

activity of I (1,2). This in turn prompted preparation of carbon-14 labeled II for further metabolism studies. This report describes the synthesis of I and II labeled with carbon-14.

EXPERIMENTAL

Radioactivity Measurements

All counting was performed with a Packard Tricarb Model 314EX2A liquid scintillation spectrometer at -8° under conditions suitable for measuring carbon-14. Appropriate aliquots of samples were dissolved in 15 ml of scintillation solvent [toluene-dioxane-methanol (350:350:210 by volume) containing 73 g of naphthalene, 4.6 g of 2,5-diphenyloxazole, and 0.08 g of 1,4-bis[2-(5-phenyloxazolyl)] benzene per liter]. The absolute counting efficiency for each sample was determined by recounting following addition of an internal standard of toluene- ^{14}C and results were then converted to mCi.

Paper chromatograms were scanned for radioactivity with a Vanguard Model 880 radiochromatogram scanner.

Paper Chromatography

Chromatograms were developed by the descending method using 86-cm lengths of Whatman No. 2 paper in the following systems: (a) Bush B-5, sheet equilibrated overnight at 34° in the vapor from a mixed solvent composed of benzene-methanol- H_2O (2:1:1 by volume) and developed in the benzene phase (R_f values for I and II were 0.74 and 0.11, respectively); and (b) Mattox I, sheet saturated with methanol-formamide (1:1 by volume), dried 15 minutes at 37° , and developed with n-butyl acetate-formamide- H_2O (100:5:5 by volume) (R_f values for I and II were 0.42 and 0.05, respectively).

Zones absorbing UV light were located as fluorescence quenching areas when the dried chromatograms were viewed with a short wavelength UV scanner.

Synthesis

Cyanogen Bromide- ^{14}C (III) - An aqueous solution of K^{14}CN^* (IV) (1.30g, 20 mM),

*Purchased from New England Nuclear Corp., Boston, Mass., U.S.A.

containing 16 mCi of carbon-14, was converted to cyanogen bromide-¹⁴C (III) by treatment with bromine as described by Graff, *et al* (3). A yield of 60% was obtained as determined by titrating the resulting ether solution of III with standard NaOH.

Cyanamide-¹⁴C (V) - The ether solution of cyanogen bromide-¹⁴C (III) was treated with 28 mM of ammonia in methanol to obtain cyanamide-¹⁴C (V) by the method of Graff, *et al* (3).

Cyanoguanidine-¹⁴C (VI) - The ether solution of cyanamide (V) was evaporated to dryness and converted to cyanoguanidine-¹⁴C (VI) by refluxing in aqueous ammonia as described by Zbarsky and Fischer(4). A yield of 0.328 g (65% based on III) of cyanoguanidine-¹⁴C (VI) was obtained; m.p. 208.5-209° (capillary, uncorrected).

2,4-Diamino-6-(diallylamino)-s-triazine-2,4-¹⁴C (I) - The cyanoguanidine-¹⁴C (VI) (0.326 g), 0.2 g of nonradioactive VI, 2 ml of methylcellosolve, 0.02 g of powdered KOH and 0.77 g of diallylcyanamide were added to a reaction flask equipped with a reflux condenser and magnetic stirring bar. The reaction mixture was stirred and heated with an oil bath. At 90-110°, the reaction mixture became homogeneous and light brown in color. The temperature was increased to 155° and the reaction mixture was refluxed for 0.25 hr. After cooling to room temperature, the reaction mixture was poured into 20 ml of ice water. The resulting product was filtered and dried to yield 0.902 g of crude product. This material was recrystallized from ethanol-H₂O with the aid of charcoal (Darco G-60) to obtain 0.856 g (67% yield based on VI) of 2,4-diamino-6-(diallylamino)-s-triazine-2,4-¹⁴C (I) having a specific activity of 1.01 mCi per mM; m.p. 142.5-143° (capillary, uncorrected). The IR [(Nujol mull) 3490, 3390, 3300, 3190, 3150 (N-H); 1645, 1615, 1550, 1515, 1490 (C=N, C=C, N-H deformation); 1315, 1280 (C-N); 970, 920, 810 cm⁻¹] and UV [(0.01N aqueous HCl) 238.5 mμ (ε_{31,550})] spectra of the product corresponded to those of standard I. The product showed single UV absorbing and radioactive zones corresponding to I in the Bush B-5 and Mattox I paper chromatography systems. *Anal.*-Calcd. for C₉H₁₄N₆: C, 52.3; H, 6.78; N,

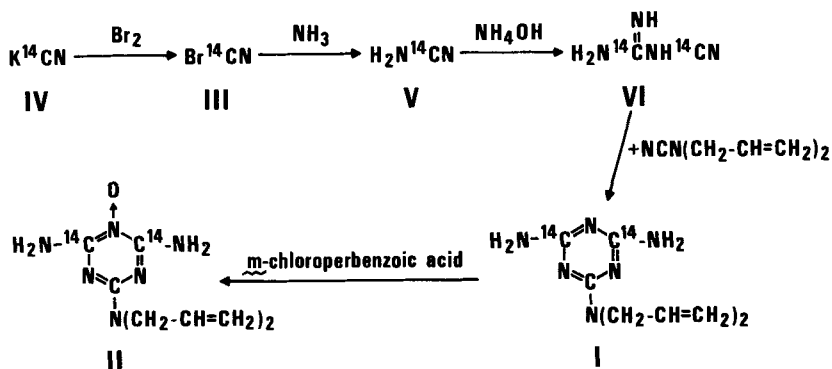
40.7. Found: C, 52.1; H, 6.60; N, 41.2.

2,4-Diamino-6-(diallylamino)-*s*-triazine-2,4-¹⁴C 3-oxide (II) - 2,4-Diamino-6-(diallylamino)-*s*-triazine-2,4-¹⁴C (I) (0.15 g, 0.72 mCi) and 0.45 g of nonradioactive I were dissolved in 12.5 ml of ethanol at 0°. The solution was stirred vigorously and 1.01 g of *m*-chloroperbenzoic acid was added in four portions over a 0.5 hr period. The reaction mixture was stirred at 0° for 16 hrs. The resulting suspension was evaporated to dryness *in vacuo* and the residue was stirred for 2 hrs with 10 ml of 1N HCl. The solid was removed by filtration and washed twice with small portions of water. The filtrate was adjusted to pH 9 and the resulting oily precipitate was removed by filtration. The filtrate was extracted five times with 15-ml portions of chloroform. The combined chloroform extracts were dried over Na₂SO₄ and evaporated to dryness. The residue was recrystallized from 25 ml of acetonitrile to yield 0.236 g (37% yield based on I) of 2,4-diamino-6-(diallylamino)-*s*-triazine-2,4-¹⁴C 3-oxide (II) having a specific activity of 0.24 mCi per mM; m.p. 181.5-182° (capillary, uncorrected). The IR [(Nujol mull) 3490, 3440, 3400, 3360, 3280, *ca* 2950b (N-H, O-H); 1665, 1615, 1585, 1510, 1485 (C=N, C=C, N-H deformation); 1310, 1285, 1200 (C-N, N-O); 930, 910, 765] and UV [(H₂O) 246 m μ (ϵ 16,050); (0.01 N HCl) 237 m μ (ϵ 26,750); (0.01N NaOH) 246 m μ (ϵ 16,150)] spectra of the product corresponded to those of standard II. The product showed single UV absorbing and radioactive zones corresponding to II in the Bush B-5 and Mattox I paper chromatography systems. *Anal.*-Calcd. for C₉H₁₄N₆O: C, 48.6; H, 6.35; N, 37.8. Found: C, 48.5; H, 6.15; N, 37.2.

RESULTS AND DISCUSSION

2,4-Diamino-6-(diallylamino)-*s*-triazine-2,4-¹⁴C (I) was prepared from K¹⁴CN by the sequence of reactions shown in Scheme 1. The conversion of K¹⁴CN to cyanoguanidine-¹⁴C was accomplished using reactions well established in carbon isotope synthesis. The yield of cyanogen bromide-¹⁴C in the first step, 60%, was significantly lower than that obtained in trial runs and reported by Graff,

Scheme 1



et al (3). The reason for the lower yield is not clear, but may be related to the possible presence of alkali in the $K^{14}CN$. The other steps leading to cyanoguanidine-¹⁴C gave the anticipated yields.

The condensation of cyanoguanidine-¹⁴C (VI) and diallylcyanamide resulted in a 67% yield of 2,4-diamino-6-(diallylamino)-s-triazine-2,4-¹⁴C (I). This product was both chemically and radiochemically pure. Oxidation of I to the N-oxide, 2,4-diamino-6-(diallylamino)-s-triazine-2,4-¹⁴C 3-oxide (II), with *m*-chloroperbenzoic acid was accomplished in 37% yield. This product, also, was shown to be both chemically and radiochemically pure.

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