are consistent with this picture of the reaction and important contributions from a *quasi*-ring intermediate are improbable. Further evidence for the ionic path was obtained from the attempted reaction of silver trifluoroacetate with 1-chloronorbornane. Bridgehead halides react but sluggishly, if at all, by ionic dissociation.[9] When solutions of 1-chloronorborane and silver trifluoroacetate in ether, benzene, or dioxane were refluxed for 58 hours, no evidence for reaction between the two solutes was obtained. This is in striking contrast to the very fast reaction between 2-bromooctane and silver trifluoroacetate.

EXPERIMENTAL

Preparation of materials. Silver trifluoroacetate was prepared by adding an ether solution of trifluoroacetic acid[10] to a suspension of silver oxide in ether. The solution was filtered from the slight excess of silver oxide, the ether was removed by distillation, and the residue was recrystallized from benzene.

(+)2-Bromooctane was prepared by treating (−)2-octanol (α_D^{25} −7.32°) with an equivalent amount of PBr_3; b.p. 75° (15 mm.), n_D^{20} 1.4507, α_D^{20} +35.24°.

1-Chloronorbornane was prepared[11] by the $AlCl_3$- catalyzed hydrogen-chlorine exchange between pentane and 2,2-dichloronorbornane.[11] The yield of twice-distilled material was 39%; b.p. 82-84° (87 mm.), n_D^{20} 1.4710, d_4^{20} 1.016.

Silver trifluoroacetate with 2-bromooctane. A solution of 33.5 g. (0.152 mole) of silver trifluoroacetate in 750 ml. of anhydrous ethyl ether was placed in a 1-l. flask equipped with a high dilution cycle.[12] While the solution was refluxed gently, a solution of 20.5 (0.106 mole) of (+)2-bromooctane in 150 ml. of ether was added through the dilution cycle during 48 hr. Openings to the atmosphere were protected with drying tubes. Precipitation of AgBr began soon after the addition of 2-bromo-octane was begun.[13] The mixture was refluxed for 4 hr. longer and about half of the ether was removed by distillation. The remaining solution was washed with water, K_2CO_3 solution, water, $Na_2S_2O_3$ solution, and water. It was dried with $MgSO_4$ and distilled. After considerable amount of forerun which decolorized $KMnO_4$ and Br_2 solutions (probably octenes) had been removed, (−)*s*-octyl trifluoroacetate[8] was obtained in 11% yield; b.p. 71–71.3° (17 mm.), n_D^{20} 1.3780, d_4^{20} 1.002. In another experiment, a 17% yield of ester was obtained. The infrared spectra of the two samples of ester were essentially identical and indicated slight contamination by alcohol. The samples of ester were combined, redistilled, and saponified by refluxing for 2.5 hr. with excess 10% NaOH solution.

The mixture was steam-distilled and (−)2-octanol was isolated in the usual way; $n_D^{20.5}$ 1.4240, d_4^{20} 0.823, α_D^{20} −2.23°.

Silver trifluoroacetate with 1-chloronorbornane. Three solutions of 1-chloronorbornane (1.0 g., 0.008 mole) and silver trifluoroacetate (1.9 g., 0.009 mole) in 30 ml. of solvent (benzene, ethyl ether, and dioxane, separately) were refluxed in the dark for 58 hr. No trace of AgCl precipitate was observed. A small amount of a brown precipitate formed in the dioxane solution. This material dissolved in aqueous ammonia but did not reappear when that solution was made acidic with HNO_3. The brown solid was probably silver oxide.

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(13) In a preliminary experiment 2-bromo-octane was poured into a solution of silver trifluoroacetate in ether. Precipitation of AgBr began immediately, the ether began to boil, and the reaction appeared to be over in less than 5 min.

Synthesis of Isotopically Labeled Medicinals. I. 2-Thiouracil-S^{35}

J. B. ZIEGLER, A. C. SHABICA, AND M. SONENBERG

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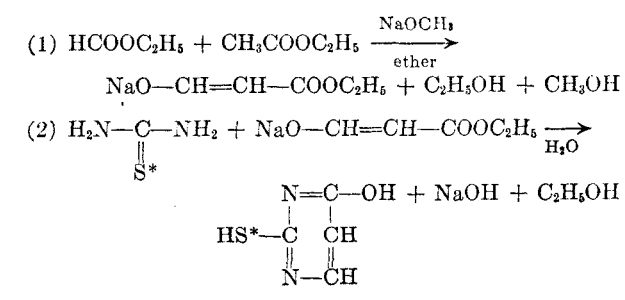
2-Thiouracil is well known as a substance with potent biological, especially antithyroid, activity,[1] and there has been considerable interest in the incorporation of various isotopic tracer atoms into this molecule. The first such preparation appears to have been that of Plentl and Schoenheimer[2] who labeled the molecule with the stable N^{15} isotope. At a later date Bennett[3] prepared this substance with the C^{14} label in the 2-position, and shortly thereafter Jeener and Rosseels[4] reported the use of this compound containing the S^{35} label in an investigation concerned with tobacco mosaic virus. However, the latter workers gave no preparative details, and since we required a sample of this particular variant for biological studies, it was necessary to work out a synthesis on a micro scale. This was accomplished, starting with S^{35}-labeled 2-thiourea, according to the following scheme.



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The sodium enolate of formylacetic acid ethyl ester was chosen for conversion of the S^{35}-labeled thiourea after preliminary experiments had shown that it was superior to both ethyl β,β-diethoxypropionate[5] and ethyl β-ethoxyacrylate[5] for this purpose. The requisite sodium enolate of formylacetic acid ethyl ester was prepared by condensation of ethyl formate with ethyl acetate. The procedure was essentially that of Wislicenus[6] except that commercially available sodium methoxide was substituted for metallic sodium as condensing agent. Conditions for the crucial second step of the synthesis of the labeled thiouracil were standardized by a series of condensations of the crude enolate of formylacetic acid ethyl ester with ordinary thiourea before proceeding to conversion of the labeled thiourea. A study of the fate of this labeled thiouracil in the rat indicated a high degree of concentration in the thyroid. Details will be published elsewhere.

EXPERIMENTAL

Sodium enolate of formylacetic acid ethyl ester. To a 1-l. 3-necked round-bottomed flask equipped with a reflux condenser (fitted with a calcium chloride tube), mechanical stirrer, and thermometer, was added 500 ml. of anhydrous ether and 27 g. (0.5 mole) of commercial sodium methoxide. With stirring, a mixture of 49 ml. (44.1 g.; 0.5 mole) of ethyl acetate, and 49 ml. (44.2 g.; 0.6 mole) of ethyl formate was added slowly. The resulting suspension was stirred for 6 hr., allowed to stand for 2 days without stirring and then stirred for 2 days more.

The solid was collected on a Büchner funnel with suction, washed well with anhydrous ether and dried *in vacuo*. The resulting crude product weighed 13.2 g. It was estimated to contain about 42% of the sodium enolate of formylacetic acid ethyl ester, based on the amount of 2-thiouracil isolated on condensation of a sample with an excess of thiourea.

2-Thiouracil-S^{35}. The reaction conditions described below are similar to those of Wheeler and Liddle.[7]

The S^{35}-labeled 2-thiourea was obtained from Tracerlab, Inc., 130 High Street, Boston 10, Mass. The sample used weighed 61 mg. (0.8 millimole) and was stated to contain 8 millicuries of S^{35} activity. It was prepared by Tracerlab to order and was delivered to our laboratory immediately after preparation and determination of activity. The experimental work described below was completed within one week after receipt of the thiourea.

The labeled thiourea was dissolved in 3 ml. of distilled water in a test tube and treated with 660 mg. (a large excess) of crude sodium enolate of formylacetic acid ethyl ester. The test tube was loosely stoppered and heated on the steam bath at 95–100° for 1.5 hr. The reddish solution was cooled to room temperature, filtered to remove a trace of insoluble material and acidified with glacial acetic acid. The precipitate was collected on a small Hirsch funnel with suction, washed with distilled water and ethanol and dried briefly on the steam bath. The product weighed 84 mg. (82% of the theoretical yield based on thiourea), dec. 315° (lit.[7] m.p. *ca.* 340° dec.). A portion was converted to the known[7] S-benzyl derivative by warming with benzyl chloride in dilute aqueous alcoholic sodium hydroxide solution. An 81% yield of product was obtained, m.p. 196–199°. A mixture with an authentic, inactive specimen of 2-benzylthiouracil, m.p. 196–199° (lit.[7] m.p. 192–193°), had m.p. 196–199°. Similar results were obtained when authentic, inactive thiouracil was benzylated. The labeled 2-thiouracil was estimated to contain (0.82 × 8 mc. =) 6.6 mc. of S^{35} activity, and therefore to have a specific activity of (6.6 mc./84 mg. =) 0.079 mc./mg. A direct β-ray count on the substance recorded 2.15 × 10^7 counts/mg./min.

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Potential Purine Antagonists XIV. Synthesis of Some 4-(Substituted amino)pyrazolo-[3,4-*d*]pyrimidines[1]

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Recent anti-tumor activity[2] exhibited by certain 4-amino- and 4-alkylaminopyrazolo [3,4-*d*]pyrimidines has prompted the preparation of additional derivatives in this series.

The new 4-(substituted amino)pyrazolo[3,4-*d*]-pyrimidines and 1-methyl-4-(substituted amino)-pyrazolo[3,4-*d*]pyrimidines which have been prepared are listed in Tables I and II. These compounds have been prepared by reaction of the corresponding 4-chloropyrazolo[3,4-*d*]pyrimidine[3,4] with various primary and secondary amines in a manner similar to that previously described.[3,4] When the higher homologs of the alkylamines were employed, it was found convenient to isolate these derivatives as the hydrochloride rather than the free base.

The anti-tumor activity of these compounds will be reported elsewhere at a later date.

EXPERIMENTAL

General method of preparation of 1-methyl-4-(substituted amino)pyrazolo[3,4-d]pyrimidines listed in Table I. Method (A). Ten grams of 1-methyl-4-chloropyrazolo[3,4-*d*]pyrimidine[4] was added to a solution of an equal molar amount of the amine dissolved in 150 ml. of absolute ethanol. The solution was heated on the steam bath for 2 hr., then boiled with charcoal and filtered. Dry hydrogen chloride gas was passed into the cooled filtrate for 20 min. The solution was then allowed to stand overnight and the precipitate filtered

(1) This investigation was supported by research grants C-2105(C2) and C-2105(C3) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

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