5-Allyluridine[1]

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As part of a program directed toward the synthesis of 5-allyldeoxyuridine for possible use in cancer chemotherapy, we prepared 5-allyluridine in 36% over-all yield from 5-allyluracil. Following the mercuri procedure[3] 5-allyluracil[4] formed a mercury salt, 5-allyluracilmercury (I), in which the pyrimidine and mercury were present in a 1:1 molar ratio. Attempts to prepare bis-(5-allyluracil)mercury or monochloromercuri-5-allyluracil were unsuccessful.[5] Accordingly I was condensed with two molar proportions of 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride in refluxing xylene to give the blocked 5-allyluridine (II) as a tan glass. Attempts to crystallize II failed and nitrogen analysis indicated the glass contained about 60% II.

When II was deacylated by heating in ethanolic ammonia crude 5-allyluridine (III) was obtained in 78% yield as an uncrystallizable gum. Partition chromatography on a Celite 545[6] column using an ethyl acetate–water system afforded crystalline 5-allyluridine. The column was operated with an aqueous immobile phase and an organic mobile phase. On the column described in the Experimental section 5-allyluridine was eluted over a narrow range after the passage of two holdback volumes of organic phase. Evaporation of these fractions gave crystalline 5-allyluridine.

The β-configuration of the anomeric center has been assumed since the formation of an α-anomer by condensation of a pyrimidine mercury salt and a benzoylated ribosyl halide has not been observed.[7] Spectral data and physical properties are in accord for 5-allyluridine.[7]

The effect of 5-allyluridine on growth of cultured mammalian cells has been studied. The system described by Eagle[8] was used and no inhibition of growth was produced in experiments with HeLa human carcinoma cells, Chang's human liver cells, or S-180 mouse sarcoma cells when 5-allyluridine was added to the medium in concentrations up to 4×10^{-4} *M*.

Two DBA/2 female mice and one DBA/1 female mouse with spontaneous breast cancers were given 5-allyluridine by intraperitoneal injection once daily at a dose of 500 mg./kg. for from 25 to 28 days. No change occurred in the previously established slope of progressive tumor growth nor did the host animals sustain evidence of drug toxicity.

Experimental[9]

5-Allyluracil was prepared according to previously published procedures.[4] Fox and co-workers have given the preparation of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride.[5] The precursor to the halogenose, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside was conveniently prepared by the procedure of Recondo and Rinderknecht.[10]

5-Allyluracilmercury (I).—5-Allyluracil (1.52 g., 0.010 mole) was dissolved in 300 ml. of ethanol with heating, then cooled to room temperature. A solution of 2.71 g. (0.010 mole) of mercuric chloride in 30 ml. of ethanol was added. Celite Analytical Filter Aid (3.52 g.) was mixed with the clear solution. As the mixture was swirled, 20 ml. of 1 *N* sodium hydroxide solution (0.020 mole) was added dropwise, causing the mercury salt to precipitate. After the addition of base was completed, 350 ml. of water was added and the mixture set aside overnight. During this time the mercury salt and Celite settled and most of the supernatant liquid could be decanted. The remaining precipitate was collected on a fine sintered glass funnel, washed with water, ethanol, and ether, and dried under an infrared lamp. The yield of mercury salt was quantitative (3.52 g.). Celite was not used in the preparation of the analytical sample.

Anal. Calcd. for $C_7H_6HgN_2O_2$: C, 23.97; H, 1.72; Hg, 57.19; N, 7.99. Found: C, 23.74; H, 2.20; Hg, 56.40; N, 7.73.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-hydroxy-5-allyl-2(1H)-pyrimidone (II).—5-Allyluracilmercury (3.52 g., 0.010 mole) mixed with 3.52 of Celite was suspended in 300 ml. of xylene by vigorous stirring and dried azeotropically by distilling 100 ml. of the xylene. The halogenose (0.020 mole) dissolved in 30 ml. of benzene was added in 3 portions to the well stirred boiling mixture at 5 min.-intervals. The mixture was refluxed for 45 min. and then all but 75 ml. of the xylene was quickly distilled. The dark greenish mixture remaining was filtered hot and the solids washed with 50 ml. of hot xylene. The solids were discarded. The cooled filtrate was mixed with 750 ml. of petroleum ether (35–60°) and the crude blocked nucleoside separated as a gum on the sides of the flask. After the supernatant liquid cleared, it was decanted and discarded. The gum was dissolved in about 50 ml. of chloroform and any solids present removed by filtration and discarded. The filtrate was washed with 30% potassium iodide solution and water and dried over magnesium sulfate. After filtration the chloroform was removed *in vacuo* and the sirup remaining heated at 100° (0.1 mm. or less) for at least 2 hr. On cooling the sirup solidified to a clear tan glass. The apparent yield was often between 100 and 125%. The blocked nucleoside resisted attempts at crystallization. Nitrogen analysis indicated the product was 50–60% pure.

Anal. Calcd. for $C_{33}H_{28}N_2O_9$: N, 4.70. Found: N, 2.80.

5-Allyluridine (III).—One-half of the crude blocked nucleoside (3–4 g., representing 0.005 mole of 5-allyluracil) obtained in the previous step was placed in a tube containing 60 ml. of absolute ethanol previously saturated with ammonia at 0°. The tube was sealed and heated at 100° overnight. After the tube was opened, the solution was brought to dryness *in vacuo* and ethyl benzoate removed by vacuum steam distillation. The residue was mixed with 50 ml. of water and extracted repeatedly with chloroform. The aqueous layer was evaporated *in vacuo* to give the crude nucleoside as a brown semisolid in 78% yield.

(1) This investigation was supported by Grant CY-2857 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) Allied Chemical Corp. Fellow, 1960–1961.

(3) J. J. Fox, N. Yung, J. Davoll, and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956).

(4) H. J. Minnemeyer, J. A. Egger, J. F. Holland, and H. Tieckelmann, *J. Org. Chem.*, **26**, 4425 (1961).

(5) J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *J. Am. Chem. Soc.*, **79**, 5060 (1957).

(6) Diatomaceous earth product.® Commercial Celite 545 was washed with 6 *N* hydrochloric acid, water, then methanol, and dried in an oven at 60° before use in partition column chromatography.

(7) See J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.*, **14**, 283 (1959), for a discussion of this point. This review article contains most of the background information and references pertinent to this work.

(8) H. Eagle, *Science*, **122**, 501 (1955); **130**, 432 (1959).

(9) Melting points are corrected and were determined on a Drechsel melting point apparatus. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn., and by Alfred Bernhardt, Mülheim, Germany. Ultraviolet absorption spectra were determined on a Beckman DK2 spectrophotometer.

(10) E. Recondo and H. Rinderknecht, *Helv. Chim. Acta*, **42**, 1171 (1959).

The only effective means for the purification of the crude 5-allyluridine was through partition chromatography on a Celite column. A column was prepared by mixing 66 g. of acid-washed Celite 545[6] with 33 ml. of the lower phase of an ethyl acetate–water mixture. The resulting powdery Celite was packed into a column 2.7 cm. wide to a height of 33 cm. The crude nucleoside (1.10 g.) was dissolved in 3.5 ml. of the lower phase and mixed with 7 g. of Celite and packed on top of the column. The column was developed with the upper phase of the ethyl acetate–water mixture. Fractions of about 20 ml. each were collected. The early fractions were colored brown and contained material absorbing ultraviolet light strongly at 226 mμ. 5-Allyluridine was eluted from fractions 23 to 29, and evaporation of these fractions gave a white crystalline solid (0.51 g., 36%), m.p. 171–174°. After recrystallization from ethyl acetate, white feathery crystals were obtained, m.p. 175–176°. The material gave a positive spray test for a 1,2-glycol.[11] The shapes of the ultraviolet absorption curves over a range of pH values appear identical with those of 1-β-D-ribofuranosylthymine,[3] with spectrophotometrically determined pK_a values of 9.9 and above 12. The following data refer to these spectra: $\lambda_{max\ (m\mu)}^{pH\ 7.01}$ 267 (ε 9230); $\lambda_{min\ (m\mu)}^{pH\ 7.01}$ 235 (ε 2090); $\lambda_{max\ (m\mu)}^{pH\ 12\ (0.01\ N\ NaOH)}$ 266 (ε 6790); $\lambda_{min\ (m\mu)}^{pH\ 12\ (0.01\ N\ NaOH)}$ 246 (ε 4530); $\lambda_{max\ (m\mu)}^{pH\ 14\ (1\ N\ NaOH)}$ 268 (ε 7020).

Anal. Calcd. for $C_{12}H_{16}N_2O_6$: C, 50.70; H, 5.67; N, 9.86. Found: C, 50.52; H, 5.82; N, 9.67.

(11) J. G. Buchanan, C. A. Dekker, and A. G. Long, *J. Chem. Soc.*, 3162 (1950).

6-Ethyl-1,2,4,2H-thiadiazine-3,5(4H,6H)dione 1,1-Dioxide

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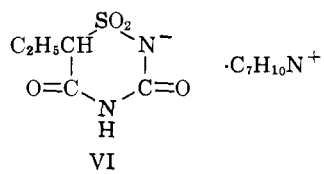
6-Ethyl-1,2,4,2H-thiadiazine-3,5-(4H,6H)dione 1,1-dioxide was synthesized as its 2,6-lutidine salt (VI) for testing as a hypnotic by reactions similar to those used for 1,2,4,2H-thiadiazine-3,5(4H,6H)dione 1,1-dioxide.[2] The reaction sequence employed is shown below.

$$Na^+C_6H_5O_3SCH_2COOC_6H_5^- \longrightarrow C_6H_5O_3SCH(C_2H_5)COOC_6H_5$$
I II

$$\xrightarrow[-33^\circ]{NH_3} C_6H_5O_3SCH(C_2H_5)CONH_2 \xrightarrow[75^\circ]{NH_3}$$
III

$$NH_2SO_2CH(C_2H_5)CONH_2 \xrightarrow{KNCO}$$
IV

$$NH_2CONHSO_2CH(C_2H_5)CONH_2 \longrightarrow$$
V



$\cdot C_7H_{10}N^+$

VI

Alkylation of the sodium derivative of diphenyl sulfoacetate (I) by means of diethyl sulfate in toluene required a period of 7–8 days. In agreement with observations made on the alkylation of malonic ester[3] it was found that the addition of dimethylformamide decreased the reaction time to less than 2 days without sacrificing the yield. Ring closure was accomplished by refluxing V in 2,6-lutidine and gave a crystalline lutidinium salt (VI). The same reaction with pyridine gave an oil which could not be crystallized but which had an infrared spectrum similar to that of the lutidinium salt. Efforts to convert the lutidinium salt to the free thiadiazine were unsuccessful. The use of aqueous systems gave the hydrolysis product, α-carbamylpropanesulfonamide (IV). Acidification in nonaqueous media gave a colorless glass which could not be crystallized. The infrared spectrum of the glass gave little information about the structure because of poor resolution. All of the bands were broad and the peaks were unresolved.

A similar synthesis of 6,6-diethyl-1,2,4,2H-thiadiazine-3,5-(4H,6H)-dione 1,1 dioxide from diphenyl α,α-diethylsulfoacetate failed in the ammonolysis step. The product, after acidification, was polymeric in nature and differed in properties from the 4,4-diethyl-3-keto-1,2-thiazetidine 1,1-dioxide reported to be formed from the diacid chloride.[4] Further work on this product is in progress.

6-Ethyl-1,2,4,2H-thiadiazine-3,5(4H,6H)dione 1,1-dioxide as the lutidine salt (VI) showed no hypnotic activity in mice. Doses of 300–750 mg./kg. intraperitoneally and 1500 mg./kg. orally produced only a slight decrease in the activity in mice.

Experimental[5]

Diphenyl α-Sulfopropionate (II).—To a suspension of sodium powder (15.42 g.) in toluene (1 l.), diphenyl sulfoacetate[2] (196 g.) was added in 20-g. increments. Each portion was added after the evolution of hydrogen had ceased from the preceding portion. The resulting mixture was treated with diethyl sulfate (206 g.) and dimethylformamide (5 ml.) and stirred at 50–75° until the solution became neutral to moist pH paper (1–2 days). Removal of half of the toluene under reduced pressure was followed by filtration of the sodium ethyl sulfate. The latter was washed with 50 ml. of toluene and the combined filtrates were heated under reduced pressure to remove the remainder of the toluene. The excess diethyl sulfate was removed by distillation (0.5 mm.) and the diphenyl α-sulfopropionate was purified by distillation; yield 155 g., b.p. 170–180° (0.15 mm.), $n^{28}D$ 1.5338.

Anal. Calcd. for $C_{16}H_{16}O_5S$: C, 60.00; H, 5.00. Found: C, 60.23; H, 5.15. The infrared spectrum of a film gave principal bands at 5.69 μ (CO), 6.3 μ (C_6H_5), and 7.21, 7.35 μ (SO_3).

Diphenyl α,α-Diethylsulfoacetate.—This ester was prepared in 81% yield from diphenyl α-sulfopropionate in a manner similar to that given for diphenyl α-sulfopropionate. The product boiled at 169–177° (0.15 mm.); $n^{28}D$ 1.5328.

Anal. Calcd. for $C_{18}H_{20}O_5S$: C, 62.05; H, 5.75. Found: C, 61.50; H, 5.62. The infrared spectrum of a film was similar to that of diphenyl α-sulfopropionate and differed only in that the peak for the SO_3 group was sharper and appeared at 7.4 μ.

Phenyl α-Carbamylpropanesulfonate (III).—Diphenyl α-sulfopropionate (2 g.) was dissolved in liquid ammonia (10 ml.) and the ammonia allowed to evaporate. The residue was extracted with cold hexane to remove phenol and then recrystallized from absolute ethanol; yield 0.95 g., m.p. 159–160°.

Anal. Calcd. for $C_{10}H_{13}NO_4S$: C, 49.40; H, 5.35; N, 5.76. Found: C, 49.45; H, 5.16; N, 5.55.

(1) Abstracted in part from the Ph.D. thesis of R. L. Abbott, submitted to the State University of Iowa, February, 1962.

(2) B. E. Hoogenboom, R. L. Abbott, L. Locatell, Jr., and R. L. Hinman, *J. Org. Chem.*, **24**, 1983 (1959).

(3) H. E. Zaugg, B. W. Horrom, and S. Borgwardt, *J. Am. Chem. Soc.*, **82**, 2895 (1960).

(4) B. J. R. Nicolaus, E. Bellasio, and E. Testa, *Helv. Chim. Acta*, **45**, 717 (1962).

(5) Melting points are corrected; boiling points are not corrected.