*UDC 547.859 : 545.84*

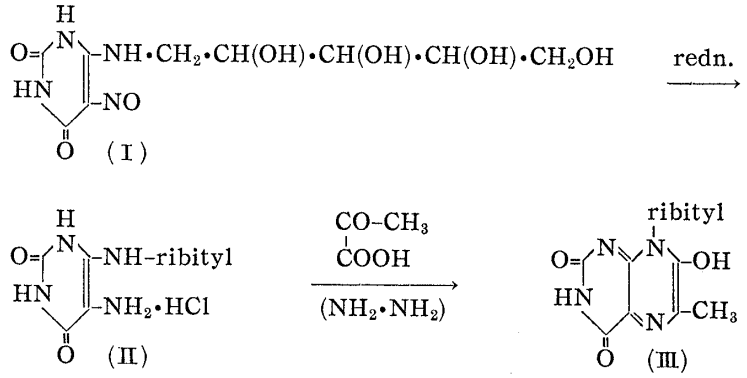
## *71.* Toru Masuda, Toyokazu Kishi, Mitsuko Asai, and Satoru Kuwada : Application of Chromatography. XXXVIII.*1 Total Synthesis of 6-Methyl-7-hydroxyribolumazine.

(*Research Laboratories, Takeda Pharmaceutical Industries, Ltd.**2)

Previously the authors isolated a purple fluorescent substance from the mycelium of *Eremothecium ashbyii* and called it first V compound[1] and later 6-methyl-7-hydroxyribolumazine.[2] From the fact that the substance gave a photodecomposition product identical with 6-methyl-7-hydroxylumazine, it was presumed to be the 8-D-ribityl derivative of the lumazine compound.[3]

Most recently the authors have succeeded in the total synthesis of V compound and thereby proved the correctness of the structure which was formerly assigned to this compound. The results are presented in this paper.

As in the previous report,*1 4-ribitylaminouracil produced from 4-chlorouracil and D-ribamine was nitrosated and resulting 4-ribitylamino-5-nitrosouracil (I) was reduced to 4-ribitylamino-5-aminouracil (II). The crystalline salt of this product was then allowed to react with pyruvic acid in the presence of hydrazine hydrate and the reaction mixture was purified by chromatography on activated carbon to give the desired lumazine compound, i.e. 6-methyl-7-hydroxy-8-D-ribityl-2(3*H*),4(8*H*)-pteridinedione (III), in crystalline form.




This product (III) was in complete agreement with the purple fluorescent V compound extracted from the mycelium of *Eremothecium ashbyii* in every respect including ultraviolet and infrared spectra, X-ray diffraction pattern, Rf-values in paper chromatography, and migration in ionophoresis, not to speak of the melting point. Thus, the structure previously assumed for the compound was positively ascertained.

Differing from the previous experiment, 4-ribitylamino-5-aminouracil was isolated as its hydrochloride or sulfite. Although these salts were far more stable than the free amine, their color intensified gradually when they were left standing in the air.

The authors are grateful to Messrs. M. Nishikawa and H. Okuto for their cooperation in physicochemical measurements, and to the members in charge of elementary analysis.

*1 Part XXXVII : This Bulletin, **7**, 361(1959).

*2 Juso-nishinocho, Higashiyodogawa-ku, Osaka (増田 亨, 貴志豊和, 浅井満子, 桑田 智).

1) T. Masuda, T. Kishi, M. Asai : This Bulletin, **5**, 598(1957).
2) S. Kuwada, T. Masuda, T. Kishi, M. Asai : *Ibid.*, **6**, 447(1958).
3) T. Masuda, T. Kishi, M. Asai : *Ibid.*, **6**, 291(1958).

NII-Electronic Library Service

## Experimental

**4-Ribitylamino-5-nitrosouracil (I)**——According to the previous method, 5.5 g. of D-ribose oxime was reduced on Pt-black and the resulting solution of D-ribamine was reacted with 2.5 g. of 4-chlorouracil in an autoclave (10 atm.) at 110~120° for 5 hr. The reaction mixture was concentrated under reduced pressure, the residue was nitrosated with 3 g. of $NaNO_2$ and 2 cc. of AcOH, and the product was purified by chromatography on activated carbon to give 0.8 g. of the nitroso compound, m.p. 105°(decomp.). *Anal.* Calcd. for $C_9H_{14}O_7N_4 \cdot H_2O$ : C, 35.07; H, 5.23; N, 18.18. Found : C, 34.99; H, 5.21; N, 18.48.

**4-Ribitylamino-5-aminouracil Hydrochloride (II)**—A solution of 0.5 g. of 4-ribitylamino-5-nitrosouracil (I) in 20 cc. of water was mixed with 0.8 cc. of *N* HCl and the mixture was subjected to catalytic reduction in the presence of 0.3 g. of 5% Pd-C. When the theoretical amount of $H_2$ (ca. 80 cc.) had been absorbed, the catalyst was separated by filtration and the filtrate was concentrated to ca. 1 cc. About 5 cc. of EtOH was added to the concentrate, the separated yellow substance was filtered off, and the filtrate, after being concentrated, was mixed with EtOH and left standing, whereupon fine crystals separated out, m.p. 90°(decomp.). The reddish color gradually intensified in the air. *Anal.* Calcd. for $C_9H_{16}O_6N_4 \cdot HCl \cdot H_2O$ : N, 16.94. Found : N, 16.92.

**4-Ribitylamino-5-aminouracil Hemisulfite**—A hot solution of 200 mg. of 4-ribitylamino-5-nitrosouracil (I) in 1 cc. of water was decolorized with $Na_2S_2O_4$ and then allowed to stand, when colorless crystals separated out, m.p. 181°(decomp.). *Anal.* Calcd. for $C_9H_{16}O_6N_4 \cdot \frac{1}{2} H_2SO_3$ : N, 17.65. Found : N, 18.06.

**6-Methyl-7-hydroxy-8-D-ribityl-2(3*H*),4(8*H*)-pteridinedione (III)**—An aqueous solution of one of the salts of 4-ribitylamino-5-aminouracil (II) was heated with pyruvic acid on a water bath for 1 hr. and the reaction mixture was developed on filter paper with a solvent of the BuOH-system, when several purple fluorescent spots were detected on the chromatogram, suggesting the formation of 6-methyl-7-hydroxy- as well as 7-methyl-6-hydroxy compounds. Therefore, attempt was made to produce only the 6-methyl-7-hydroxy compound by conducting the reaction in the presence of hydrazine as reported previously.[3]

An aqueous solution of one of the salts of (II) or a mixture of a solution of 200 mg. of 4-ribitylamino-5-nitrosouracil in 5 cc. of water and 1 g. of $Na_2S_2O_4$ was heated with 0.3 cc. of $NH_2NH_2 \cdot H_2O$ and 0.5 g. of pyruvic acid at 80° for 2 hr. and the reaction mixture was poured through a column of 20 g. of activated charcoal. The column was washed first with 250 cc. of water and 200 cc. of 2% pyridine-water, then eluted with 5% pyridine-water, and ca. 500 cc. of the eluate, purple fluorescent under ultraviolet ray, was collected and concentrated to ca. 1.0 cc., whereupon colorless needles separated out. The product, after recrystallization from 80% EtOH, melted at 263°(decomp.) and showed no depression in m.p. when melted with 6-methyl-7-hydroxyribolumazine (m.p. 263°) isolated from the mycelium of *Er. ashbyii*. *Anal.* Calcd. for $C_{12}H_{16}O_7N_4$ : C, 43.90; H, 4.91; N, 17.07. Found : C, 44.19; H, 5.00; N, 17.55.

Table I. Rf Values of Paper Chromatography and Migration Distance of Paper Ionophoresis

| Solvent system for development | 6-Methyl-7-hydroxyribolumazine | |
|---|---|---|
| | Natural | Synthesized |
| AcOH•BuOH•$H_2O$ (1:4:5) | 0.15 | 0.15 |
| EtOH•BuOH•$H_2O$ (15:50:35) | 0.23 | 0.23 |
| Pyridine•BuOH•$H_2O$ (3:4:7) | 0.43 | 0.43 |
| Migration distance in paper ionophoresis (pH 7, 300 V, 3 hr.) | +4.0 cm | +4.0 cm |

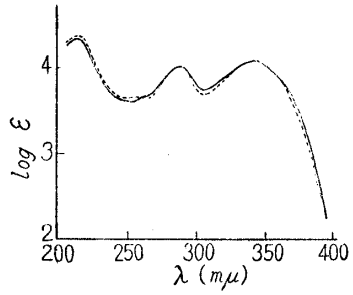



Fig. 1.
Ultraviolet Absorption Spectra of 6-Methyl-7-hydroxyribolumazine from *Er. ashbyii* and Synthesized Product

—— Synthesized 6-methyl-7-hydroxyribolumazine (in $H_2O$)
------ 6-Methyl-7-hydroxyribolumazine from *Er. ashbyii* (in $H_2O$)

NII-Electronic Library Service

**Comparison of the Product with 6-Methyl-7-hydroxyribolumazine isolated from *Er. ashbyii*—** The synthetic product was compared with the lumazine compound extracted from *Er. ashbyii* and it was found that they are in complete agreement in UV (Fig. 1) and IR spectra (Fig. 2), X-ray diffraction pattern (Fig. 3), Rf value in paper chromatography, and migration in ionophoresis.

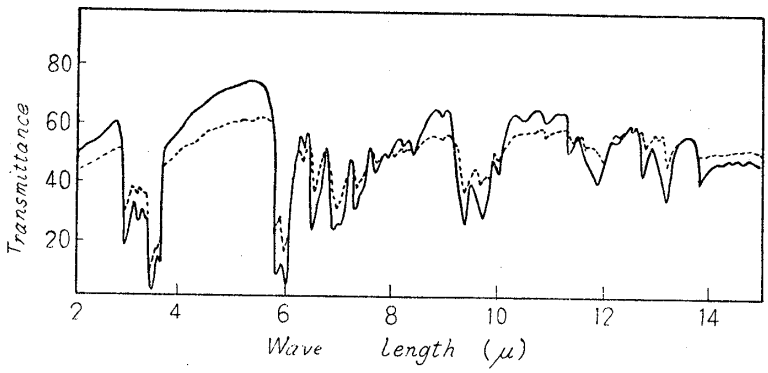
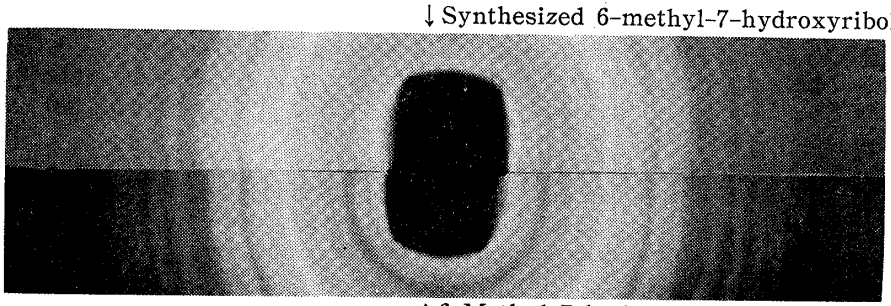



Fig. 2. Infrared Absorption Spectra of 6-Methyl-7-hydroxyribolumazine from *Er. ashbyii* and Synthesized Product
—— Synthesized 6-Methyl-7-hydroxyribolumazine
------ 6-Methyl-7-hydroxyribolumazine from *Er. ashbyii*

↓ Synthesized 6-methyl-7-hydroxyribolumazine



↑ 6-Methyl-7-hydroxyribolumazine from *Er. ashbyii* (natural)

Fig. 3. X-Ray Diffraction Powder Patterns of Natural and Synthesized 6-Methyl-7-hydroxyribolumazine

## Summary

According to the method reported previously*[1] 4-ribitylaminouracil was produced from 4-chlorouracil and D-ribamine, and its nitroso derivative was reduced to 4-ribitylamino-5-aminouracil, which was isolated as hydrochloride or sulfite. Reaction of the salt with pyruvic acid in the presence of hydrazine afforded 6-methyl-7-hydroxy-8-D-ribityl-2(3*H*),4(8*H*)-pteridinedione, m.p. 263°(decomp.), which was in complete accord in every property with purple fluorescent 6-methyl-7-hydroxyribolumazine isolated from the mycelium of *Eremothecium ashbyii*.

(Received February 16, 1959)

NII-Electronic Library Service