[CONTRIBUTION FROM SCHOOL OF MEDICINE, YALE UNIVERSITY]

A Synthesis of "6-Azauracil" (1,2,4-Triazine-3,5(2H,4H)-dione), an Analog of Uracil[1]

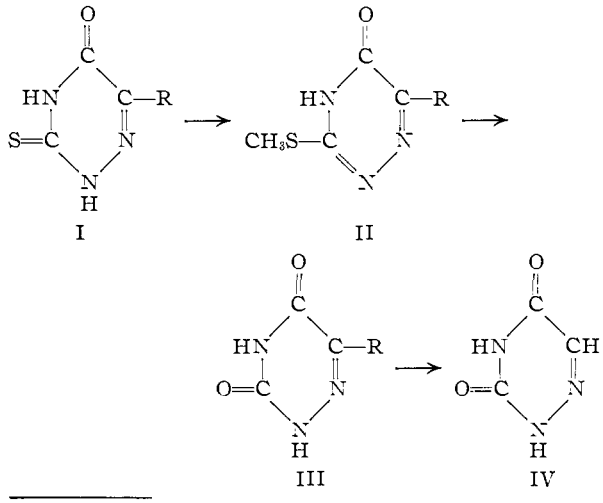
BY RICHARD B. BARLOW[2,3] AND ARNOLD D. WELCH[3]

RECEIVED OCTOBER 3, 1955

6-Azauracil (1,2,4-triazine-3,5-dione), an analog of uracil described by Seibert, has now been prepared by a series of reactions starting from sodium mesoxalate. When heated with thiosemicarbazide, the latter formed 1,2,4-triazine-3-thione-5-one-6-carboxylic acid, presumably by ring closure of the thiosemicarbazone, which was not isolated. From this the 3-methylthiol was obtained by treatment with methyl iodide and alkali. When refluxed with a mixture of concentrated hydrochloric and glacial acetic acids, the methylthiol yielded 1,2,4-triazine-3,5-dione-6-carboxylic acid and this was decarboxylated, giving 6-azauracil, by sublimation at 230–235° (10 mm.).

Seibert[4] has described the synthesis of 1,2,4-triazine-3,5-dione (6-azauracil) from the semicarbazone of glyoxylic acid. At that time, however, the pharmacological potentialities of this compound do not seem to have been appreciated. Recent investigation of the biological properties of 6-azathymine[5–8] have led to a revival of interest in the synthesis of 6-azauracil. This paper describes a synthesis from more convenient starting materials than those used by Seibert. The method, which is very similar to that used for preparing 6-azathymine (III, R = CH_3) from thiosemicarbazone of pyruvic acid,[6–9] is outlined below.

The condensation with thiosemicarbazide occurs more readily with sodium mesoxalate than with the free acid. The diethyl ester (diethyloxomalonate) may be used alternatively in the synthesis of 6-azauracil, but the yield is poorer. Whether the ester or the sodium salt is used, the condensation in the synthesis of 6-azauracil proceeds directly to the cyclic compound (I, R = COOH or COOEt); the thiosemicarbazone was not isolated. The cyclic acid (I, R = COOH) was smoothly and rapidly methylated in alkali, yielding the expected methylthioether (II, R = COOH). The methylthioether link was readily broken by acid (a mixture of glacial acetic and concentrated hydrochloric acids was used), yielding 1,2,4-triazine-3,5-dione-6-carboxylic acid (III, R = COOH). When this was heated at 230° (10 mm.), decarboxylation occurred and 6-azauracil (IV) sublimed.[10]



Experimental[11]

1,2,4-Triazine-3-thione-5-one-6-carboxylic Acid (I, R = COOH).—The disodium salt of mesoxalic acid (51 g., 0.283 mole), dissolved in water (250 ml.), was refluxed for 6 hours with thiosemicarbazide (26 g., 0.286 mole). Traces of insoluble matter were removed by filtration, the filtrate was adjusted to *p*H 1 with concentrated hydrochloric acid and left overnight at 4°. The product (I, R = COOH), which crystallized out, was recrystallized from water; it was yellowish-green, and melted (dec.) at 244–246°; the yield was 82% (40 g.). After further crystallization from glacial acetic acid the material sintered at 220° and melted at 247° dec.

Anal. Calcd. for $C_4H_3N_3O_3S$: C, 27.8; H, 1.75; N, 24.3; S, 18.5. Found: C, 28.0; H, 1.82; N, 24.2; S, 18.4.

This substance also was obtained in the following manner: ethyl oxomalonate (14.5 g., 0.083 mole), thiosemicarbazide (10 g., 0.11 mole) and water (30 ml.) were heated in an oil-bath under reflux for 5 hours and then left overnight at 4°. **The ethyl ester** (I, R = COOEt) **of 1,2,4-triazine-3-thione-5-one-6-carboxylic acid** crystallized out and was recrystallized from water. The product, composed of greenish-yellow needles, melted at 202–204°, yield 26% (4.3 g.). After further crystallization from water the material had m.p. 206–207°.

Anal. Calcd. for $C_6H_7N_3O_3S$: C, 35.8; H, 3.57; N, 20.9; S, 15.9. Found: C, 35.9; H, 3.63; N, 20.5; S, 15.6.

This ester was hydrolyzed either by acid (concentrated hydrochloric acid) or alkali (1 *N* sodium hydroxide) giving an acid which was shown, by its m.p., by a mixed m.p. test, and by its ultraviolet absorption spectrum in water (maximum at 269 mμ, minimum at 234 mμ), to be identical with the material obtained by starting with the sodium salt of mesoxalic acid.

3-Methylmercapto-1,2,4-triazine-5-one-6-carboxylic Acid.—The thione above (I, R = COOH; 17.7 g., 0.102 mole) was methylated by dissolving in 1 *N* sodium hydroxide (356 ml.) and adding methyl iodide (6.9 ml., 15.7 g., 0.111 mole); the mixture was stirred mechanically. The methyl iodide reacted rapidly, but stirring was continued for about 10 more minutes after a homogeneous solution was formed. The solution was adjusted to *p*H 1 with concentrated hydrochloric acid, the volume reduced (to 125 ml.) under partial vacuum (water-pump), and left overnight at 4°. The product which crystallized out was filtered off and dried on

(1) This work was supported by a grant from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) Alexander Coxe and Squibb Post-doctoral Fellow in Pharmacology, Yale University.

(3) Department of Pharmacology, School of Medicine, Yale University, New Haven, Conn.

(4) W. Seibert, *Ber.*, **80**, 498 (1947).

(5) W. H. Prusoff and W. L. Holmes, *Federation Proc.*, **11**, 271 (1952).

(6) W. H. Prusoff, W. L. Holmes and A. D. Welch, *Cancer Research*, **14**, 570 (1954).

(7) W. H. Prusoff, *J. Biol. Chem.*, **215**, 809 (1955).

(8) W. H. Prusoff and A. D. Welch, *ibid.*, in press.

(9) G. H. Hitchings, personal communication.

(10) 6-Azauracil has exhibited inhibitory activity for the growth of several bacterial species (R. E. Handschumacher and A. D. Welch, *Fed. Proc.*, **15**, in press (1956)) as well as marked inhibitory activity for the growth of sarcoma 180 and several lymphomas with negligible toxicity in mice (M. T. Hakala, L. W. Law and A. D. Welch, *Proc. Amer. Assoc. Cancer Research, Cancer Research*, in press).

(11) Microanalyses were done by Huffman Laboratories, Wheatridge, Colorado. Melting points were uncorrected.

a steam-bath. The material was almost white and sintered at 156–158°, m.p. 210° dec. The yield was 77% (14.7 g.). After recrystallization from glacial acetic acid the substance effervesced at 176°, resolidified, and then melted at 212–214°.

Anal. Calcd. for $C_5H_5N_3O_3S$: C, 32.1; H, 2.70; N, 22.5; S, 17.1. Found: C, 32.1; H, 2.80; N, 22.4; S, 17.1.

1,2,4-Triazine-3,5-dione-6-carboxylic Acid.—The methylthiol above (14.7 g., 0.078 mole) was heated with concentrated hydrochloric acid (30 ml.) and glacial acetic acid (55 ml.) under reflux for 5 hours. The solution was filtered; the insoluble matter was washed with a little boiling glacial acetic acid and the washings were added to the filtrate. The product which crystallized out on standing overnight at 4°, decomposed with evolution of gas at 237–238°; yield 55% (6.75 g.). After further recrystallization from glacial acetic acid, gas was evolved from the material at 241°.

Anal. Calcd. for $C_4H_3N_3O_4$: C, 30.6; H, 1.93; N, 26.8. Found: C, 31.0; H, 1.89; N, 26.4.

When concentrated, the original mother liquors yielded more material which effervesced at 227–229°. When this was recrystallized from glacial acetic acid, gas was evolved at 233–234°; the amount (1.3 g.) brought the total yield up to 65%.

1,2,4-Triazine-3,5-dione (6-Azauracil).—When the above carboxylic acid (III, R = COOH) was heated at 230–250° (10 mm.) in a sublimation tube, a vigorous effervescence occurred and almost the theoretical yield of 6-azauracil sublimed onto the cold inner surface of the apparatus. This material sintered at 272°, lit.[4] m.p. 272°.

Anal. Calcd. for $C_3H_3N_3O_2$: C, 31.9; H, 2.68; N, 37.1. Found: C, 31.7; H, 2.64; N, 36.9.

Since paper chromatography revealed the presence of a trace of impurity, the sublimed product was recrystallized twice from water; its m.p. remained unchanged.

Anal. Found: C, 31.8; H, 2.93; N, 37.0.

NEW HAVEN, CONN.

COMMUNICATIONS TO THE EDITOR

THE STRUCTURES OF TETRAPHYLLICINE, AJMALIDINE AND RAUVOMITINE[1]

Sir:

Recently, there has been described[2] the isolation from *R. tetraphylla L.* of a new *Rauwolfia* alkaloid, tetraphyllicine, with the apparent empirical formula $C_{20}H_{26}N_2$ (calcd.: C, 81.58; H, 8.90; N, 9.52). The similarity of this alkaloid, especially along spectroscopic lines, with ajmaline led to the suggestion that tetraphyllicine might be the oxygen-free parent substance of the ajmaline group.

The accumulation of additional quantities of this alkaloid has now permitted further degradation experiments. Selenium dehydrogenation furnished ind-N-methylharman, a characteristic degradation product of ajmaline,[3] thus providing further support for the supposed structural similarity of the two alkaloids. Tetraphyllicine was found to contain one active hydrogen atom and in order to determine whether N(b) was secondary, the alkaloid was acetylated. The resulting product was clearly an O-acetate (infrared spectrum) and mild saponification regenerated the parent alkaloid. These results raised serious doubt as to the correctness of the original $C_{20}H_{26}N_2$ formula which had been based on seven combustion analyses from three different analytical laboratories. Three of these analyses showed C, 81.24–81.52; H, 8.29–8.51, while the remaining ones ranged from C, 78.23–78.76. These latter analyses were ignored since they were assumed to be due to incomplete combustion of the high melting alkaloid. Repeated analyses,[4] including direct oxygen determinations (found: C, 77.97, 78.23; H, 7.89, 8.08; N, 8.91; O, 5.28, 5.20) now clearly show that tetraphyllicine possesses the empirical formula $C_{20}H_{24}N_2O$ (calcd.: C, 77.88; H, 7.84; N, 9.08; O, 5.19). The infrared spectrum closely resembles that of desoxyajmaline[5] and the rotatory dispersion curves between 700–320 mμ are very similar in shape except that the values for desoxyajmaline are consistently higher by *ca.* 100° over the 375–320 mμ range. Catalytic hydrogenation of tetraphyllicine yielded dihydrotetraphyllicine which proved to be identical by mixture melting point determination, infrared comparison and virtual coincidence of the rotatory dispersion curves[6] (700–320 mμ) with desoxyajmaline. The position of the double bond was proved by ozonolysis of tetraphyllicine which yielded 55% of acetaldehyde. These results coupled with the recently established structure[7] of ajmaline require the expression I for tetraphyllicine.

Ajmalidine, a new alkaloid ($C_{20}H_{24}N_2O_2$) isolated[8] in trace amounts together with ajmaline and tetraphyllicine from *Rauwolfia sellowii* shows striking similarities to ajmaline but contains a cyclopentanone ring as demonstrated by its infrared spectrum; it is likely, therefore, that ajmalidine is best represented by structure III.

Quite recently, two groups[9] have announced the isolation of a new ester alkaloid, rauvomitine ($C_{30}H_{34}N_2O_5$)[9a] from *R. vomitoria* Afz., which on hydrolysis yielded trimethoxybenzoic acid and a new base, $C_{20}H_{24}N_2O$. Direct comparison of this cleavage product (kindly provided by Dr. E. Haack,[9a] C. F. Boehringer, Mannheim-Waldhof) with tetraphyllicine has now established the identity of the two substances. Consequently, rauvo-

(1) Paper XI in the Wayne series "Alkaloid Studies."

(2) C. Djerassi and J. Fishman, *Chem. and Ind.*, 627 (1955).

(3) F. A. L. Anet, D. Chakravarti, R. Robinson and E. Schlittler, *J. Chem. Soc.*, 1242 (1954); *cf.* A. Chatterjee and S. Bose, *Experientia*, **9**, 254 (1953).

(4) Carried out by Mr. G. M. Maciak (Eli Lilly and Co.) and Dr. A. Bernhardt (Mülheim).

(5) R. Robinson, *Chem. & Ind.*, 285 (1955).

(6) These will be published in a detailed paper.

(7) R. B. Woodward, *Angew. Chem.*, **68**, 13 (1956).

(8) S. C. Pakrashi, C. Djerassi, R. Wasicky and N. Neuss, THIS JOURNAL, **77**, 6687 (1955).

(9) (a) E. Haack, A. Popelak and H. Spingler, *Naturwiss.*, **42**, 627 (1955); (b) J. Poisson, R. Goutarel and M. M. Janot, *Compt. rend.*, **241**, 1840 (1955).