SUMMARY

1. The conditions for the acid hydrolysis of industrial wastes from a ginseng tissue culture have been studied. The temperatures, the liquor ratios, and the concentrations of acids permitting the maximum yield of MSCs at the optimum degree of hydrolysis to be obtained have been selected.

2. The qualitative and quantitative compositions of the MSCs of hydrolysates have been studied with the aid of gas-liquid chromatography; they can find use as nutrient media or additives for such media.

LITERATURE CITED

1. E. P. Kukhta, I. V. Aleksandrova, V. N. Paukov, and M. A. Lyal'chenko, Khim. Prir. Soedin. 342, (1988) [in this issue].
2. P. Andrews, L. Hough, and D. B. Powell, Chem. Ind. (London), 658 (1956).
3. I. F. Saeman, I. L. Buhe, and E. E. Harris, Ind. Eng. Chem. Anal. Ed., 17, 35 (1945).
4. N. K. Kochetkov and A. F. Bochkov, Carbohydrate Chemistry [in Russian], Khimiya, Moscow (1976).
5. R. M. McCready, in: Methods in Carbohydrate Chemistry, Academic Press, New York, Vol. 2, (ed. R. L. Whistler and M. L. Wolfrom) (1963), p. 27.
6. E. P. Kukhta, V. Ya. Chirva, M. P. Norovskii, M. A. Tolmacheva, and I. V. Eroshenko, Khim. Prir. Soedin., 275 (1982).
7. V. V. Elkin, O. B. Lyubavina. and V. N. Paukov, Khim Drev., No. 6, 60 (1979).
8. E. P. Kuhta, R. G. Butenko, S. P. Afanaseyv, and I. V. Alexandrova, in: Abstracts at the IInd Bratislava Symposium on Saccharides. Czechoslovakia (1984), p. 35.

DETECTION OF PROSTAGLANDIN $F_{2\alpha}$ IN TISSUES OF *Populus Balsamifera* AND *Larix Sibirica*

É. D. Levin, V. E. Cherepanova, T. O. Sedlova, I. A. Zimovtseva, and S. V. Zinchenko

UDC 581.192.2:674.032.475.3

The identification of prostaglandin $F_{2\alpha}$ isolated from the buds and cambial zone of *Populus balsamifera* and the buds of *Larix sibirica* Ledeb, is described. Identification was performed with the aid of TLC, GLC, HPLC, chromatomass fragmentography, and PMR spectroscopy. This is the first time that the presence of prostaglandin $F_{2\alpha}$ in the living tissues of higher plants has been demonstrated.

In papers published previously, the identification of prostaglandins (PGs) $F_{1\alpha}$, E_1, and E_2 isolated from the living tissues of *Populus balsamifera* and *Larix sibirica* Ledeb. was described [1, 2]. In the present paper we describe the identification of $PGF_{2\alpha}$, also isolated from these tissues. The living tissues are the buds and cambial zone of the trumk of *Populus balsamifera* and the buds and cambial zone of the shoots of *Larix sibirica*. The PGs were separated and identified by TLC, GLC, high-performance liquid chromatography (HPLC), chromatomass fragmentography, and PMR spectroscopy.

On TLC using system 1, the R_f value of the PG isolated and of standard $PGF_{2\alpha}$ were identical at 0.18, and in system 2 they both had R_f 0.50.

Two different instruments and different phases were used for the GLC analysis of the PGs. The PGs were converted into volatile derivatives: the carboxy groups were methylated with diazomethane, and the alcoholic hydroxy groups were converted into trimethylsilyl ether groups. The corresponding derivatives of standard $PGF_{2\alpha}$ were used as markers. The $PGF_{2\alpha}$ was estimated quantitatively with the aid of an internal standard, as which the marker was used. A conformation of the correctness of the identification was obtained by means of a mixed melting point (the same marker was used). On chromatograms, the reten-

Siberian Technological Institute, Krasnoyarnsk. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 351-353, May-June, 1988. Original article submitted July 9, 1987; revision submitted December 24, 1987.

tion time of the sample under investigation coincided with that of the standard.

For HPLC, the extract and the standard $PGF_{2\alpha}$ were treated with bromophenacyl bromide. Analysis showed that the retention time of the $PGF_{2\alpha}$ isolated from the buds of Populus balsamifera was 18.61 min, that from the cambial zone of the trunk of Populus balsamifers 17.90 min, and that from the buds of Larix sibirica 17.90 min, while that of standard $PGF_{2\alpha}$ was 18.20 min. The variations in the retention times of the standard $PGF_{2\alpha}$ with different injections of the sample amounted to 0.8 min.

When chromato-mass fragmentography was performed it was found that the molecular ion of the PG under investigation had m/z 552, which is characteristic for $PGF_{2\alpha}$ [4].

EXPERIMENTAL

The prostaglandins were isolated by known methods [3]. The preliminary purification of the crude extract of PGs by the CC method and the isolation of fractions containing predominantly type F PGs has been described in [1]. Their final purification was also described in that paper. After final purification, samples with a predominant content of $PGF_{2\alpha}$ were obtained.

PMR spectra were recorded on Bruker WP-200 sy instrument with a working frequency for ^1H of 200.13 MHz. The samples consisted of solutions of 10 mg of prostaglandin in $CDCl_3$. The parameters of the PMR spectrum of prostaglandin $F_{2\alpha}$ were as follows: 0.86 (3H, m, H-20); 1.26–1.66 (12H, m, H-3, H-4, H-7, H-17, H-18, H-19); 2.13-2.30 (8H, m, H-2, H-8, H-10, H-12, H-16); 3.90 (1H, m, H-10a); 4.05-4.13 (2H, m, H-15, H-10b, H-10b), 5.38-5.49 (4H, m, H-5, H-6, H-13 H-14); 4.40 (3H, br. OH). The chemical shifts are given relative to TMS. The spectrum of standard $PGF_{2\alpha}$ recorded under the same conditions was identical with that described.

The chromato-mass fragmentography* of the $PGF_{2\alpha}$ extract was performed on a LKB-2109 instrument (Sweden). A 0.35 mm × 25 m capillary column with the deposited stationary phase SE-30 was used, with helium as the carrier gas. The pressure in the system was 10.8×10^4 Pa. The temperature was programmed to rise from 20 to 300°C at the rate of 10 K/min. The temperature of the separator was 300°C and that of the ion source 250°C, and the energy of the ionizing electrons was 22.5 eV. The instrument was fitted with a system for the dry introduction of the sample without splitting the flow. The preparation was methylated with a freshly prepared solution of diazomethane and was silylated by treatment with a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide and piperidine (1:1) at 60°C for 1 h [5].

HPLC† was performed with the use of Beckman Instruments chromatographic equipment on a column of Ultrasphere-ODS with a diameter of 5 μ. The PGs were treated with bromophenacyl bromide (5 mg/ml) in the presence of triethylamine (28 mg/ml) in anhydrous acetonitrile at room temperature for 30 min. The calculated amounts of esterifying components were 190 ml of bromophenacyl bromide and 10 ml of triethylamine for the treatment of 6-9 mg of PGs. The eluent was methanol–water (80:20) at a rate of passage of 1 ml/min, and the substances were detected spectrophotometrically at 254 nm.

The GLC analysis of the PGs was performed with the aid of Chrom-5 (Czechoslovakia) and Biokhrom-1 chromatographs. In the first case, separation was effected in a 3 mm x 3.5 m silanized glass column filled with Chromaton N-AW-DMCS (100-125 mm) impregnated with 1% of SE-30. Flame-ionization detector. Programmed rise in the temperature of the column from 140 to 300°C at the rate of 7.5 K/min: carrier gas helium at a rate of passage of 35 ml/min. In the second case, separation was performed in a 0.25 mm x 54 m glass capillary column with the deposited stationary liquid phase OV-101. Flame-ionization detector. Programmed heating of the column from 80 to 170°C at the rate of 4 K/min, the carrier gas being helium passed at the rate of 37 ml/min.

*Chromato-mass fragmentography was carried out in the laboratory of prostaglandin and steroid chemistry of the Institute of Experimental Endocrinology and Hormone Chemistry of the USSR Academy of Medical Sciences under the direction of K. K. Pivnitskii.

†HPLC was performed in the laboratory of the Belozerskii Moscow state University under the direction of Prof S. D. Varfolomeev. Standard $PGF_{2\alpha}$ was obtained from the experimental biopreparations factory of the ESSR Academy of Sciences.

The TLC of the extract was carried out on Silufol UV-254 plates using the solvent systems chloroform–methanol–acetic acid–water (90:8:1:0.8) (system 1) and on glass plates coated with silica gel L (5-40 μm) containing 5% of gypsum in the slovent system benzene–dioxane–acetic acid (20:20:1) (system 2). A 10% ethanolic solution of molybdophosphoric acid and a 2% ethanolic solution of vanillin were used as revealing agents.

SUMMARY

The presence of prostaglandin $PGF_{2\alpha}$ in the living tissues of higher plants has been shown for the first time by TLC, GLC, HPLC, chromato-mass fragmentography, and PMR spectroscopy.

LITERATURE CITED

1. E. D. Levin, Sh. T. Alaudinov, and V. E. Cherepanova, Khim. Drev., No. 5, 94 (1983).
2. E. D. Levin, Sh. T. Alaudinov, and V. E. Cherepanova, Khim. Prir. Soedin., 567 (1984).
3. B. Samuelsson and S. Bergstrom, J. Biol. Chem., 238, 3229 (1963).
4. V. I. Melnikova and K. K. Peivnitsky, Biomed. Mass Spectrom., 10, 399 (1983).
5. S. Middleditch and D. M. Desiderio, Anal Biochem., 55, No. 2, 509 (1973).

PHOSPHOLIPID AND FATTY ACID COMPOSIITON OF ECHINODERMATA.

II. THE CLASS ASTEROIDEA

V. M. Dembitskii

UDC 593.43:591.1+547.953:661.732

The phospholipid composition of starfish belonging to the class Asteroidea has been investigated. It has been shown that starfish may be promising objects for the study of the metabolism of alkoxylipids and a source for the preparative isolation of these compounds.

The present communication is a continuation of investigations of the phospholipid (PL) and fatty acid (FA) composition of ophiuroids [1].

The first analyses of the unspaonifiable lipids of starfish were carried out by Karnovskii et al. [2, 3] more than 30 years ago. In the screening analysis of the phospholipids of starfish we have detected a large amount of plasmalogenic forms in the phosphatidylethanolamine (PE) [4, 5]. The mean content of PE plasmalogens amounted to more than 80% of the total forms. Further work on the study of the plasmalogens of starfish has been carried out in Kostetskii's laboratory [6, 7]. The phospholipid compositions of starfish in the winter and summer periods were studied and it was observed that there were no appreciable differences in the composition of the PLs.

In the papers mentioned above, the results are given of investigations of the plasmalogenic and of the combined acyl and alkyl forms of PE and of phosphatidylcholine (PC). We have recently published an improved method of determining the plasmalogenic, acyl, and alkyl forms in the main classes of PLs [8], by using which we have studied the PLs of six species of the starfish of northern seas.

The analysis showed that the plasmalogenic form in the PE ranged from 84 to 69%, the alkyl form from 29 to 12%, and the acyl form from 10 to 0% (Table 1). In the PC, the amount of plasmalogenic form was lower than in the PE, and the amount of alkyl form higher: the bulk of the PC consisted of the 1,2-diacyl analogs. Karnovsky and Brumm [2] have previously shown that the amount of alkyl ethers in the total lipid extract rises during the life of the starfish. The amount of phosphatidylserine in lipid extracts of the starfish ranges from 8.4 to 15.1% and corresponds to the level found in Far Eastern starfish [6].

Institute of Ecology of the Volga Basin, USSR Academy of Sciences, Togliatti.

Translated from Khimiya Prirodnykh Soedinenii, No.3, pp. 353-357. May-June, 1988. Original article submitted July 28, 1987; revision submitted November 16, 1987.