BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 45, 2198—2199(1972)

The Structure of a New Pteridine Compound Produced by *Pseudomonas ovalis*

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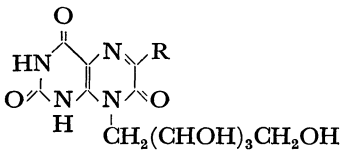
(Received September 28, 1971)

The following ribityllumazines were hitherto isolated from microorganisms: 6,7-dimethyl-8-(1-D-ribityl)-2,4-dioxohexahydropteridine (Masuda's G-compound),[1] 6-methyl-8-(1-D-ribityl)-2,4,7-trioxohexahydropteridine (Masuda's V-compound)(II),[2] 6-(3-indolyl)-8-(1-D-ribityl)-2,4,7-trioxohexahydropteridine,[3] 6-(*p*-hydroxyphenyl)-8-(1-D-ribityl)-2,4,7-trioxohexahydropteridine and 6-(2-carboxyethyl)-8-(1-D-ribityl)-2,4,7-trioxohexahydropteridine (Putidolumazine)(III).[4,5]

This paper describes the isolation of D-*erythro*-neopterin and three ribityllumazine compounds from cultures of *Pseudomonas ovalis*. Two of them have been identified as compounds II and III, but the last compound (I) has not been isolated from natural sources. The structure of this compound was deduced to be 8-(1-D-ribityl)-2,4,7-trioxohexahydropteridine from UV-spectra and chemical degradation. The compound was synthesized by condensation of 2,6-dihydroxy-5-amino-4-D-ribitylaminopyrimidine and glyoxylic acid, and its identity with the natural compound I was confirmed.



(I) R: -H (II) R: $-CH_3$
(III) R: $-CH_2CH_2COOH$

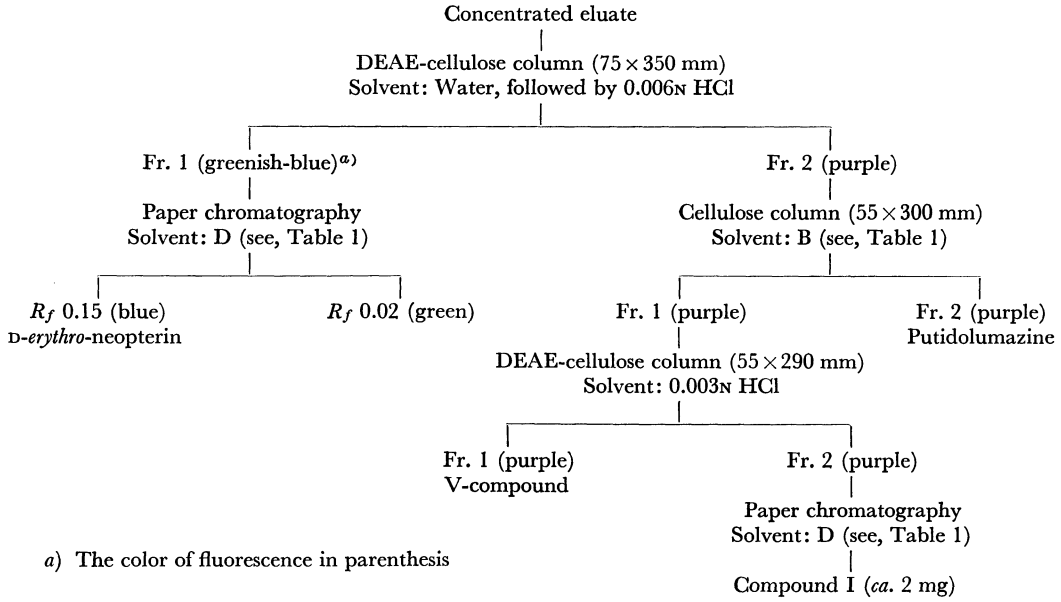
Experimental

Isolation of Compound I. *Pseudomonas ovalis* (IAM 1506) was cultivated for 10 days in six fermentation jars (total quantity: 60 liters) at 30°C with aeration. The medium for cultivation was as follows: sodium glutamate 200 g, glucose 50 g, $Na_2HPO_4 \cdot 12H_2O$ 56 g, KH_2PO_4 2.5 g, $MgSO_4 \cdot 7H_2O$ 1.0 g, $FeSO_4 \cdot 7H_2O$ 50 μg, $Ca(NO_3)_2 \cdot 4H_2O$ 14.4 mg and water 10 liters. The pH was adjusted to 7.6. After cells were removed by centrifuging at 13000 rpm, fluorescent compounds were adsorbed on charcoal (240 g), eluted with 50% ethanol–4% ammonia (1:1) solution and the concentrated eluate was subjected to chromatography as shown in Fig. 1.

Characterization of Compound I. The aqueous solution of this compound shows strong purple fluorescence. R_f-values and UV-spectra are given in Table 1 and Fig. 2.

Periodate Oxidation: One milliliter of aqueous solution of I (4.56×10^{-5}M) was treated with 2 m*l* of aqueous potassium metaperiodate solution (11.1×10^{-5}M, pH 3.5) at room temperature. At intervals the amount of undestroyed periodate was estimated from UV-absorption (222 mμ) by the method of Dixon and Lipkin.[6] The compound (M, 314) consumed 2.77 mol of periodate per mole.

$NaBH_4$ Reduction of the Periodate Oxidation Product: I (*ca.* 0.3 mg) was dissolved in 0.3 m*l* of water and 0.4 mg of potassium metaperiodate was added. After 1 hr, the oxidation product was purified by paper chromatography using 1-butanol–acetic acid–water (4:1:1) and 2-propanol–water



a) The color of fluorescence in parenthesis

Fig. 1. Isolation of compound I

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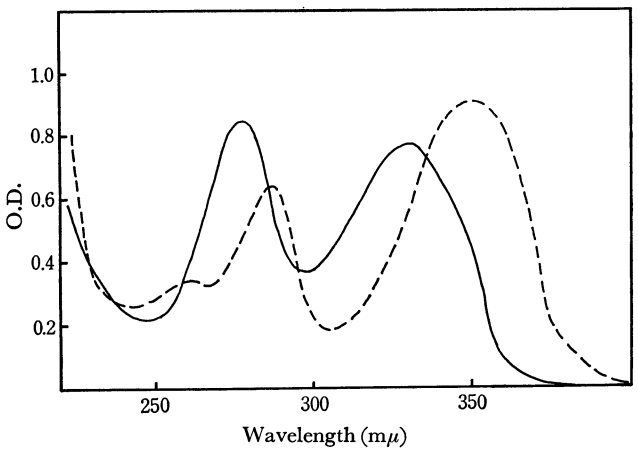

Fig. 2. Ultraviolet spectra of compound I.
——: in 0.1N HCl, ------: in Water

(2: 1) as solvents. The oxidation product was then reduced with a small amount of sodium borohydride in 0.2 m*l* of water. The solution was kept standing at room temperature for 30 min, then adjusted to pH 1 with hydrochloric acid. The product was again purified by paper chromatography using a solvent of 2-propanol–1% ammonia (2: 1). The final product was identified as 8-(2-hydroxyethyl)-2,4,7-trioxohexahydropteridine (IV)[7] by comparison with synthetic material. (Table 1)

Table 1. Paper chromatography and electrophoresis of pteridines

Substance	R_f values[a]				Electrophoresis[b]
	A	B	C	D	
Compound I	0.49	0.52	0.64	0.08	22
After KIO_4 oxidation and $NABH_4$ reduction	0.55	0.60	0.62	0.19	29
Synthetic material (I)	0.49	0.52	0.64	0.08	22
Synthetic material (IV)	0.55	0.60	0.62	0.19	29
V-compound	0.55	0.56	0.64	0.13	18
Putidolumazine	0.42	0.50	0.72	0.11	28
D-*erthro*-neopterin	0.52	0.47	0.63	0.15	−8

a) Solvents: A. 2-propanol:2% ammonium acetate (1:1)
B. 2-propanol:1% ammonia (2:1)
C. 3% aqueous ammonium chloride
D. 1-butanol:acetic acid:water (4:1:1)
b) Distance (in mm) to anode after paper electrophoresis at pH 4.65 (sodium acetate buffer) for 60 min at 25V/cm.

Synthesis of 8-(1-D-Ribityl)-2,4,7-trioxohexahydropteridine (I). 2,6-Dihydroxy-5-nitro-4-D-ribitylaminopyrimidine[8] (625 mg) in water (80 m*l*) was hydrogenated over a platinum oxide catalyst (300 mg). After the theoretical amount of hydrogen was taken up, the catalyst was removed by filtration. The solution was acidified with acetic acid (3 m*l*). Glyoxylic acid (400 mg) was added and the mixture was heated for 5 hr on a water bath. The solution was neutralized with aqueous ammonia and concentrated *in vacuo* to about 30 m*l* below 40°C. The product was purified by chromatography using a DEAE–cellulose column (5.5×32 cm; elution: 0.005N HCl) and a cellulose column (7.5×30 cm; elution: 2-propanol–1% ammonia (2: 1)). The eluate was concentrated to dryness *in vacuo* below 40°C. The residue was recrystallized from water, yield, 76 mg, mp 262—263°C (decomp.).

Found: C, 40.65; H, 4.52; N, 16.79%. Calcd for $C_{11}H_{14}O_7N_4 \cdot 1/2H_2O$: C, 40.86; H, 4.64; N, 17.33%.

The UV-absorption spectrum of the substance in 0.1N HCl was as follows: $\varepsilon_{min}^{250m\mu}$ 3410, $\varepsilon_{max}^{278m\mu}$ 12800, $\varepsilon_{min}^{298m\mu}$ 5520, $\varepsilon_{max}^{332m\mu}$ 11800; in H_2O: $\varepsilon_{min}^{243m\mu}$ 3930, $\varepsilon_{max}^{261m\mu}$ 5190, $\varepsilon_{min}^{267m\mu}$ 4940, $\varepsilon_{max}^{287m\mu}$ 9680, $\varepsilon_{min}^{305m\mu}$ 2860, $\varepsilon_{max}^{350m\mu}$ 13700.

Discussion

Four pteridines have been isolated from the culture of *Pseudomonas ovalis*. Three of them were characterized as D-*erythro*-neopterin, 6-methyl-8-(1-D-ribityl)-2,4,7-trioxohexahydropteridine (II) and 6-(2-carboxyethyl)-8-(1-D-ribityl)-2,4,7-trioxohexahydropteridine (III).

The fourth compound (I) was a new pteridine. UV-spectra of this compound suggests that it is a 2,4,7 (1H,3H,8H) pteridinetrion with alkyl side chains at N-8 and C-6 position (Fig. 2). I is stable against Al–Hg reduction suggesting that it has –H or $-CH_2-$ bound to carbon-6.[9] It is stable against esterification, suggesting that it has no carboxyl group. One mole of compound I consumed 3 mol of KIO_4 and reduction of the product with $NaBH_4$ yielded a substance identical with 8-(2-hydroxyethyl)-2,4,7-trioxohexahydropteridine (IV);[7] this was identified by cochromatography with the synthetic material (Table 1). From these results the structure of 8-(1-D-ribityl)-2,4,7-trioxohexahydropteridine (I) was postulated for I. The compound was synthesized by condensation of 2,6-dihydroxy-5-amino-4-D-ribitylaminopyrimidine and glyoxylic acid and its identity with the natural product was confirmed.

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