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Synthesis of 6-Hydroxymethyl-1,3-dimethyllumazine by Rearrangement of the Corresponding 6-Methyllumazine 5-Oxide (1)

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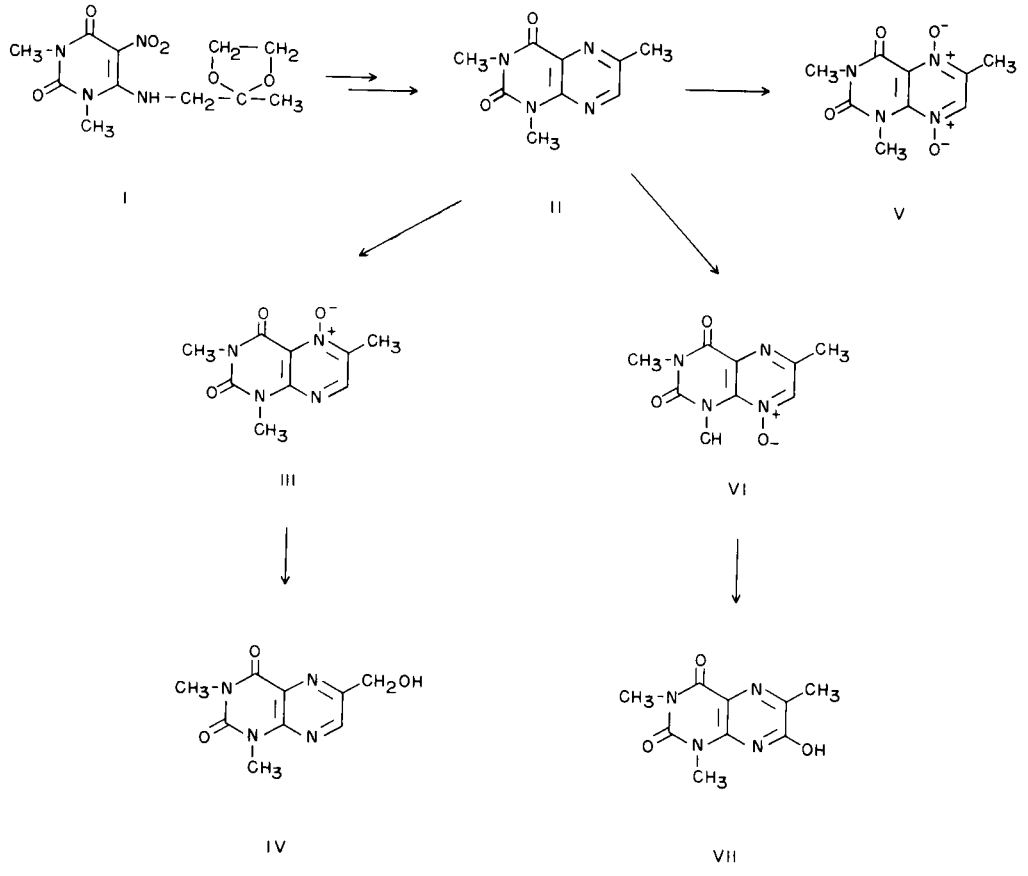
Starting with a chloronitrouracil, the synthesis of 1,3,6-trimethyllumazine has been achieved. Oxidation to the 5-oxide and subsequent rearrangement gave 6-hydroxymethyl-1,3-dimethyllumazine. Because of the method of synthesis, the product is uncontaminated with the 7-isomer.

The synthesis of 6-substituted pteridines which are free from contamination with the corresponding 7-isomers is notoriously difficult. Generally, these compounds are prepared from 5,6-diaminopyrimidines via the Isay reaction, and many modifications of the procedure have been devised to ensure at least a preponderance of one or the other isomer. For example, Baugh and Shaw (2) have recently claimed the synthesis of "pure" 2-amino-3,4-dihydro-6-hydroxymethyl-4-oxopteridine from dihydroxyacetone and 2,4,5-triamino-6-hydroxypyrimidine, using cysteine as a reducing agent; however, in our hands, the reaction product always contains a small amount of the 7-isomer. In a different approach, Viscontini and Nasini (3) have synthesized what appears to be exclusively 2-amino-3,4-dihydro-



6-hydroxymethyl-4-oxopteridine by allowing hydrazine hydrate and dimethylformamide to react with 2-amino-3,4,5,6,7,8-hexahydro-4-oxopteridine, to give, after oxidation, the hydrazone of 2-amino-3,4-dihydro-6-formyl-4-oxopteridine, which can be converted into the corresponding 6-hydroxymethyl compound. However, this unusual synthesis is of only very limited application.

Since the biological properties of members of this group (*e.g.*, biopterin or 2-amino-3,4-dihydro-6-hydroxymethyl-4-oxopteridine) might be seriously affected by contamination with the corresponding 7-isomers, as well as introducing potential complications in evaluation of the observed effects, we have attempted to devise new methods of synthesis which yield exclusively one isomer. This paper describes the synthesis of one such model compound, 6-hydroxymethyl-1,3-dimethyllumazine.

Starting with 4-chloro-1,3-dimethyluracil, nitration and subsequent reaction with 2,2-ethylenedioxypropylamine (4) yielded 4-(2',2'-ethylenedioxypropyl)-amino-1,3-dimethyl-5-nitrouracil (I). Reduction of I gave a 4,5-diamino derivative which after hydrolysis of the protecting ketal and ring closure, afforded 7,8-dihydro-1,3,6-trimethyllumazine. Mild oxidation then gave 1,3,6-trimethyllumazine (II) which was further oxidized with trichloroperoxyacetic acid to the corresponding 5-oxide (III). The 5-oxide was readily purified by chloroform extraction of the oxidation mixture; treatment of the 5-oxide with acetic anhydride brings about its rearrangement (5) to 6-acetoxymethyl-1,3-dimethyllumazine, which yields the corresponding 6-hydroxymethyl-1,3-dimethyllumazine (IV) on hydrolysis. If chloroform extraction is omitted, a small amount of the 5,8-dioxide (V) can be obtained from the reaction mixture. When no attempt was made to isolate the oxidation products before rearrangement with acetic anhydride, two compounds were separated by column chromatography, the expected 6-hydroxymethyl-1,3-dimethyllumazine (IV), and 7-hydroxy-1,3,6-trimethyllumazine (VII). Compound VII is identical in all respects with that prepared by Pfleiderer (6), and its isolation proves that the oxidation of 1,3,6-trimethyllumazine yields the 8-oxide (VI) in addition to the 5- and 5,8-dioxides.

Spectral and chromatographic data on these compounds are summarized in Table I.

TABLE I

Physical Constants

	Ultraviolet absorption spectra, λ max, mμ (log ε) (a)		Rf values (b)			
	*p*H 1	*p*H 13	A	B	C	D
6-(2',2'-Ethylenedioxypropyl)amino-1,2,3,4-tetrahydro-1,3-dimethyl-5-nitro-2,4-dioxopyrimidine (I)	333 (3.95), 243 (4.21)	340 (4.01), 285 sh (3.48), 221 (4.17)	0.85	0.81	0.82	0.89
7,8-Dihydro-1,3,6-trimethyllumazine (c)	355 (3.68), 271 (4.11), 239 (4.06)	292 (4.09)	---	---	---	---
1,3,6-Trimethyllumazine (II)	337 (3.82), 240 (4.17)	333 (3.72), 285 (3.58), 238 (4.02)	0.85	0.80	0.80	0.83
1,3,6-Trimethyllumazine 5-oxide (III)	354 (3.72), 289 (3.79), 241 (4.44)	305 (3.51), 267 (3.96), 228 (4.12)	0.80	0.73	0.83	0.86
1,3,6-Trimethyllumazine 5,8-dioxide (V)	333 (3.87), 243 sh (3.92), 248 (4.10)	332 (3.74), 290 (3.70), 263 (3.80), 243 (3.90)	0.57	0.47	0.79	0.85
6-Acetoxymethyl-1,3-dimethyllumazine	335 (3.84), 240 (4.19)	330 (3.64), 285 (3.57), 239 (3.95)	0.82	0.72	0.82	0.92
6-Hydroxymethyl-1,3-dimethyllumazine (IV)	337 (3.91), 239 (4.27)	332 (3.73), 285 (3.59), 240 (4.06)	0.81	0.72	0.79	0.82
7-Hydroxy-1,3,6-trimethyllumazine (d) (VII)	327 (4.24), 288 (3.97), 273 (3.99)	328 (4.37), 278 (4.10)	0.69	0.81	0.55	0.69
Isoxanthopterin (e)	---	---	0.22	0.21	0.34	0.34

(a) Determined using a Cary Model 14 recording spectrophotometer. (b) Ascending chromatography on Whatman No. 1 paper. A = *n*-Propanol-1% ammonia (2:1), B = *n*-butanol-5 *N* acetic acid (2:1), C = 4% sodium citrate, D = 3% ammonium chloride. (c) Rf values not reported because compound oxidized to 1,3,6-trimethyllumazine during development of the chromatograms. (d) The following values have been reported (6) λ max, mμ and (log ε) given: *p*H 1.5, 327 (4.09), 284 (3.79), 269 (3.82); *p*H 6.0, 329 (4.23), 279 (3.91). (e) Reported for comparison of Rf values.

EXPERIMENTAL

4-(2',2'-Ethylenedioxypropyl)amino-1,3-dimethyl-5-nitrouracil (I).

To a solution of 6.98 g. of 4-chloro-1,3-dimethyluracil in 20 ml. of concentrated sulfuric acid was added 7 ml. of fuming nitric acid, maintaining the temperature below 20°. The reaction mixture was poured over ice, and the product extracted with chloroform. The chloroform extract was dried and concentrated *in vacuo* to *ca.* 30 ml.; 5.8 g. of 2,2-ethylenedioxypropylamine (4) was then added, followed by the slow addition of a solution of 4 ml. of triethylamine in 15 ml. of chloroform. The whole was refluxed for 10 minutes and then evaporated to dryness *in vacuo*. The residue was recrystallized from

50% aqueous ethanol to give I (7.72 g., 64%), m.p. 146°.

Anal. Calcd. for $C_{11}H_{16}N_4O_6$: C, 44.0; H, 5.4; N, 18.7. Found: C, 44.0; H, 5.5; N, 18.7.

7,8-Dihydro-1,3,6-trimethyllumazine Hydrochloride.

Compound I (5.52 g.) was suspended in 100 ml. of ethanol and hydrogenated using 10% palladium on charcoal as catalyst. The catalyst was removed by filtration, and 5 ml. of concentrated hydrochloric acid was added to the filtrate. When the resulting red solution was heated on the steam bath it turned yellow and a solid separated. After one hour the precipitate (2.94 g., 61%) was collected, washed with ethanol and dried.

Anal. Calcd. for $C_9H_{12}N_4O_2 \cdot HCl$: C, 44.2; H, 5.4; N, 22.9. Found: C, 44.4; H, 5.6; N, 22.6.

The free base was obtained by neutralization of an aqueous solution of the hydrochloride (730 mg.) with 1 *N* sodium hydroxide, evaporation to dryness *in vacuo*, and extraction of the residue with 100 ml. of boiling 95% ethanol. The product, which separated on cooling, was recrystallized twice from 95% ethanol, yield 660 mg. (90%).

Anal. Calcd. for $C_9H_{12}N_4O_2 \cdot 2H_2O$: C, 44.25; H, 6.6; N, 22.9. Found: C, 44.6; H, 5.5; N, 23.0.

1,3,6-Trimethyllumazine (II).

A solution of 2.5 g. of 7,8-dihydro-1,3,6-trimethyllumazine hydrochloride in 25 ml. of water was treated with 1.5 ml. of 30% hydrogen peroxide. After two days at 4°, II (1.9 g., 88%) was collected by filtration and recrystallized from water.

Anal. Calcd. for $C_9H_{10}N_4O_2$: C, 52.4; H, 4.9; N, 27.1. Found: C, 52.8; H, 4.9; N, 26.7.

1,3,6-Trimethyllumazine 5-Oxide (III).

Compound II (1.88 g.) was stirred for 40 hours at room temperature with a solution of 2.5 g. of trichloroacetic acid in 10 ml. of 30% hydrogen peroxide. The clear solution was concentrated to *ca.* 5 ml. *in vacuo* and extracted with chloroform. The chloroform extract was dried (magnesium sulfate), evaporated *in vacuo* and the resulting syrup induced to crystallize by addition of 20 ml. of water. The product (0.96 g., 48%) was recrystallized from water, m.p. 150°.

Anal. Calcd. for $C_9H_{10}N_4O_3$: C, 48.6; H, 4.5; N, 25.2. Found: C, 48.5; H, 4.6; N, 25.5.

In another experiment, the clear oxidation mixture (from 1.5 g. of II) was evaporated *in vacuo* and the oily residue taken up in 18 ml. of water (instead of extracting with chloroform). Upon cooling, the 5,8-dioxide (V) (114 mg., 6.6%) separated from the aqueous solution; the 5-oxide (III) could be isolated from the filtrate. Compound V was recrystallized from ethanol, m.p. 241°.

Anal. Calcd. for $C_9H_{10}N_4O_4$: C, 45.4; H, 4.2; N, 23.5. Found: C, 45.4; H, 3.7; N, 23.4.

6-Acetoxymethyl-1,3-dimethyllumazine.

A solution of III (492 mg.) in 7 ml. acetic anhydride was refluxed 7 hours and evaporated *in vacuo*. The resulting syrup was taken up in ethanol and the solution boiled for a short time and again evaporated *in vacuo*. The residual syrup was then boiled with 110 ml. of water and filtered. On cooling, the product slowly separated from the filtrate; concentration of the mother liquors gave additional product. The total yield was 390 mg. (66%). An analytical sample was prepared by recrystallization from ethanol-cyclohexane, m.p. 125-126°.

Anal. Calcd. for $C_{11}H_{12}N_4O_4$: C, 50.0; H, 4.6; N, 21.2. Found: C, 49.5; H, 4.6; N, 21.2.

The 6-acetoxymethyl compound undergoes surprisingly ready hydrolysis to give the corresponding 6-hydroxymethyllumazine (IV); for example, the product obtained on repeated recrystallization from water was IV (demonstrated by elemental analysis), not the acetylated derivative. Because of this, the 6-acetoxymethyl compound was usually hydrolyzed directly without isolation.

6-Hydroxymethyl-1,3-dimethyllumazine (IV).

The above acetyl derivative (100 mg.) was brought into solution by boiling with 1 *N* hydrochloric acid (1 ml.); upon cooling crystalline IV (71 mg., 85%) separated. Recrystallization from ethanol gave a product which melted at 208-209°; after sublimation in high vacuum ($< 10^{-4}$ mm.) the compound melted at 209-210°.

Anal. Calcd. for $C_9H_{10}N_4O_3$: C, 48.6; H, 4.5; N, 25.2. Found: C, 48.2; H, 4.5; N, 24.9.

In one experiment, 180 mg. of the crude product obtained from trichloroperoxyacetic acid oxidation was treated with acetic anhydride to effect rearrangement and the rearrangement product was hydrolyzed without isolation of the intermediate. The reaction mixture was evaporated *in vacuo* to give a solid residue (180 mg.). The residue was taken up in *ca.* 20 ml. of 1% aqueous ammonia, the insoluble material removed by centrifugation and the supernatant added to a column (13 x 2 cm.) of Dowex 1 anion-exchange resin in the chloride form. The column was washed with water (1000 ml.); the eluate contained a compound (IV) absorbing at 338 mμ, reaching maximum absorption after 300 ml. and decreasing essentially to zero after 800 ml. of eluate had passed through the column. Elution with 0.01 *N* hydrochloric acid was then started, after about 600 ml. had passed through the column, the absorption at 300 mμ began to rise, reached a maximum and began to decline. The fractions containing the 330 mμ absorbing material were combined (1000 ml.) and evaporated *in vacuo*; the residue was recrystallized from water (charcoal) to give 7-hydroxy-1,3,6-trimethyllumazine (VII), white needles, m.p. > 300° dec. Yields were estimated from spectroscopic data as 33 mg. IV and 32 mg. VII.

Anal. Calcd. for $C_9H_{10}N_4O_3$: C, 48.6; H, 4.5; N, 25.2. Found: C, 48.5; H, 4.6; N, 25.4.

REFERENCES

(1) This work was supported by U. S. Public Health Grants GM-12323, AM-09973, and career development award 5-K3-GM-22,684 (JML), and by the Robert A. Welch Foundation, Houston, Texas.

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Received November 14, 1966 Austin, Texas 78712