of water and was extracted three times with 360-ml portions of hexane. The extract obtained was filtered through a paper filter and was evaporated, and the residue was separated by chromatography on a column (1 × 30 cm) of silica gel in the hexane–ethyl acetate (95:5) system to eliminate degradation products and traces of hexametapol. The total yield of dideoxysugars was 0.59 g, R_f 0.42 in the hexane–ethyl acetate (1:1) system. The mixture of dideoxysugar acetates obtained (0.59 g) was dissolved in 6 ml of absolute methanol, and 0.2 ml of a 0.4 N solution of sodium methanolate in methanol was added. The mixture was kept at 60°C for 5 min (with TLC monitoring). The resulting solution was cooled, deionized with KU-2 ion-exchanger (H^+), and filtered and evaporated. The yield of syrupy product was 0.39 g. This mixture was deposited on a column (1 × 30 cm) containing silica gel and was eluted with the methanol–chloroform (1:50) system.

The following products were obtained as a result: Methyl 4,6-Dideoxy-α-L-lyxo-hexopyranoside. Yield 0.12 g (31.3%), R_f 0.24; mp 97-98°C, $[\alpha]_D^{20}$ −95.4° (c 0.6; methanol). According to the literature: mp 99-100.5°C, $[\alpha]_D^{20}$ −83° (chloroform) [3].

Methyl 2,6-Dideoxy-α-L-arabino-hexopyranoside. Yield 0.16 g (41%). R_f 0.21; syrup, $[\alpha]_D^{20}$ −170.5° (c 0.6; methanol). According to the literature: syrup, $[\alpha]_D^{23}$ −146° (acetone) [4].

Methyl 3,6-Dideoxy-α-L-arabino-hexopyranoside. Yield 0.06 g (15%) R_f 0.15; mp 85-86°C; $[\alpha]_D^{20}$ −143.8° (c 0.7; methanol). According to the literature: mp 82-84°C, $[\alpha]_D^{20}$ −127.7° (chloroform) [5].

LITERATURE CITED

1. P. M. Collins and V. R. Z. Munasinghe, J. Chem. Soc. Chem. Commun., 927 (1977).
2. W. Hartwig, Tetrahedron, 39, 2609 (1983).
3. V. Pozsgay and A. Neszmelyi, Carbohydr. Res., 85, 143 (1980).
4. F. Sztaricskai, J. Pelyvas, L. Szilagyi, R. Bognar, J. Tamas, and G. Czira, Carbohydr. Res., 65, 193 (1978).
5. D. R. Bundle and S. Josephson, Can. J. Chem., 56, 2686 (1978).

PHOSPHOLIPID COMPOSITION OF PROSTAGLANDIN EXTRACTS OF SOME MARINE INVERTEBRATES WITH DIFFERENT DEGREES OF PROSTAGLANDIN-LIKE ACTIVITY

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UDC 551.464.791.5

The qualitative and quantitative compositions of the phospholipids of eight species of marine invertebrates have been determined. A correlation has been shown between the amounts of particular phospholipids and the prostaglandin-like activities of the extracts. The activities of the extracts have been expressed quantitatively as a function of the set of phospholipids in them.

It is known that phospholipids (PhLs) are synergists of the prostaglandins (PGs) [1-3]. The creation of drugs is based upon this property of them both in the Soviet Union and abroad. In 1983, Japanese workers created an antitumoral drug consisting of a complex of PGs of group A with PhLs [4]. An improvement in the pharmacological action of PGE_1 on its inclusion into lipid microspheres containing egg PhLs has been shown [2, 5]. L. M. Braginsteva and her colleagues have proposed a drug consisting of PGE_2 and $PGF_{2\alpha}$ enriched with PhLs and with unsaturated fatty acids and tocopherol [6-8]. The capacity of the PGs for forming complexes with phosphatidylcholine (PhC) and cholesterol has been established [9].

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Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 322-325, May-June, 1990. Original article submitted February 9, 1988; revision submitted December 18, 1989.

0009-3130/90/2603-0264$12.50

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TABLE 1. Amounts of Phospholipids and Prostaglandins in the PG Extracts of Marine Invertebrates

Sample	Activity	Organic substance in the PG extract, % on the tissue	Amount of PGB, μg/g of tissue	Amounts of PhLs in the PG extract, %						
				PC	LPC	PE	NPPL	PS	PGly	PI
Soft coral Dendronephthya sp. (Australia)	+	0,137	0,60	—	0.93	0,44	1,25	—	3,96	2,12
Soft coral Dendronephthya sp. (Viet Nam)	+	0.035	0,87	0,92	1,01	—	0.65	—	0,47	0.73
Soft coral Plexaura homomalla (forma typica) (Cuba)	+	1,690	2,00	0,63	—	0,20	0,45	—	0,28	—
Soft coral Plexaura homomalla (forma kukenthali) (Cuba)	+	2,000	1,01	—	0,18	0,35	0 29	—	—	0,28
Sponge Coscinoderma sp. Great Barrier Reef	+	0,103	1,70	1,09	1.45	—	1,63	—	1,45	3,45
Sponge Spongia officinales Great Barrier Reef	—	1,774	10,00	—	0,26	—	—	—	—	0,16
Soft coral Alcyoniidae sp. (Australia)	—	0.578	3,20	0.93	—	—	—	0,20	—	0,73
Soft coral Litophyton sp. (Singapore Reefs)	—	0,187	35,00	0,41	0,54	0,32	—	0,32	0,38	—

*The symbol + denotes a high activity of the extract, and — the absence of activity.

In an investigation of the PGs of marine organisms, we found that PG extracts from them differed substantially with respect to PG-like activity. In order to determine whether a relationship exists between high PG-like activity and the PhL composition of the PGs of the extracts, we have investigated the PhL composition of the PG extracts of a number of marine invertebrates in accordance with their biological activities. Among those investigated there were six species of soft corals gathered in various geographical zones, and two species of Australian sponges. The objects were selected on the basis of biotrials performed previously [10, 11], which permitted related organisms possessing different degrees of PG-like activity to be formed into groups.

It might be assumed that the multistage extraction procedure that we used to obtain the PG extracts [12] should have eliminated the bulk of the PhLs; however, analysis showed that the amount of ThLs in the extract was considerable. This may indicate the existence of complexes of PGs with PhLs [9].

The results obtained on the PhL composition of the PG extracts are given in Table 1.

Samples possessing a high activity differed from the inactive ones by an increased amount of PhLs and a qualitative difference in them. Thus, the active samples contained a comparatively high level of phosphatidylglycerol (PhG) while PhG was not detected in the inactive ones, with the exception of the soft coral Litophyton sp. In characterizing the PhLs of the active samples, it was found that, in contrast to the inactive ones, they contained an unidentified ninhydrin-positive PhL (NPPhL). It was also observed that the extracts with PG-like activity lacked phosphatidylserine (PhS). The majority of the active samples were rich in choline-containing PhLs which were represented by PhC and LPhC (lysophosphatidylcholine).

The study of the inactive samples was undertaken in order to show that none of them contained the PhL which we have designated as NPPhL and which, according to Table 2, increased the activity of the sample by 50%.

At the same time, if the amount of active form of the PGs in the sample was vanish-

TABLE 2. Change in Activity of the Extract in the Absence of Particular PhLs

PhL isolated from the extract	Activity of of PGs of the extract after the eliminated of the PhL, %
Lysophosphatidylcholine	77,50
Phosphatidylcholine	88,14
Lysophosphatidylethanolamine	82,60
Phosphatidylethanolamine	68,80
Ninhydrin-positive phospholipid	51,20
Phosphatidylinositol	61,70
Phosphatidylglycerol	48,29

ingly small, then it was not manifested at all, since the extract did not contain those PhLs that can substantially enhance the action of the PGs.

As the biotesting of standard PhLs and the PhLs isolated from the PGs of the extract showed, by themselves the PhLs possessed no PG-like activity. It is just for this reason that the activity of the samples with a comparatively low percentage of organic matter and a high percentage of PhLs in the PG extract can be explained as the result of the fact that the PhLs are capable of enhancing the action of the PGs. Table 1 shows that PhLs do in fact enhance the action of PGs.

The amounts of PGBs shown in Table 1 include directly two types of PGs – groups A and E (as the result of a reaction they were both transformed into group B [14]). The amount of PGBs in the inactive species was considerably greater than in the active ones. Thus, an inactive sponge contained a large amount of PGs but these were of type A which, in the biotests used should not exhibit its activity. In this biotest, the PGs of groups E and F, which were obviously not present in this sample, are responsible for activity.

It then appeared necessary to determine precisely how the PhLs affect the degree of activity of the PGs. An extract of the soft coral Plexaura homomalla kukenthali, distinguished by a particularly high activity, was taken. Each of the PhLs present in it was isolated from equal amounts of this extract with the aid of two-dimensional TLC and each was tested for its degree of activity. In the course of the investigation it was found that a substantial influence on the activity of the extract was exerted by silica gel after contact with which the activity fell fourfold. It is just this residual activity that was taken as 100% in the subsequent experiments.

In themselves, the PhLs exhibited no activity, but after the isolation of each of them a fall in the activity of the extract was observed. In the literature there is information on the synergism of the action of PhLs on PGs only for PEs. Our results show that synergism is possessed by many PhLs, but they differ in the degree of their synergism. The highest capacity for enhancing the action of PGs was observed for PhG. It is also interesting that of all the samples investigated only those were distinguished by a high activity the PG extract of which contained more than two of the PhLs the absence of which particularly lowered activity, namely: NPPhL, PhI, and PhG.

EXPERIMENTAL

Samples of the animals studied were fixed with acidified ethanol immediately after trapping. The samples of sponges were collected in the region of the Great Barrier Reef (Australia) in January 1980, during the expedition of the Scientific Research Vessel Professor Bogorov, and two samples of soft corals – one from the family Alcyoniidae and the other from the genus Dendronephthya – were obtained from the same area. A soft coral from the same genus, Dendronephthya, was collected in May, 1982, in Viet Nam. Corals from the genus Litophyton were collected, March 1980, on the Singapore Reefs. Both corals from the genus Plexaura were collected in Cuba in January, 1986, during an expedition on the Scientific Research Vessel Akademik Oparin.

The PG extracts were obtained by multistage extraction using ethanol, diethyl ether, and hexane [12]. Pande's method [13] was used for the quantitative determination of the total PGs. PGBs were determined by Andersen's method [14]. The PG-like activity was determined by the biotrials method on rat uterus smooth musculature [15]. The PhL composition of the PG extracts was determined by a method proposed by V. I. Svetashev and V. E. Vas'kovskii [16]. To analyze the qualitative composition of the PhLs we used the systems proposed by Rouser et al. [17]: in the first direction – chloroform–methanol–28% ammonia (65:25:5, by volume), and in the second direction – chloroform–acetone–methanol–acetic acid–water (30:40:10:10:5). The PLs were detected with the molybdate reagent [18], and before spraying the universal reagent was diluted 4-fold with distilled water. The choline-containing compounds were detected with the Dragendorff reagent [19]. Compounds with a free amino group were detected with a 0.1% solution of ninhydrin in acetone. For the quantitative analysis of the PhLs we used V. E. Vas'kovskii's method [18], involving the preliminary spraying of plates for nonspecific detection with a solution of sulfuric acid in methanol followed by heating the plates on an electric hotplate. The amount of phosphorus was determined from a calibration curve plotted with the aid of a standard solution of monopotassium phosphate.

To isolate individual PhLs from the extract, plates with the same amounts of deposited extract were passed through Rouser's system and were sprayed with Vas'kovski's reagent, after which the individual PhLs were extracted. Then the residual matter on the plate was extracted with a mixture of chloroform and methanol, and the extract was filtered, evaporated, and subjected to biotesting.

LITERATURE CITED

1. Y. Mizushima, K. Hoshi, H. Aihara, and M. Kubahi, J. Pharm. Pharmacol., 35, No. 6, 397 (1983).
2. Y. Mizushima, Y. Shiokawa, M. Homma, S. Kashiwazaki, Y. Ichikawa, H. Hashimoto, and A. Sakuma, J. Rheumatol., 14, No. 1, 97.
3. D. V. McGivern and G. S. Basran, Eur. J. Pharmacol., 102, 183 (1984).
4. Y. Mizushima, A. Yanagawa, and K. Hoshi, J. Pharm. Pharmacol., 35, No. 10, 666 (1983).
5. Y. Mizushima, Y. Shosi, T. Kato, M. Fukushima, and S. Kurozumi, J. Pharm. Pharmacol., 38, No. 2, 132 (1986).
6. L. M. Bragintseva, T. K. Ustynyuk, N. R. Ievleva, V. A. Kovalenko, N. B. Grishina, and H. S. Orekhova, in: Abstracts of an All-Union Symposium on Synthesis and Research of Prostaglandins [in Russian], Tallinn (1986), p. 164.
7. A. V. Nikolaev, T. K. Ustynyuk, L. M. Bragintseva, L. A. Mamedov, and M. V. Belova, in: Abstracts of an All-Union Symposium on Synthesis and Research of Prostaglandins [in Russian], Tallinn (1986), p. 167.
8. M. R. Belova, T. K. Ustynyuk, L. M. Bragintseva, and G. A. Osipov, Farmatsiya, No. 5, 25 (1984).
9. A. D. Sorokina, G. A. Deborin, Yu. É. Lille, and N. É. Samel', in: Abstracts of an All-Union Symposium on Synthesis and Research of Prostaglandins [in Russian], Tallinn (1986), p. 135.
10. N. P. Dobrii, S. V. Isai, and T. Ya. Mishchenko, in: Abstracts of an All-Union Symposium on Synthesis and Research of Prostaglandins [in Russian], Riga (1982), p. 76.
11. S. V. Isay, O. D. Korotchenko [Korochenko], T. Ya. Mishchenko, and L. G. Zemeleva, in: Symposium on Synthesis and Research of Prostiglandins. Abstracts, Tallinn (1986), p. 69.
12. O. D. Korotchenko [Korochenko], T. Ya. Mishchenko, and S. V. Isay, Comp. Biochem. Physiol., 74, No. 1, 85 (1983).
13. S. V. Pande, R. Parvin Khan, and T. A. Venkitasubramanian, Anal. Biochem., 6, 415 (1963).
14. N. H. Andersen, J. Lipid Res., 10, 320 (1969).
15. J. R. Weeks, J. R. Schultz, and M. E. Brown, J. Appl. Physiol., 25, No. 6, 788 (1968).
16. V. I. Svetashev and V. E. Vaskovsky [Vas'kovskii], J. Chromatogr., 67, No. 1, 376 (1972).
17. G. Rouser, G. Kritchevsky, and A. Yamamoto, in: Lipid Chromatographic Analysis, Marcel Dekker, New York, Vol. 1 (1967), p. 99.
18. V. E. Vaskovsky [Vas'kovskii], E. Ya. Kostetsky [Kostetskii], and I. M. Vasendin, J. Chromatogr., 114, No. 1, 129 (1975).
19. M. Kates, Techniques of Lipidology, American Elsevier, New York (1972) [Russian translation, Mir, Moscow (1975), p. 140].