IDENTIFICATION OF 2-AMINO-4-HYDROXY-6-METHYLPTERIDINE AS A NATURALLY-OCCURRING COMPOUND IN TWO METHANE·OXIDIZING BACTERIA

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Summary: Four pteridines have been isolated from two species of methane-oxidizing bacteria. They are: 2-amino-4-hydroxy-6-carboxypteridine, D-erythro-neopterin, 2-amino-4-hydroxypteridine, and 2-amino-4-hydroxy-6-methylpteridine. This latter compound has not previously been shown to occur naturally.

Anthony and Zatman (1) have described an alcohol dehydrogenase from Pseudomonas sp. M27 which is involved in the oxidation of methanol and which appears to have an unidentified pteridine cofactor. Similar alcohol dehydrogenases occur in the methane·oxidizing bacteria, Pseudomonas methanica (2), Methylococcus capsulatus (3), and in some new isolates (4). We have isolated and characterized the pteridines present in two of these new isolates, Methylosinus sporium strain 5, and Methylosinus trichosporium strain PG. As described in this note, these pteridines are all known compounds, but one of them, 2-amino-4-hydroxy-6-methylpteridine has not previously been isolated from natural sources.

EXPERIMENTAL

Organisms. Methylosinus sporium strain 5 and Methylosinus trichosporium strain PG were isolated by Whittenbury et. al. (4). Cultures were grown on the basal medium of Foster and Davis (5) under an atmosphere of methane and air (1:1 by volume) at 30C. Cultures were grown from a 10% inoculum for 4 days in 21. erlenmeyer flasks containing 300 ml medium. Cells were

harvested by centrifuging for 10 min. at 16,000 xg and were washed twice in 60mM phosphate buffer pH 7. Freshly-harvested, wet, packed cells were obtained by courtesy of Dr. D. S. Hoare and R. Patel, Microbiology Department, University of Texas.

Isolation of compounds. Wet cells (2 g) were processed, as described previously (6) by boiling with dilute acetic acid and manganese dioxide. This technique ensures rapid oxidation of any unstable, reduced pteridines to the stable, aromatic derivatives. The compounds actually isolated are not implied to be in the aromatic form in the living cell; they probably occur as dihydro or tetrahydro derivatives. Pteridines present in the acetic acid-manganese dioxide extract were adsorbed on charcoal, eluted with ethanol-ammonium hydroxide (1:1) and the concentrated eluate was subjected to chromatography on Whatman No. 1 paper using Solvent II (Table 3). Four fluorescent compounds were isolated from Methylosinus trichosporium, strain PG; five were separated from Methylosinus sporium, strain 5, four of which were identical with these from Methylosinus trichosporium, strain PG. The relative abundance of these compounds was estimated by assuming that each exhibits the same fluorescence spectrum, in which case the relative fluorescence intensities are approximate measures of their relative abundance. Fluorescence data were obtained on 1% ammonium hydroxide eluates from paper chromatograms using a Farrand MK1 spectropfluorometer, (activating wavelength, 368 nm; fluorescence wavelength, 455nm). The results are shown in Table 1.

Table 1

Relative abundance of pteridines from methane-oxidising bacteria

Species	Compound				
	1	2	3	4	5
Methylosinus sporium, strain 5	0.50	0.59	1.00	0.50	0.25
Methylosinus trichosporium, strain PG	0.50	0.49	1.00	0.55	----

Table 2

Oxidation of isolated pteridines

Compound	$NaIO_4$	Alkaline $KMnO_4$	Oxidation Product	Side chain in position 6 prior to oxidation
1	no reaction	no reaction	---	-COOH
2	+	+	-6-COOH	-CHOHCHOHCH$_2$OH (D-erythro)
3	no reaction	no reaction	---	-H
4	no reaction	+	-6-COOH	$-CH_3$

The eluted compounds were further purified by paper chromatography in Solvent I (Table 3). This procedure eliminated a few minor fluorescent constituents which were not present in sufficient amounts to permit identification.

Characterization of the Compounds. Compounds 1, 2, 3, and 4 (designated in order of increasing R_f values in Solvent II) were identified by a combination of diagnostic tests and direct comparison with authentic materials. The diagnostic tests were 1) oxidation with sodium metaperiodate at room temperature in neutral solution for 24 hours, and 2) oxidation with alkaline potassium permanganate, followed, in both cases, by identification of the reaction product. The results are summarized in Table 2. The compounds identified were: 1, 2-amino-4-hydroxy-6-carboxypteridine; 2, D-erythro-neopterin; 3, 2-amino-4-hydroxypteridine; 4, 2-amino-4-hydroxy-6-methylpteridine. R_f values and electrophoretic mobilities of these pteridines and authentic materials are compared in Table 3. Compound 5 was not obtained in sufficient quantity for identification.

DISCUSSION

Synthetic 2-amino-4-hydroxy-6-methylpteridine has been used, in

Table 3

Chromatographic and Electrophoretic Comparison

of isolated compounds

Compound	R_f values[a]					Electrophoretic mobility[b]
	I	II	III	IV	V	
1	0.14	0.15	0.31	0.02	0.59	35
2-Amino-4-hydroxy-6-carboxypteridine	0.14	0.15	0.31	0.02	0.59	35
2	0.15	0.26	0.52	0.16	0.66	-3
D-erythro-Neopterin[c]	0.15	0.26	0.52	0.16	0.66	-3
3	0.35	0.34	0.58	0.28	0.57	-3
2-Amino-4-hydroxy-pteridine	0.35	0.34	0.58	0.28	0.57	-3
4	0.40	0.41	0.64	0.35	0.57	-3
2-Amino-4-hydroxy-6-methylpteridine[d]	0.40	0.41	0.64	0.35	0.57	-3

[a]Solvents: I, 1-butanol-acetic acid-water (4:1:1); II, 1-propanol-1% ammonium hydroxide (2:1); III, 1-propanol-2% ammonium acetate (1:1); IV, 2-propanol-5% boric acid (4:1): V, 3% ammonium chloride.

[b]Distance (in mm) compound migrated from origin toward the anode or cathode (negative values) after electrophoresis at pH 4.70 (sodium acetate buffer) for 1 hour at 20 V/cm.

[c]Prepared from 2,4,5-triamino-6-hydroxypyrimidine and D-ribose.

[d]Prepared from 2,4,5-triamino-6-hydroxypyrimidine and hydroxyacetone, or by decomposition of pteroylglutamic acid.

reduced form, as an artificial cofactor for phenylalanine hydroxylase (7), but its isolation from natural sources raises the possibility that it may be a cofactor in its own right. Its relation to the cofactor for the alcohol dehydrogenase described by Anthony and Zatman (1) remains to be determined.

It is interesting to note, however, that this compound has not been isolated from Pseudomonas species in which the pteridines have been characterized e.g., the Pseudomonas sp. ATCC 11299a studied by Guroff and coworkers (8, 9) in relation to its inducible phenylalanine hydroxylase, which is considered to have a pteridine cofactor. Also L-threo-neopterin has been isolated by these workers from this same species and from Pseudomonas roseus fluorescens (10). However, the neopterin isomer described herein is the D-erythro isomer. There thus appears to be considerable diversity in pteridine production in methane-oxidizing bacteria and related species, which may be a reflection of functional diversity of these compounds.

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