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FATTY ACIDS AND PROSTAGLANDINS OF THERMAL CYANOBACTERIA

T. V. Kafanova, N. G. Busarova, S. V. Isai, and T. Ya. Zvyagintseva

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The compositions of the fatty acids and prostaglandins of extracts from thermophilic cyanobacteria of the taxa Oscillatoria *and* Microcystidaceae *have been investigated. The fatty acids include not only saturated but also polyunsaturated species, including 20:3, 20:4, and 22:4 acids with a known pharmaceutical effect. It has been shown for the first time that the lipid fraction of thermophilic cyanobacteria includes a set of prostaglandins of groups A, E, and F, both in the free form and in the form of esters — MeA_1, AcA_1, $AcMeA_1$, MeA_2, AcA_2, $AcMeA_2$, MeA_3, AcA_3, $AcMeA_3$, 6-ketoE$_1$, MeE_1, AcE_1, $AcMeE_1$, MeE_2, AcE_2, $AcMeE_2$, MeE_3, AcE_3, $AcMeE_3$, 6-ketoF$_{1\alpha}$, $MeF_{1\alpha}$, $AcF_{1\alpha}$, $AcMeF_{1\alpha}$, 6-ketoF$_{2\alpha}$, $MeF_{2\alpha}$, $AcF_{2\alpha}$, and $AcMeF_{2\alpha}$. The latter, together with other previously known compounds, undoubtedly make a contribution to the healing properties of thermal springs.*

It is known that thermal springs are used for medicinal purposes. Judging from the literature, their medicinal effects are connected with the mineral composition of the water and its heat regime and with cyanobacteria [1, 2].

We have investigated the fatty acid (FA) and prostaglandin (PG) compositions of thermal cyanobacteria (TCBs) of Kamchatka species of *Oscillatoria* and *Microcystidaceae*. We worked with a prostaglandin extract (PGEx) of the TCBs, since a PG-like and bactericidal activity had been found in it previously [3, 4].

By TLC we isolated from the TCB PGEx eight fractions, five of which possessed PG-like activity. Biotesting was conducted by the method of [5]. The TCB PGEx caused contractions of smooth musculature identical with the action of $5 \cdot 10^{-8}$ g/ml of $PGF_{2\alpha}$; fraction 3 exhibited a smaller contractile activity — $2 \cdot 10^{-8}$ g/ml of $PGF_{2\alpha}$; and fractions 4-7 caused contractions identical with the contractions under the action of $5 \cdot 10^{-8}$ g/ml of $PGF_{2\alpha}$.

Thus, the results of biotests have shown a prostaglandin-like activity of five fractions isolated from TCBs that is similar to the action of a standard specimen of $PGF_{2\alpha}$.

When the above-mentioned fractions were treated with 1 N KOH an increase in absorption at 278 nm was observed, which is characteristic for PGs of group B. Since PGBs are formed under the action of alkali on PGAs and PGEs, it may be assumed that the fractions investigated contained PGs of groups A and E.

We then investigated the fatty acid (FA) compositions of the PGEx. It is known that a deficiency of certain FAs leads to skin diseases, and that individual FAs are precursors of PGs [6, 7]. There is literature information on the composition of the FAs of cyanobacteria [8-10], including thermophilic types [11, 12], which shows variations in the FA content from 2 to 12% of the dry matter. According to literature sources, for all genera of cyanobacteria, including *Oscillatoria* and *Microcystidaceae*, a set of FAs from 10:0 to 18:3 is characteristic, the 16:0 and 16:1 species almost always predominating [8, 9]. Exceptions are certain marine cyanobacteria in which the main acid is the 14:0 variety [10]. Furthermore, polyunsaturated fatty acids (PUFAs) with 3-5 double bonds have been found in cyanobacteria but not in other microorganisms [13].

The cyanobacteria that we have studied contain a broader spectrum of FAs, and, in particular, FAs with up to 26 carbon atoms, including PUFAs (Table 1). The results that we obtained agree with those in the literature.

Glycolipids are more characteristic for cyanobacteria, while phospholipids (PhLs) are practically absent [11]. Phosphatidylglycerol and phosphatidylcholine (PhC) have been detected in some species of cyanobacteria [11]. Our results on

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TABLE 1. Compositions and Amounts of Fatty Acids in a Prostaglandin Extract of Thermal Cyanobacteria from Kamchatka Relative to the Total FAs

Fatty acid	%	Fatty acid	%	Fatty acid	%
12:0	0.5	*iso*16:0	4.3	*anteiso*19:0	Tr.
13:0	0.6	16:0	63.9	18:2	0.7
*iso*14:0	0.1	16:1	8.0	18:3	0.1
14:0	6.0	16:2	1.8	20:3	Сл.
14:1	0.1	*anteiso*17:0	0.1	20:4	0.9
*anteiso*15:0	2.1	18:0	2.1	22:4	1.3
15:0	2.2	18:1	0.1	26:0	5.1

the determination of PhLs in TCBs showed that they are present in only trace amounts. In the PGs of an extract from native TCBs we found phosphatidylglycerol, PhC, phosphatidylethanolamine, and phosphatidylinositol in small amounts. It must be mentioned that PhC, which we have detected in TCBs, is not found in the majority of bacterial species [11] but is the main lipid of plant and animal tissues. The presence of PhC in TCBs can be explained by the fact that the material investigated was a symbiosis of bacteria with algae.

The TCB prostaglandins were identified mainly by EDIAP mass spectrometry (MS) [14], which permits the determination in the material under investigation of not only free PGs but also their esters. It is quite likely that in the analysis of the fractions by EDIAP mass spectrometry the FAs in them may interfere with the determination of the PGs. In order to exclude a contribution of FAs in the MS of the fractions, we calculated theoretically the *m/z* values of the main high-mass ions of individual FAs and PGs. It was found that the *m/z* values of the quasimolecular ions of the main TCB FAs (M^+ 200-396) and the quasimolecular ions of the PGs (M^+ 332-412) and their derivatives do not coincide, except for components with M^+ 332 (the 22:4 FA and PGA_3) and M^+ 396 (the 26:0 FA, AcE_1, and $AcF_{2\alpha}$).

Table 2 gives the experimental results of the MS analysis of the fractions. The masses of the FAs that we had determined previously by the GLC method (Table 1) have been excluded from Table 2. These results require explanation. Each of the fractions investigated consisted of a mixture of several PGs and their derivatives, with a predominance of some one type of PG. The separation of the PGs into fractions that we achieved took place in accordance with the rules of single-stage TLC. To obtain individual PGs required repeated fractionation of an extract.

Table 2 shows that there were only a few molecular ions the *m/z* values of which could be assigned both to FAs and PGs. However, the chromatographic behaviors of FAs and PGs are different. Some *m/z* values may correspond to more than one type of PGs. For example, we assigned the ion with *m/z* 393 $[M + K]^+$ in fraction 4 to $PGF_{2\alpha}$. It could simultaneously relate to 15-$AcMeA_1$ $[M + H]^+$, PGE_1 $[M + K]^+$, $PGE_{2\alpha}$ $[M + K]^+$, 6-keto-$F_{1\alpha}$ $[M + Na]^+$, and to another three PGs.

According to TLC, the esters of PGA_1 and PGE_1 have different R_f values: $R_fF_{2\alpha}$ 0.29; R_fE_1, E_2 0.40; R_fA_1, A_2 0.49-0.51; the R_f values of the methyl esters of all groups of PGs are 0.86-0.93 and, consequently, they can be excluded from the fraction under consideration. The PG G-keto-$F_{1\alpha}$ is concentrated mainly in the preceding fraction. Thus, it is most likely that the PG has the structure of $PGF_{2\alpha}$. In addition to this, fraction 4 contained, in the form of impurities, PGs of a third series: A_3 and E_3 and their esters. An additional confirmation of the presence of PGs of the third series is the finding of the 22:4 acid in the material investigated (Table 1) as a precursor of the biosynthesis of this type of PG. There is information that some bacteria stimulate the biosynthesis of PGs of the E series, including E_3 [15].

Fraction 5 was represented by PGA_2 and the methyl and acetate–methyl esters of PGs of groups F and E, which agrees with the TLC results. Fractions 6 and 7 contained the methyl, acetate, and acetate–methyl esters of PGs of groups A_1, A_2, A_3, $F_{1\alpha}$, $F_{2\alpha}$, E_1, E_2, and E_3.

To identify the PGs additionally, each of the fractions we had obtained previously (3-7) was analyzed by the HPLC method (Fig. 1) followed by the MS of each peak (Table 3). However, we give the results only for fraction 5. The results of EDIAP MS showed that fraction 5 consisted of a mixture of the PGs given in Table 3. The characteristics of peaks 1-3 are not shown in this table because the quasimolecular ions of these substances do not correspond to PGs and their esters. For technical reasons, the EDIAP MSs were obtained in the range of mass numbers from 300 to 400, and the table therefore does not include ions with *m/z* 413 $[AcMeF_{1\alpha} + H]^+$ and 451 $[AcMeF_{1\alpha} + K]^+$, which relate to $AcMeF_{1\alpha}$.

While almost all the peaks of fraction 5 were represented by a mixture of PGs, peak 7 corresponded to an individual PG, PGA_2; i.e., HPLC enabled PGA_2 to be isolated in the individual form. Thus, on comparing the EDIAP MS results for fraction 5, which are shown in Table 2, with the MS results for this fraction analyzed by the HPLC method (Table 3) we can see the same set of PGs: A_2, E_1, $F_{1\alpha}$, MeE_1, and MeE_2.

TABLE 2. Mass-Spectrometric Analysis of the Fractions Isolated from the TCB PGEx

Fraction number	*m/z* (intensity in %, type of quasimolecular ion)
3	359(15.4), 361(4.5), 365(35.7), 367(11.9), 391(9.5, [6-Keto-$F_{2\alpha}$+Na]$^+$), 393(9.5, [6-Keto-$F_{1\alpha}$+Na]$^+$), 405(9.5, [6-Keto-E_1+K]$^+$), 407(11.9, [6-Keto-$E_{2\alpha}$+K]$^+$)
4	347(8.5, [MeA_3+H]$^+$), 349(5.1, [MeA_2+H]$^+$), 351(2.5, [E_3+H]$^+$), 389(3.9 [E_3+K]$^+$), 391(4.2, [$MeF_{2\alpha}$+Na]$^+$, [E_2−K]$^+$), 393(6.8, [$F_{2\alpha}$+K]$^+$)
5	335(6.25, [A_2+H]$^+$), 367(5.0, [MeE_2+Na]$^+$), 377(3.5, [E_1+Na]$^+$), 379(3.1 [$F_{1\alpha}$+Na]$^+$), 391 (3.12, [MeE_1+Na]$^+$), 393(3.13, [E_1+K]$^+$), 405(4.2, [MeE_2+K]$^+$), 413(4.2, [$AcMeF_{1\alpha}$+H]$^+$), 451(6.25, [$AcMeF_{1\alpha}$+K]$^+$)
6	357(22.1 [A_2+Na]$^+$), 359(4.7, [A_1+Na]$^+$), 365(100), 371(9.5, [MeA_2+Na]$^+$ [$MeF_{1\alpha}$+H]$^+$), 375(15, [A_1+K]$^+$), 382(15.0, [A_1Na+Na]$^+$), 387(7.9, [MeA_2−K]$^+$), 389(7.9, [MeE_2+Na]$^+$, [$AcMeA_3$+H]$^+$), 391(4.7, [$AcMeA_2$+H]$^+$), 394(15.8), 396(7.9), 399(19.0 [AcA_2+Na]$^+$), 400(16.5), 401(6.3, [AcA_1+Na]$^+$), 403(7.9, [MeE_3+K]$^+$), 409(15.0, [$MeF_{1\alpha}$+K]$^+$, [$AcMeE_2$+H]$^+$), 413(20.0, [$AcMeA_2$+Na]$^+$, [AcA_3+K]$^+$, [$AcMeF_{1\alpha}$+H]$^+$), 415(10.2, [AcA_2+K]$^+$, [$AcMeA_1$+Na]$^+$, [AcE_3+Na]$^+$), 417(7.0, [AcA_1+K]$^+$), 422(7.0, [AcA_2Na+Na]$^+$), 424(6.03, [AcA_1Na+Na]$^+$), 427(14.2, [$AcMeA_3$+K]$^+$), 429(6.0, [$AcMeE_3$+Na]$^+$, [$AcMeA_2$+K]$^+$), 431(16.5, [$AcMeE_2$+Na]$^+$, [AcE_3+K]$^+$), 433(9.5, [$AcMeF_{2\alpha}$+Na]$^+$, [AcE_2+K]$^+$, [$AcMeE_1$+Na]$^+$), 435(4.7, [$AcF_{2\alpha}$+K]$^+$, [$AcMeF_{1\alpha}$+Na]$^+$, [AcE_1+K]$^+$), 437(3.9, [$AcF_{1\alpha}$+K]$^+$), 441(3.9, [$AcMeE_3$+Na]$^+$), 445(6.0, [$AcMeE_3$+K]$^+$), 447(8.7, [$AcMeE_2$+K]$^+$), 449(5.5, [$AcMeF_{2\alpha}$+K]$^+$), 454(13.4, [$AcMeE_2$Na+Na]$^+$, 456(5.5, [$AcMeE_1$Na−Na]$^+$, [$AcMeF_{2\alpha}$Na+Na]$^+$)
7	365(100), 373(11.1, [A_2+K]$^+$, [MeA_1+Na]$^+$), 377(8.0, [AcA_2+H]$^+$), 380(17.6, [A_2Na+Na]$^+$), 382(8.8, [A_1Na+Na]$^+$), 383(25.6), 384(39.2), 385(9.6), 386(8.0), 394(4.8), 396(5.6), 397(15.2, [$AcF_{2\alpha}$+H]$^+$), 399(5.6, [AcA_2+Na]$^+$), 401(5.6, [AcA_1+Na]$^+$), 405(6.4, [MeE_2+K]$^+$), 407(6.4, [MeE_1+K]$^+$), 419(8.8, [$AcF_{2\alpha}$+Na]$^+$), 420(5.6), 421(4.8. [$AcF_{1\alpha}$+Na]$^+$), 424(4.0, [AcA_1Na+Na]$^+$), 431(13.6, [$AcMeE_2$+Na]$^+$), 433(8.0. [AcE_2+K]$^+$, [$AcMeE_1$+K]$^+$), 436(6.4, [$MeAcA_2$Na+Na]$^+$), 437(4.8 [$AcF_{1\alpha}$+K]$^+$), 444(3.2, [$AcF_{1\alpha}$Na+Na]$^+$), 445(8.0, [$AcMeE_3$+K]$^+$), 447(4.0, [$AcMeE_2$+K]$^+$), 449(7.2, [$AcMeF_{2\alpha}$−K]$^+$, [$AcMeE_1$+K]$^+$)

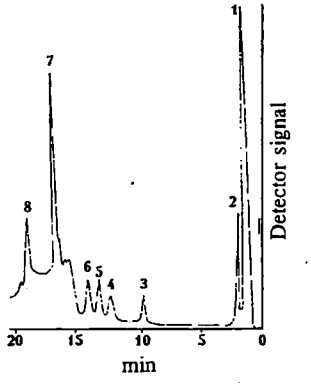

Fig. 1. HPLC chromatogram of fraction 5 of an extract of the prostglandins of thermal cyanobacteria.

EXPERIMENTAL

The material was collected from mountain springs (+42°C) of the spa Paratunk in the environs of Petropavlovsk-Kamchatskii.

The PGEs were obtained from the TCBs by a procedure described previously [16]. As standards we used PGE_1 and PGE_2 (Tallin), $F_{2\alpha}$ (Upjohn Co., USA), and PGA_1 and PGA_2 (Serva).

The preliminary purification of the extract was carried out by TLC on type KSK silica gel with the ethyl acetate—benzene—formic acid (25:75:1) system. The following fractions were obtained: start; 1; 2; 3) R_f 0.3-0.4; 4) R_f 0.4-0.53; 5) R_f 0.53-0.66; 6) R_f 0.66-0.8; 7) R_f 0.83-0.93.

UV spectra in ethanol and methanol were obtained on a Specord spectrophotometer (Germany).

TABLE 3. Results of the MS Analysis of the Peaks (according to the results of HPLC)

Peak No.	*m/z* (intensity in %, type of quasimolecular ion)
4	335(20[A_2+H]$^+$), 355(24[E_1+H]+), 367(53.1[MeE_2+H]$^+$), 373(48[A_2+K]$^+$), 389(100[MeE_2+Na]$^+$).
5	377(100[E_1+Na]$^+$), 379(21.4[$F_{1\alpha}$+Na]$^+$, 391(79[MeE_1+Na]$^+$), 393(61.4[E_1+K]$^+$).
6	316(100), 391(28.6[E_2+K]$^+$)
7	335 (100[A_2+H])
8	345(100[$C_{20:3}$+K]$^+$), 389(72.3[MeE_2+Na]$^+$)

The mass-spectrometric analysis was conducted on an experimental specimen of the instrumental complex KhZh-MKh 3303 constructed in NTO RAN [Scientific and Technical Division, Russian Academy of Sciences] (St. Petersburg). Conditions for recording the spectra: U_{drop} = 2.5 kV, U_{nozzle} = 350 V, rate of feed of the solution to be analyzed 0.2 μl/min, time of recording one spectrum 3 min. The recording covered the range of mass numbers from 300 to 400.

HPLC was conducted on a Milikhrom microcolumn liquid chromatograph with a 2 × 62 mm^2 stainless column filled with Nucleosil 5-C18 (Macherey-Nagel, Germany). The eluent used was acetonitrile (ultrapure)—H_2O in the gradient regime with a 0-100% change in concentration, at a rate of elution of 100 μl/min. Detection at 220 nm with a sensitivity of 1.6.

Analysis of the FAs was made by the GLC method on a Tsvet-100 instrument with a flame-ionization detector. The carrier gas was argon at a rate of flow of 30 ml/min. Analysis was conducted under isothermal conditions at 180 and 197°C in 4 mm × 3 m steel columns filled with 2.5% of poly(ethylene glycol) succinate and 2.5% of poly(ethylene glycol) adipate on Chromaton N-AW-HMDS 0.160-0.200 mm.

Biotests were carried out for each fraction obtained. For this purpose we used the smooth musculature of an isolated section of rat uterine horn, which, possessing a high sensitivity to the action of PGs, permits them to be detected in concentrations of not less than 10^{-9} g/ml. Semiquantitative analysis was carried out relative to a standard solution of $PGF_{2\alpha}$. In the experiment we used puberal nulligravida female random-bred white rats weighing 150-200 g. From each uterine horn we isolated sections 1.5 cm long, which were placed in aerated Tyrode solution at 37°C and were equilibrated under these conditions for an hour [5]. Muscular contractions were recorded on the blackened paper of a kymograph.

PhLs were determined by two-dimensional microchromatography using the method of V. E. Vaskovskii et al. [17].

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