%.

5-Methyl-4-(β-hydroxyethyl)thiazole-S^{35}. 2-Amino-5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} hydrochloride (220 mg.) was dissolved in 5.8 ml. of concentrated hydrochloric acid, with cooling in a methanol–ice bath at −5°. Sodium nitrite (1.8 ml. of a 1*N* solution, precooled to 0°) was added dropwise. The mixture was allowed to stand for 30 min. at 0° to −5°. Then 5.8 ml. of water (also precooled to 0°) was slowly added in order to reduce the concentration of hydrochloric acid to 6*N*. During approximately 10 min., 2.6 ml. of cold 32% hypophosphorous acid was added, with the temperature maintained at −5°. The solution was stirred rapidly during all of the above additions. The reaction mixture was placed in the refrigerator at 0° to 2° for 12–15 hr., during which time nitrogen was evolved. The solution was cooled to −5° and neutralized by the addition of 30% sodium hydroxide. Excess base was then added to bring the *p*H to 11–12, and the solution was washed into a continuous extractor and extracted for 16 hr. with ether.[5] After removal from the extractor, the aqueous phase was extracted with several portions of ether in a separatory funnel. The combined ethereal extracts were dried over anhydrous magnesium sulfate, and the ether was removed by distillation from the steam bath. The yield of 5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} was 70 mg. (50%). The reduced thiazole was obtained in yields ranging from 50–70% when 150–250 mg. of the 2-aminothiazole were used. The crude thiazole-S^{35} was identified by the conversion of a small amount to the picrate, which melted at 159–161° when precipitated from anhydrous ether.

Since the condensation of the crude thiazole with the pyrimidine moiety gave very low yields of thiamine, we found it necessary to purify the crude material by distillation under reduced pressure. The pure thiazole-S^{35} was obtained in recoveries of 85–90%, when 50–100 mg. of the crude material were distilled under reduced pressure (b.p. 126–128° at 2–3 mm. and 128–130° at 3–4 mm. pressure).

Thiamine-S^{35} bromide hydrobromide. The 5-methyl-4-(β-hydroxyethyl)thiazole-S^{35} (25 mg.) and 60 mg. of 2-methyl-4-amino-5-bromomethylpyrimidine hydrobromide (1:1 molar ratio) were dissolved in 2 ml. of anhydrous ethanol[6] in a 20-ml. pear-shaped flask fitted with a reflux condenser,

(1) This investigation was aided, in part, by Contract No. AT (30-1)-1056 between the U.S. Atomic Energy Commission and Fordham University.

(2) D. L. Williams and A. R. Ronzio, *J. Am. Chem. Soc.*, **74**, 2409 (1952).

(3) Obtained from Abbott Laboratories, Division of Radio-Pharmaceuticals, North Chicago, Ill.

(4) A. R. Todd, F. Bergel, H. L. Fraenkel-Conrat, and A. Jacob, *J. Chem. Soc.*, 1601 (1936).

(5) The extraction period can be shortened from 40 hr. without any decrease in the yield of thiazole.[2]

(6) Williams and Ronzio[2] carried out the condensation in butanol at 125°. The side reaction (ether formation between the butanol and the β-hydroxyethyl group of the thiazole moiety) which occurs under those conditions was completely avoided when anhydrous ethanol was used as the solvent.

and the mixture was heated on the steam bath for 1 hr. After the reaction mixture was allowed to stand in the refrigerator overnight, the precipitated thiamine-S^{35} bromide hydrobromide was filtered. Additional material was obtained by dilution of the mother liquor with anhydrous ether. The crude reaction product melted at 239–240° with decomposition.[7] The crude thiamine-S^{35} was recrystallized by dissolving in 2 ml. of anhydrous methanol and diluting the solution with 20 ml. of anhydrous ether. The yield was 81 mg. (90%) of thiamine-S^{35} bromide hydrobromide with a m.p. 229–231° (with dec.).

It was found that condensation of 25–75 mg. of thiazole with an equimolar amount of pyrimidine gave thiamine yields from 74–93% of once-recrystallized material, the average yield being 87%. We observed that refluxing for more than 1 hr. did not improve the yield, and that the best yields were obtained when the condensation was allowed to take place in the minimum amount of ethanol necessary to dissolve the pyrimidine.

A sample of thiamine bromide hydrobromide prepared from nonradioactive thiourea according to the above procedure was analyzed.

Anal. Calcd. for $C_{12}H_{18}Br_2N_4OS$: N, 13.4; Br, 37.50. Found: N, 13.04; Br, 37.50.

When thiourea-S^{35} with a radioactivity content of 6 mc. per 100 mg. was used, the thiamine-S^{35} bromide hydrobromide obtained had a specific activity of about 1.4 mc. per 100 mg.

DEPARTMENT OF BIOCHEMISTRY
FORDHAM UNIVERSITY
NEW YORK 58, N. Y.

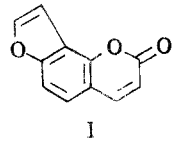
(7) With respect to the melting points of thiamine preparations, see Williams and Ronzio.[2]

Synthetic Experiments in the Furocoumarin Series

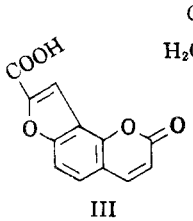
R. M. NAIK AND V. M. THAKOR

Received March 1, 1957

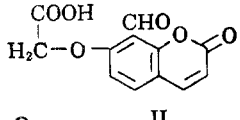
In the course of our investigations on the synthesis of some naturally occurring furocoumarins, we required angelicin (I) as the starting material. In our effort to prepare it according to Späth and Pailer,[1] we observed the melting point of 7-(8-formylcoumarinoxy)acetic acid (II) to be much higher (248–249°) than what they stated (178–181°). Moreover, this acid on heating with fused sodium acetate and acetic anhydride, gave a good yield of 2′-carboxyangelicin (III) along with angelicin (I). The authors do not appear to have isolated the acid III. This acid underwent smooth decarboxylation with quinoline and copper powder to furnish more of angelicin (I).



I



III

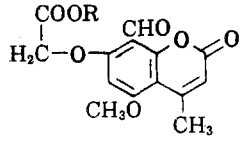


II

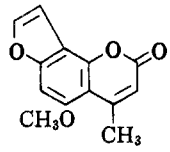
(1) E. Späth and M. Pailer, *Ber.*, **68B**, 941 (1935).

The Elbs persulfate oxidation of angelicin under the usual conditions[2] met with failure. Similarly a parallel oxidation of 3′,4-dimethyl-furo-7,8-(4′,5′)-coumarin prepared according to Limaye[3] also failed indicating the improbability of successfully effecting Elbs oxidation on coumarins with furan ring in the 7,8-position. An attempt to introduce a hydroxyl group in 6-position by Elbs oxidation of II was unsuccessful. A stable coumarinic acid derivative[4] was isolated, which regenerated the parent coumarin (II) on crystallization from acetic acid.

The interaction of 5-methoxy-7-hydroxy-4-methyl-8-formylcoumarin[5] with ethyl bromacetate gave poor yield of ethyl 7-(5-methoxy-4-methyl-8-formylcoumarinoxy)acetate (IV, R = C_2H_5) which furnished the corresponding acid (IV, R = H) on hydrolysis with 5% methanolic potassium hydroxide. On refluxing this acid with fused sodium acetate in acetic anhydride, 4-methylisobergaptene (V) was obtained in poor yield. Some other dark, alkali-soluble material was obtained, which failed to give a definite product. This indicates an alternative method of synthesis of isobergaptene.



IV



V

EXPERIMENTAL

All melting points are uncorrected.

Ethyl 7-(8-formylcoumarinoxy)acetate. A mixture of 7-hydroxy-8-formyl-coumarin[1] (2 g.), anhydrous potassium carbonate (6 g.) and ethyl bromoacetate (2.0 cc.) in dry acetone (100 c.c.), was gently refluxed on a water bath for 48 hr. The contents were filtered and the fitrate, on evaporation of acetone, yielded a white product along with the residual ethyl bromoacetate which was removed by closed steam distillation. The product crystallized from alcohol as shining flakes, m.p. 163°. Yield 1.6 g. Späth and Pailer[1] give m.p. 157° in a vacuum capillary.

7-(8-Formylcoumarinoxy)acetic acid (II). The preceding ester (1 g.) was kept at room temperature with sodium hydroxide (8 cc., 5%) for 3 hr. when the ester slowly went into solution. The product which separated on acidification, crystallized from acetic acid as thin white needles, m.p. 248–249° (decomp.) Yield 0.7 g. The hydrolysis could also be brought about by 80% sulfuric acid at room temperature or boiling with 5% alcoholic potassium hydroxide.

Since the previous authors[1] gave m.p. 178–181° (dec.) in a vacuum capillary, it was thought desirable to analyze the acid.

Anal. Calcd. for $C_{12}H_8O_6$: C, 58.1; H, 3.2. Found: C, 57.9; H, 3.3.

(2) S. Sethna and co-workers, *J. Ind. Chem. Soc.*, **27**, 369 (1950); **28**, 366 (1951); **30**, 610 (1953).

(3) D. B. Limaye, (a) *Ber.*, **65B**, 375 (1932); (b) with D. D. Gangal, *Rasāyanam*, **1**, 15 (1936); *Chem. Abstr.*, **31**, 2207 (1937).

(4) *Cf.* R. M. Naik and V. M. Thakor, *J. Org. Chem.*, **22**, 1626 (1957).

(5) R. M. Naik and V. M. Thakor, *J. Org. Chem.*, **22**, 1630 (1957).