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THE STRUCTURE OF PTERIDINES FROM *PHOTOBACTERIUM PHOSPHORIUM*

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Three pteridines were isolated from the mycelium of *Photobacterium phosphorium*. One of them, photopterin-A, is a new compound and its structure was deduced as 6-(DL-1,2-dihydroxyethyl)-8-D-ribityl-2,4,7-trioxopteridine. Other two pteridines were identified as 2 and 7. The configuration of the tetrahydroxypentyl group in those pteridines was confirmed unambiguously by comparison of their ORD spectra with those of all possible isomers synthesized.

Hitherto several derivatives of 8-D-ribityl-2,4,7-trioxopteridine have been isolated from microorganisms. These are 8-D-ribityl-2,4,7-trioxopteridine (7)[2] itself, its 6-methyl (2),[3] 6-(2-carboxyethyl),[4] 6-(3-indolyl),[5] and 6-(p-hydroxyphenyl)[5] derivatives. It was reported that the 6-methyltrioxopteridine (2), known as V-compound,[3] functions as an inhibitor in the biosynthesis of riboflavin.[6] In this paper, we describe the isolation and characterization of a new pteridine from *Photobacterium phosphorium*, as well as two more pteridines, configuration of which is unambiguously confirmed. In 1960, Kometani[7] first obtained a blue-violet fluorescent compound from the same bacterium, and suggested it to be an analogue of biopterin because of their similarity in the UV spectra, but now it turned out that the similarity had occurred incidentally because of the impurities contaminated with the fluorescent compound.

The mycelium was extracted with hot water and the fluorescent substances were adsorbed on activated charcoal, which were then eluted with a mixture of 0.01M ammonia and n-butanol (100:7.5). The concentrated eluate was subjected to chromatographic separations successively on DEAE-cellulose (eluant: 0.3 - 0.5% sodium chloride; gradient), on Florisil (water),[8] and on Dowex 1-X2 column (0 - 0.5M

Table 1. Physical Properties of the Pteridines

Compd.	pKa[a]	UV[b] pH	UV[b] λ_{max} (nm)	NMR[c] δ (ppm)	Rf[d] i	Rf[d] ii	Rf[d] iii	yield[e]
A	3.36	1.0	204, 279, 333	3.6-4.5(8-9H)	0.034	0.59	0.70	7
	12.44	8.0	212, 259, 289, 351	4.94 (1H)				
		14	262, 285*, 364					
B	3.30	1.0	204, 280, 332	3.6-4.4(7H)	0.059	0.55	0.67	17
	12.46	8.0	214, 259, 287, 349	7.68 (1H)				
		14	262, 285*, 363					
C	3.78	1.0	214, 283, 326	3.6-4.4(7H)	0.100	0.54	0.67	100
	12.91	8.0	212, 252, 290, 346	2.26 (3H)				
		14	259, 283*, 359					

a) at 20°C by spectroscopic method.

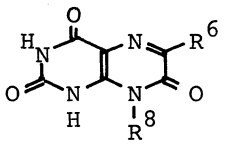
b) at 20°C; at pH 1.0 for neutral species, at pH 8.0 for monoanions, and at pH 14 for dianions. * : shoulder.

c) in D_2O at pH 8, relative to DSS.

d) by descending method at 20°C; solvent systems, (i): 50% acetic acid/n-butanol (1:1), relative Rf from 2 (Rf 0.100); (ii):4% sodium citrate; (iii): 4% ammonium chloride.

e) relative to compound C, whose yield was 14 mg from 1 kg of the dry mycelium.

Table 2. pKa and Rf Values of Synthetic Pteridines



Compd.	R^6	R^8	pKa[a]		Rf[d] i	Rf[d] ii	Rf[d] iii
1	CH_3	CH_2CH_2OH			0.26	0.51	0.64
2	CH_3	D-ribityl	3.71	12.94	0.100	0.55	0.67
3	CH_3	D-arabityl	3.60	12.85	0.106	0.62	0.73
4	CH_3	L-arabityl	3.60	12.92	0.104	0.61	0.73
5	CH_3	D-xylityl	3.67	12.75	0.093	0.60	0.72
6	CH_3	D-lyxityl	3.76	13.01	0.102	0.59	0.69
7	H	D-ribityl	3.28	12.40	0.060	0.55	0.67
8	H	D-arabityl	3.16	12.54	0.060	0.59	0.69
9	H	L-arabityl	3.17	12.42	0.060	0.59	0.69
10	H	D-xylityl	3.18	12.45	0.054	0.58	0.69
11	CH_2OH	CH_2CH_2OH			0.12	0.50	0.59
12	(L)-CH(OH)-CH_2OH	D-ribityl	3.36	12.41	0.034	0.59	0.70
13	(D)-CH(OH)-CH_2OH	D-ribityl	3.37	12.40	0.034	0.57	0.68
14	(DL)-CH(OH)-CH_2OH	D-ribityl			0.034	0.59	0.70

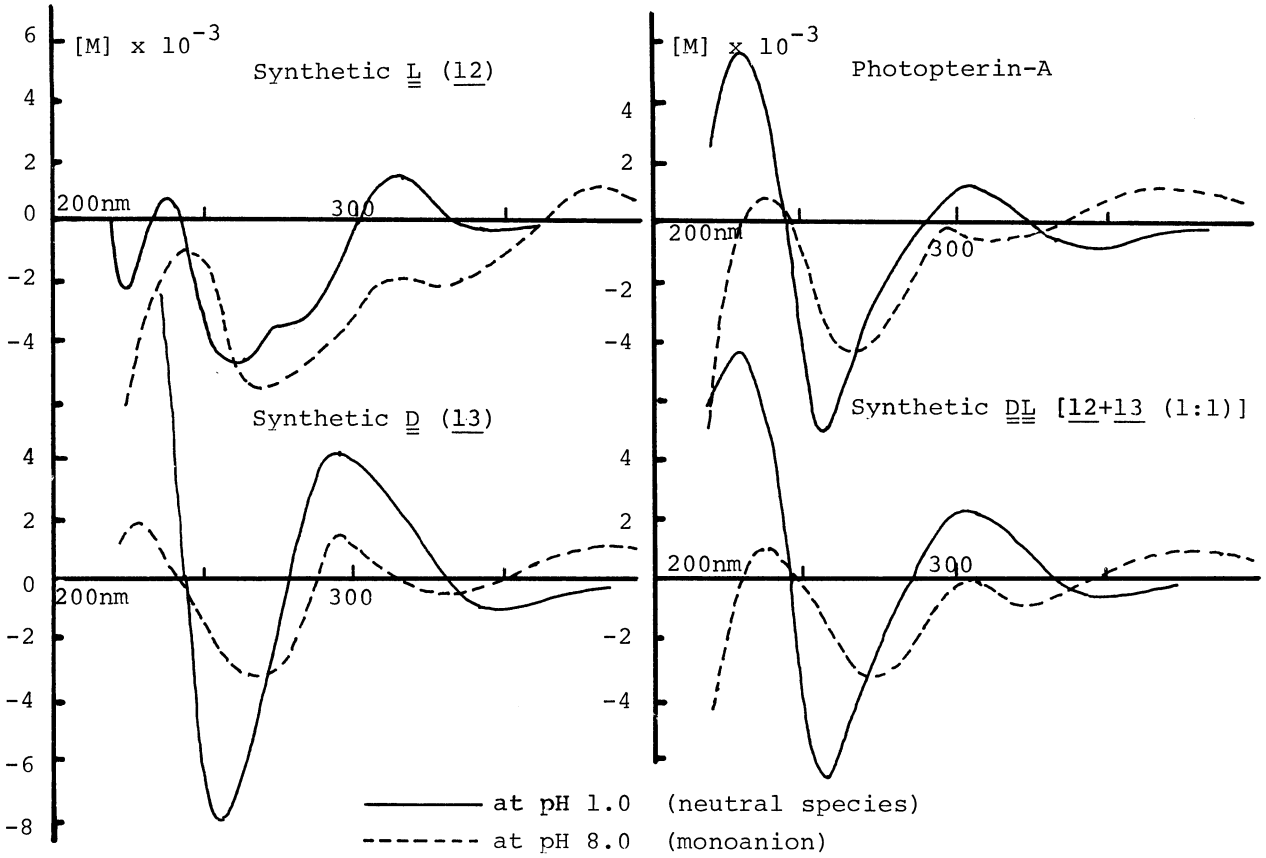
a) and d) see footnotes in Table 1.

formic acid; gradient). Three fluorescent compounds, A, B, and C, thus isolated were finally purified by paper chromatography [solvent: 50% acetic acid/n-butanol (1:2)]. The Rf values, yields, pKa values, UV and NMR data are shown in Table 1.

Compound C was identified as 6-methyl-8-tetrahydroxypentyl-2,4,7-trioxopteridine from its pKa values, UV and NMR spectra, and the facts that its periodate oxidation (consumed three moles of the reagent) and subsequent reduction with potassium borohydride gave 8-(2-hydroxyethyl)-6-methyl-2,4,7-trioxopteridine (1).[9] In order to confirm the configuration of the tetrahydroxypentyl group, we synthesized all of the possible tetrahydroxypentylpteridines (2 - 6)[9] having different configurations, and compared with compound C in respect of their ORD spectra at pH 1.5 (neutral species) and pH 8.0 (monoanion). This allowed an unambiguous confirmation of the natural product as 6-methyl-8-D-ribityl-2,4,7-trioxopteridine (2). Although it was claimed that the tetrahydroxypentyl group of the above mentioned natural product is D-ribityl group,[2-5] no such unambiguous confirmation of its configuration has been carried out before. Compound B was identified as 8-D-ribityl-2,4,7-trioxopteridine (7) in the same manner by comparison with synthetic pteridines (7 - 10).[9]

The structure of the third compound (A), which is named as photopterin-A, was deduced to be 6-(1,2-dihydroxyethyl)-8-D-ribityl-2,4,7-trioxopteridine (12 or 13) for the following reasons: (a) its UV spectra suggest its nucleus to be 8-substituted 2,4,7-trioxopteridine, (b) its NMR spectra has no signal corresponding to $C-CH_3$, $C-CH_2-C$, or vinyl protons, but has a signal (1H) at 4.94 ppm, (c) it consumed four moles of potassium periodate, and subsequent reduction of the oxidation product with potassium borohydride gave 8-(2-hydroxyethyl)-6-hydroxymethyl-2,4,7-trioxopteridine (11), identified with a material prepared by an unambiguous method,[9] and (d) on reduction with aluminum amalgam[10] it gave 7 by losing the side chain at the 6-position. In order to confirm the structure of photopterin-A, we prepared 6-(L-1,2-dihydroxyethyl)-8-D-ribityl-2,4,7-trioxopteridine (12) by condensation of 5-amino-4-D-ribitylamino-2,6-dihydroxypyrimidine[11] and barium α-keto-L-erythronate; the latter being obtained by oxidation of the corresponding osone[12] with iodine.[13] Photopterin-A was identical with 12 in their Rf and pKa values and UV spectra, but different from 12 in their ORD spectra (see Fig 1). The ORD spectra of photopterin-A also differed from those of the 6-(D-dihydroxyethyl)pteridine (13) which was prepared by an analogous manner to its L-isomer, but were identical with those of a 1:1 mixture of the two isomers. Thus we concluded that photopterin-A is 6-(DL-1,2-dihydroxyethyl)-8-D-ribityl-2,4,7-trioxopteridine.

Fig. 1 ORD Spectra of the Pteridines



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