the most informative characteristics of the separation of the class of epimers are differences in the CSs of the C_{13} and C_{15} carbon atoms due to stereochemical interactions of the groups of atoms at the C_{12} and C_{15} chiral centers in the 15β epimers. Characteristic features of the cis isomers of the α- and ω-chains relative to the cyclopentane ring in the homologues of 11-deoxy-PGE_1 are upfield shifts of the signals of the C_8 and C_{12} atoms of the ring and of the C_7 and C_{13} atoms of the α- and ω-chains.

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PREPARATION OF TRITIUM-LABELED PROSTAGLANDIN E_3

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Conditions for the biosynthesis of PGE_3 from eicosa-5,8,11,14,17-pentaenoic acid using an enzyme system isolated from ram seminal vesicles have been worked out. Tritium-labeled PGE_3 has been obtained in good yield with a molar radioactivity sufficient for performing many biological investigations.

The possibility of obtaining tritium-labeled prostaglandin E_2 has been shown previously [1]. Enzyme systems converting eicosa-5,8,11,14,17-pentaenoic acid into various prostaglandins of the 3 series are known [2, 3]. The aim of the present work was to obtain tritium-labeled prostaglandin E_3 (PGE_3) from [^{3}H]eicosa-5,8,11,14,17-pentaenoic acid using an enzyme system isolated from ram seminal vesicles. To optimize the conditions for the biosynthesis of PGE_3 we performed a series of experiments at various concentrations of enzyme, polyunsaturated acid, epinephrine, hemin and hydroquinone, and we also investigated the kinetics of the conversion of the polyunsaturated acid into PGE_3. In each case the yield of prostaglandin was determined after alkaline isomerization [1] and measurement of the absorption at λ 278 nm. With increases in the concentrations of polyenic acid, enzyme, and hemin and with a lengthening of the time of incubation the yield of PGE_3 rose to a definite value and then did not change further, while the curves reflecting the dependence of the yield of product on the concentrations of epinephrine and of hydroquinone had maxima. In the investigation of the stability of the enzyme with time it was found that the enzyme did not lose its initial activity for a period of two months.

As a result of the experiments performed, the following conditions were selected for biosynthesis using the enzyme system in the form of an unpurified supernatant (per 2 ml of supernatant with a concentration of 22 mg of protein/ml): 0.5 mg (1.65 μmole) of eicosa-5,8,11,14,17-pentaenoic acid, 0.56 mg (3.06 μmole) of epinephrine or 0.32 mg (2.9 μmole) of hydroquinone, 1 μg (1.5 nmole) of hemin, and 1.2 mg (3.9 μmole) of reduced glutathione, the reaction being performed at 32°C for 8 min. If 50-100 mg of eicosa-5,8,11,14,17-penatenoic acid was used in the reaction with observance of the same ratios of the other ingredients

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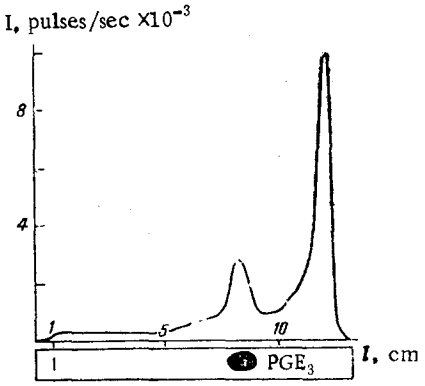
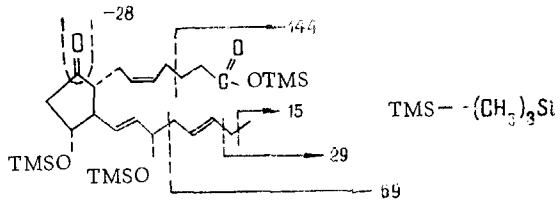


Fig. 1. Distribution of radioactivity along a Silufol plate after the performance of the biosynthesis of [^{3}H]PGE_3 from [^{3}H]eicosa-5,8,11,14,17-pentaenoic acid.

the yield of PGE_3 fell to 10-15%. This is apparently due to an inadequate efficiency of the aeration of the incubation mixture.

The PGE_3 was characterized by thin-layer chromatography (TLC) and by gas–liquid chromatography (GLC). In addition, mass spectra of this compound were obtained. In thin-layer chromatography and GLC the mobility of the product of biosynthesis coincided with that of PGE_2, but in TLC on argentized silica gel it differed. A study of the mass spectrum permitted the compound obtained to be identified as PGE_3. PGE_3 differs from PGE_2 only by the presence of a C_{17}=C_{18} bond in its molecule, and it is known [4] that the fragmentation of the molecular ions of olefinic hydrocarbons takes place with high probability at the ß bond on relation to the multiple bond as the result of a McLafferty rearrangement. The formation of $(M - 15)^+$ ions is also typical, particularly in those cases where a methyl group is present in the β position to the multiple bond. In fact, the most probable primary act of the interaction of the molecule of the prostaglandin obtained with an ionizing electron proved to be the cleavage of the C_{15}–C_{16} and C_{19}–C_{20} bonds with the formation of $(M - 15)^+$ and $(M - 69)^+$ ions:



[^{3}H]Eicosa-5,8,11,14,17-pentaenoic acid labeled with tritium as described by Mikhailov et al. [5] was incubated under the conditions given above, which enabled [^{3}H]PGE_3 with a molecular radioactivity of 59.2 GBq/mmole to be obtained in a yield of 10-15% (see Fig. 1).

EXPERIMENTAL

Methyl eicosa-5,8,11,14,17-pentaenoate was kindly provided by B. E. Vas'kovskii and N. A. Romashina (Institute of Marine Biology of the Far-Eastern Scientific Center of the Academy of Sciences of the USSR) and PGE_2, reduced glutathione, epinephrine, hemin, and hydroquinone were commercial preparations. Solvents were purified by standard methods. [^{3}H]Eicosa-5,8,11,14,17-pentaenoic acid was obtained by the method of Mikhailov et al. [5] with a molar radioactivity of 0.1 TBq/mmole.

Radioactivity was measured on a scintillation counter with an efficiency for the recording of tritium of ~30% in a dioxane scintillator [6]. GLC was performed on a Chrome-5 chromatograph (Czechoslovakia) fitted with a 2000 × 3 mm glass column containing 5% of SE-30 on Chromaton N-AW, 100-125 μm, with nitrogen as the carrier gas at the rate of 25 ml/min; 240°C. UV spectra were recorded on a Hitachi-220 spectrometer.

Mass spectra were recorded both on a Varian CH-5 instrument (GFR) using direct introduction of the sample into the ion source and also on an LKB-9000A chromato-mass spectrometer (Sweden) with a two-stage jet separator. The rate of flow of helium was 30 ml/min.

Column containing 1.5% of OV-17 on Gas Chrom Q, 100-120 mesh, 230°C, injector temperature 250°C. The energy of electron impact was 70 eV.

Protein was determined by Lowry's method [7]. Alkaline isomerization was performed as described in [1]. Analytical TLC of the prostaglandins obtained was carried out in the chloroform–methanol–acetic acid (90:9:1) system on Silufol plates (Czechoslovakia), the R_f values of PGE_2 and PGE_3 being 0.5-0.6, and on Silufol plates impregnated with silver nitrate [8] in the benzene–dioxane–acetic acid (10:5:2) system, when the R_f value of PGE_2 was 0.40-0.48 and that of PGE_3 0.10-0.15. The prostaglandins were subjected to preparative purification as described by Chevchenko et al. [1] on Merck silica gel plates (GFR), the R_f value of PGE_3 being 0.6 in the chloroform–methanol–acetic acid (90:9:1) system.

The samples were prepared for GLC and mass spectroscopy in the following way: 200 μg of PGE_3 was dissolved in 10 μl of absolute pyridine, and 30 μl of N,O-bis(trimethylsilyl)trifluoroacetamide was added, after which the mixture was left at 40°C for 1 h.

Isolation of the Enzyme System. Prostaglandin synthetase was isolated from ram seminal vesicles, for which purpose ~20 g of tissue frozen in liquid nitrogen was homogenized in a tissue mill for 5 min with 60 ml of 20 mM potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 5000 rpm, and the supernatant obtained was stored at −70°C. The mean protein content in this fraction was ~22 mg/ml. The change in the biological activity of the enzyme was studied over 6 months.

Conditions for the Biosynthesis of PGE_3. The unpurified supernatant was tested with the use of arachidonic acid as described in [1] and was used if the yield of PGE_2 was not less than 45-55%. In the course of determining the optimum conditions of biosynthesis, the concentration of eicosa-5,8,11,14,17-pentaenoic acid was varied from 0.06 to 0.66 mM, that of epinephrine from 0.3 to 1.8 mM, that of hydroquinone from 0.18 to 1.8 mM, and that of hemin from 0.03 to 0.9 μM, and an amount of enzyme containing from 11 to 66 μg of protein was added; the reaction time was varied from 1 to 15 min.

Preparation of PGE_3 and of [^{3}H]PGE_3. The sodium form of eicosa-5,8,11,14,17-pentaenoic acid was obtained by treating 0.5 mg of the acid with 1.5 ml of 30 mM Na^+-EDTA buffer (pH 8.0) at room tempeature for 30 min. Then this solution and 1 μg of hemin were added to a mixture of 1.2 mg of reduced glutathione, 0.56 mg of epinephrine. and 2 ml of the enzyme system in 1.5 ml of the same buffer that had previously been incubated for 10 min. The reaction mixture was stirred at 32°C for 8 min. The reaction was stopped by the addition of a 2 M solution of citric acid (to pH 3), and the PGE_3 was extracted with chloroform (3 × 10 ml). The combined extracts were dried with sodium sulfate, filtered, and evaporated, and the residue was dissolved in 1.5 ml of ethanol. The yield of PGE_3 ranged from 15 to 20% depending on the activity of the enzyme under the conditions selected. The silyl derivatives of PGE_3 had the following characteristic peaks in their mass spectrum: m/z $(M - CH_3)^+$ 551 (intensity 7%); $(M - CO)^+$ 538 (4); $(M - C_2H_5)^+$ 537 (4); $(M - CH_3 - CO)_+$ 523 (9); $(M - C_2H_5 - CO)^+$ 509 (5); $(M - C_5H_9)^+$ 497 (52); $(M - C_5H_9 - H_2CO)^+$ 467 (31); $(M - CH_3$ Y− TMSOH$)^+$ 461 (17); $(M - C_5H_9 - TMSOH)^+$ 407 (58); $(M - C_5H_9 - C_2H_3CO_2TMS)^+$ 353 (85); $(M - C_5H_9 - 2TMSOH)^+$ 317 (52).

In working with [^{3}H]eicosa-5,8,11,14,17-pentaenoic acid we used a procedure developed for the biosynthesis of 0.5 mg of an unlabeled polyunsaturated acid. The yield and molar radioactivity are given in the text. The radiochemical purity of the preparation after chromatographic purification was not less than 95-97%.

SUMMARY

Conditions have been worked out for the biosynthesis of prostaglandin E_3 from eicosa-5,8,11,14,17-pentaenoic acid using an enzyme system isolated from ram seminal vesicles. Tritium-labeled PGE_3 has been obtained with a good yield and with a molar radioactivity sufficent for the performance of many biological investigations.

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LIPIDS OF THE SEEDS OF *Paliurus spina-christi*

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The composition of the unsaturated and oxidized fatty acids and steroids of the lipids of the seeds of *Paliurus spina-christi* has been determined. Among the unsaturated acids the 20:1 (11) was detected, while the 18:1 and 18:2 acids predominate. The oxidized acids are represented by the sum of the 9,10(12,13)-epoxy-12(9)-18:1, the 9,10-epoxy-18:0, the oxo-18:1, the oxo-18:2, the 12-hydroxy-9-18:1, and the 9-hydroxy-10,11-18:2 acids. The main component of the sterols is β-sitosterol. It has been found that the composition of the acylglycerols of the lipids of the seeds of *P. spina-christi* gathered in different years is variable.

Paliurus spina-christi Mill. (Christ-thorn paliurus) (family Rhamnaceae) grows throughout the territory of the Georgian SSR and other regions of the Soviet Union [1]. The fruit of this plant has long been used in folk medicine for the most diverse diseases. Recently, it has been found in the Institute of Pharmacochemistry of the Academy of Sciences of the Georgian SSR that the lipids of the seeds of *P. spina-christi* exhibit an interesting biological activity in experiments on animals.

In the literature there are only general references to the lipids of the seeds of *P. spina-christi* [3], and we therefore set ourselves the aim of making a detailed analysis of the composition and structure of the lipid components of the seeds of this plant growing in Georgia.

The yield of total neutral lipids from the seeds amounted to 20%. A number of physicochemical indices of the total lipids and of the fatty acids isolated from them were determined by methods generally adopted:

Index	Total lipids	Acids
Density, d_4^{20}m g/cm^3	0.9227	0.9100
Refractive index, n_D^{20}	1.4778	1.4662
Iodine No., % I_2	102.6	109.2
Acid No., mg KOH	4.5	–
Amount of unsaponifiables, %	0.2	–

The IR spectra of the lipids and of the methyl esters (MEs) of the fatty acids had absorption bands in the region of conjugated double bonds at 230-234 nm and of a carbonyl group at 265-280 nm. The IR spectrum showed absorption bands of an epoxy group at 850, 875, 920, and 1280 cm^{-1} and of a trans-olefinic bond at 970 cm^{-1}, a broadened band of an ester carbonyl at 1750 cm^{-1} with inflections at 1670, 1690, and 1705 cm^{-1}, and the absorption of hydroxy groups at 3480 cm^{-1}.

On TLC analysis (in system 1) of the total lipids, they were found to contain hydrocarbons (HCs), sterol esters (SEs), triacylglycerols (TAGs), epoxyacylglycerols (EAGs), free fatty

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