borohydride. The oxidation of (I) was carried out with potassium permanganate to give the triol (XIV), m.p. 121.5~122°. When oxidized with periodate, (XIV) consumed only one equivalent of the reagent and did not produce acetone. From these facts it follows that (I) possesses the structure of 2,6-dimethyl-9-(1′-methyl-1′-hydroxyethyl)-bicyclo[5.3.0]dec-7-ene. Examinations on the configuration of compounds of this series are in progress.

Faculty of Pharmacy,
University of Osaka,
Toneyama, Toyonaka, Osaka-fu.
Itiro Yosioka (吉岡一郎)
Yasuko Sasaki (佐々木靖子)

Pharmaceutical Institute,
Faculty of Medicine,
Tohoku University,
Kita-4-bancho, Sendai.
Hiroshi Hikino (ヒキノヒロシ)

December 6, 1960.

UDC 577.164.12 : 577.159

On the Mechanism of Enzymic Conversion of 6,7-Dimethylribolumazine to Riboflavin

The writers previously reported that a crude enzyme prepared from the mycelium of *Eremothecium ashbyii* reacted with 6,7-dimethylribolumazine to produce 6-methyl-7-hydroxyribolumazine besides riboflavin[1] and that the reaction yielded a considerable amount of riboflavin without any carbon donor.[2] The greater part of the products in this reaction were identified by paper partition chromatography and, of the two unidentified spots, the bluish green fluorescent one at Rf 0.10 was found to resemble the polymer consisting of two moles of 4-ribitylamino-5-aminouracil[3] reported by the present writers.

Recently, Plaut[4] discussed the conversion of 6,7-dimethylribolumazine to riboflavin by means of the enzyme of *Ashbya gossypii*, the gist of which is as follows: 1) The enzyme acts on 6,7-dimethylribolumazine to produce riboflavin without any carbon donor; 2) a C_4 compound is split off from the pyrazine ring of 6,7-dimethylribolumazine and then reacts with another 6,7-dimethylribolumazine to produce riboflavin. In other words, 6,7-dimethylribolumazine acts as a carbon donor as well as a carbon acceptor; 3) one mole of riboflavin is produced from two moles of 6,7-dimethylribolumazine; 4) when the methyl groups at the 6- and 7-positions of 6,7-dimethylribolumazine are labeled with ^{14}C, the molar specific radioactivity of the resulting riboflavin becomes twice as strong as that of 6,7-dimethylribolumazine; 5) decompositon by the Kuhn-Roth method of the riboflavin thus obtained gives two moles of acetic acid containing ^{14}C, thereby revealing the distribution of radioactivity in riboflavin. In another paper,[5] Plaut stated that formation of 1 mole of riboflavin requires 3 moles of 6,7-dimethylribolumazine and that 6-methyl-7-hydroxyribolumazine was a product other than riboflavin, but did not mention the amount of the resulting 6-methyl-7-hydroxyribolumazine in the above-mentioned report.[4]

The writers prepared an enzyme solution from *Er. ashbyii* and allowed the product to react with 6,7-dimethylribolumazine as reported before. A part of the reaction mixture

---

[1] S. Kuwada, T. Masuda, T. Kishi, M. Asai: This Bulletin, **6**, 618 (1958).
[2] S. Kuwada: Vitamins (Kyoto), **14**, 933 (1958).
[3] S. Kuwada, T. Masuda, T. Kishi, M. Asai: This Bulletin, **8**, 798 (1960).
[4] G.W.E. Plaut: J. Biol. Chem., **235**, PC 41 (1960).
[5] *Idem*: Federation Proc., **19**, 312 (1960).

was taken out three times at intervals of 1 hour and the unchanged 6,7-dimethylribolumazine, the resulting riboflavin, and 6-methyl-7-hydroxyribolumazine were separated by paper partition chromatography. The results of determinations are shown in Table I. As the amounts of the unused substrate and products are shown in the table in percentage against the substrate, formation of riboflavin ought to consume 6,7-dimethylribolumazine in the amounts shown in parentheses, according to the theory of Plaut, and therefore, their total amount comes to about 100% as shown outside the table. Addition of diacetyl in this reaction, however, increased the quantity of the unchanged 6,7-dimethylribolumazine and decreased that of the resulting 6-methyl-7-hydroxyribolumazine, but the amounts of all the substances detected as such totaled nearly 100%. This may be explained by considering that a uracil compound formed by the decomposition of 6,7-dimethylribolumazine undergoes condensation with diacetyl, regenerating the original compound, and therefore formation of 6-methyl-7-hydroxyribolumazine and polymerization of the uracil compound decreases. As for the mechanism for the formation of 6-methyl-7-hydroxyribolumazine, there are still many points to be clarified, but it seems reasonable to think that the compound is produced by condensation of the uracil compound with a C_3-compound rather than by direct conversion of the 7-methyl group in 6,7-dimethylribolumazine.

TABLE I. Progress of the Enzymic Reaction *in vitro* under Various Conditions

	Enzyme soln. + 6,7-dimethylribolumazine (3.0×10^{-6} mole)					
	1 hr.		2 hr.		3 hr.	
	$\times 10^{-6}$ mole	%	$\times 10^{-6}$ mole	%	$\times 10^{-6}$ mole	%
6,7-Dimethylribolumazine	2.18	72.7	1.78	59.3	1.47	49
Riboflavin	0.282	9.4 (18.8)	0.410	13.65 (27.30)	0.427	14.23 (28.46)
6-Methyl-7-hydroxyribolumazine	0.298	9.93	0.458	15.75	0.61	20.3
Total	2.76	92.03 (101.4)	2.648	88.7 (101.35)	2.507	83.53 (97.76)

	Enzyme soln. + 6,7-dimethylribolumazine (3.0×10^{-6} mole) + diacetyl (1.96×10^{-5} mole)					
	1 hr.		2 hr.		3 hr.	
	$\times 10^{-6}$ mole	%	$\times 10^{-6}$ mole	%	$\times 10^{-6}$ mole	%
6,7-Dimethylribolumazine	2.75	91.7	2.84	94.6	2.75	91.7
Riboflavin	0.129	4.3	0.136	4.53	0.19	6.33
6-Methyl-7-hydroxyribolumazine	0.068	2.26	0.016	0.53	0	0
Total	2.947	98.26	2.992	99.96	2.94	98.03

Many more experiments under various conditions would be necessary to confirm whether the same reaction as *in vitro* is effected in the living body of *Er. ashbyii.*

Research Laboratories,
Takeda Pharmaceutical
Industries, Ltd.,
Juso-nishino-cho,
Higashiyodogawa-ku, Osaka.

Mitsuko Asai (浅井満子)
Toru Masuda (増田 亨)
Satoru Kuwada (桑田 智)

December 9, 1960.