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PROSTAGLANDINS: IDENTIFICATION AND CONTENT IN HIGHER PLANTS

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Key Word Index—*Populus balzamifera*; Salicaceae *Larix sibirica*; Pinaceae prostaglandins; identification; content; annual cycle.

Abstract—It has been established for the first time that prostaglandins of groups F, E, A and B are present in fresh tissue of higher plants (*Populus balzamifera* and *Larix sibirica*). The prostaglandins were isolated in pure form and the changes in the tissue levels determined over the course of one year.

INTRODUCTION

For a long time it was considered that prostaglandins (PG) were absent from plants. The first indication that they were constituents of plants was provided by the reports of the presence in plants of compounds similar to prostaglandins or having similar structural features to prostaglandins [1–4]. This was followed by reports of the identification of prostaglandins in onion (*Allium cepa*) and *Kalanchoe blossfeldiana* var Pollen [5, 6]. However, in none of these studies were prostaglandins isolated in pure form. Prior to most of these reports on the presence of prostaglandins in higher plants, R. P. Gregson and his colleagues had isolated prostaglandins E_2 and $F_{2\alpha}$ from the red algae *Gracilaria lichnenoioles* and had identified the latter by physico-chemical methods [7].

The presence of prostaglandins in higher plants was established for the first time by Levin and Alautdinov [8, 9], who with their colleagues then went on to isolate prostaglandins of groups E, F, A and B in pure form from the cambial zone and buds of *Larix sibirica* and *Populus balzamifera* [10–12].

RESULTS AND DISCUSSION

The buds and cambial zone of *P. balzamifera* and *L. sibirica* were sampled in the last week of every month for two years. Four trees, always from the same area, were taken for analysis since a previous study [13] had shown that for a statistically meaningful result two trees are needed for determination of prostaglandins F, E, A and four for PGB. On bioassay of extracts from cambial zone, buds, bark, heartwood and sapwood of young and mature samples, by an *in vitro* procedure using rat uterine horn only the extracts of cambial zone and buds showed prostaglandin activity.

TLC of the prostaglandins isolated was performed by a procedure similar to that used for the isolation of these substance from animals tissues [14]. The R_f values of the spots obtained coincided with or were very close to those of prostaglandins present in human semen (Table 1).

The UV spectrum of prostaglandin E was recorded before and after alkali treating. A characteristic bathochromic shift of the maximum absorption was obtained (λ_{max}^{EtOH} 278 nm). Prostaglandins of group B and A had maximum absorption in ethanol at 278 and 217 nm respectively.

The IR spectra of the prostaglandins isolated from the cambial zone and buds contained a number of characteristic bands. PGE_1: $\nu_{max}^{CHCl_3}$ cm^{-1}: 1728 (>C=O), 964 (*trans* double bond); $PGF_{1\alpha}$: 1697 (>C=O), 967 (*trans* double bond); PGB_1: 1726 (>C=O), 1635 (C=C), 970 (*trans* double bond); PGA_1: 1720 (>C=O), 1586 (C=C), 962 (*trans* double bond).

GC of prostaglandins was carried out on two phases. Authentic samples of prostaglandins $F_{1\alpha}$, $F_{2\alpha}$, E_1 and E_2 were used for identification by the mark method. Prosta-

Table 1. R_f values of prostaglandins

	P. balzamifera				*L. sibirica*					
	Cambial zone		Buds		Cambial zone		Buds		Standards	
Type	I	II	I	II	I	II	I	II	I	II
$PGF_{2\alpha}$	0.18	0.33	0.19	0.31	0.18	0.33	0.18	0.32	0.18	0.31
PGE_2	0.24	0.47	0.23	0.46	0.26	0.46	0.23	0.48	0.23	0.48
PGD_2	0.27	0.61	—	—	0.29	0.62	—	—	0.27	0.63
PGB_2	0.59	0.75	0.59	0.76	0.59	0.76	0.59	0.76	0.60	0.76
PGA_2	0.70	0.78	0.72	0.78	0.72	0.78	0.70	0.80	0.70	0.78

glandins of type F, E, A, B were identified. They were quantitated by the internal standard method and normalization of peak areas.

The GC-MS (probe, 70 eV) of the methyl ether of the trimethylsilyl derivative of $PGF_{1\alpha}$ showed that the isolated substance was really prostaglandin $F_{1\alpha}$.

Prostaglandin E_1 and E_2 were identified by GC-MS of their tris-trimethylsilyl and tetrakis-trimethylsilyl derivatives. The fragmentation pattern and the relative intensity of the various ions were similar to those of PGE_1 and PGE_2 isolated from animal sources [15].

GC-SIMS of derivatized prostaglandin concentrates provided further evidence for the presence of prostaglandins E_1 (*m/z* 552, E_1–TMS), E_2 (550, E_2–TMS), $F_{1\alpha}$ (554, $F_{1\alpha}$–TMS), $F_{2\alpha}$ (552, $F_{2\alpha}$-TMS) in the concentrate, as did HPLC of the bromophenacyl derivatives of the prostaglandins (Table 2).

The melting points of the prostaglandins $F_{1\alpha}$ (102°), E_1 (115°) and E_2 (70°) were the same as those of authentic prostaglandins isolated from animal sources. On recrystallization the melting points were unchanged. Finally, the 200 MHz ^{1}H NMR spectrum of $PGF_{2\alpha}$ was recorded and found to be identical to that of authentic $PGF_{2\alpha}$ [18].

The above results prove that fresh tissues of higher plants contain prostaglandins $F_{1\alpha}$, $F_{2\alpha}$, E_1 and E_2. Identification of prostaglandins A and B is unnecessary, since it is known that on dehydrogenation prostaglandins E are converted into PGA and PGB. It was shown earlier by us, that by changing the pH of the medium, 74% of prostaglandin E is converted into prostaglandin A [12].

In addition to identifying the prostaglandins, it was important to determine how the tissue levels of these compounds change in the course of the annual cycle. This was followed by GC (Table 3) over a period of two years. Prostaglandins were absent from leaves and needles at all stages of their development.

As seen from Table 3, the prostaglandin content of buds is not stable and depends on the phenological condition of the tree. The changes have no obvious connection with the surrounding air temperature and depend on, apparently, ongoing biochemical processes in living tissue, which are connected with the different phases of the annual cycle.

It is impossible yet to give any exhaustible explanations for these changes. It may be supposed, however, that prostaglandins are bioregulatore of reproductive processes, and of those biochemical processes which occur in winter and allow the living tissue to survive for a long period at low temperatures. Combining Chailakxyan's theory [16], Groenewald's experiments [17] and the results of this study we are led to propose that prostaglandins are the unknown blossoming stimulator, which was called 'florigen' by Chailakxyan. Recently, Groenewald, treated plants with prostaglandins (of animal origin) and achieved active blossoming without growth intensification. However, our studies provide the first direct evidence that prostaglandins are present in higher plants and that they show the seasonal changes expected of 'florigen'.

EXPERIMENTAL

Bioassay. Plant tissue was extracted immediately with EtOH (10 × 24 hr at −15/−17°). The assay was conducted on isolated rat's uterine horn. Miograms were recorded by means of a plethysmograph PG-2. Just before introducing the preparation the Et_2O was evapd from the sample, and 1.5 ml of physiological soln (Ringer–Lock soln) poured immediately on to the dry residue. 0.1 ml and then 1 ml of soln were introduced into the apparatus.

Analytic methods. UV: EtOH then EtOH–2M NaOH; IR: Me ethers prepared by treating sample with CH_2N_2. Double

Table 2. Identification of prostaglandins by HPLC

R_f of prostaglandin derivatives (min)						
P. balzamifera buds	*P. balzamifera* cambial zone	*L. sibirica* buds	Standards D_2	E_2	$F_{2\alpha}$	B_2
	14.0		14.3			
16.8	16.2	16.2		16.6		
18.6	17.9	17.9			18.2	
25.01	24.9	24.9				25.0

Table 3. Prostaglandin content of *P. balzamifera* and *L. sibirica* buds

Month	Temperature of surrounding air (°)	Prostaglandin content (μg/g dry wt tissue) *P. balzamifera*	*L. sibirica*
Oct	0	20.3	
Nov	−9	20.5	9.1
Dec	−36	17.9	8.8
Jan	−20	9.4	1.5
Feb	−20	5.5	2.0
Mar	−10	9.5	5.3
Apr	+10	70.0	17.9

beam instrument used with solvent blank in reference beam. TLC: Silufol UV-254 with $CHCl_3$–MeOH–HOAc–H_2O (450:40:5:4) (system 1); silica gel L (5–40 μ)–gypsum (20:1) with C_6H_6–dioxane–HOAc (20:20:1) (system II). Detection with 10% soln of phosphomolybdic acid and 2% alcohol soln of vanillin; GC: 2 m × 2.5 mm glass silanized column, packed with either cramaton N-Super with 3% OV-225 or chromaton N-AW-DMCS with 1% SE-30. 3% OV-225: resonance-ionization detector, 220°, N_2 37 ml/min. 1% SE-30: FID detector, 180 to 280° at 5°/min, He 35 ml/min; GC-MS (LKB-9000 and LKB-2109): Me-TMS-$PGF_{1\alpha}$: 70 eV, accelerating voltage 3.6 kV, *m/z* (rel. int.); 586 $[M]^+$ (1.8), 571 $[M-Me]^+$ (8), 515 $[M-71-(C_{16}–C_{20})]^+$ (60), 496 $[M-(Me)_3\text{-}SiOH]^+$ (60), 425 $[M-71-(Me)_3SiOH]^+$ (100), 406 $[M-2(Me)SiOH]^+$ (10), 335 $[M-71-2(Me)_3SiOH]^+$ (14), 316 $[M-3\ (Me)_3SiOH]^+$ (76); Me–TMSi–PGE_1 and Me–TMSi–PGE_2: 2.5 m × 0.3 mm quartz capillary column, packed with SE-30, He $P = 10.8 \times 10^4$ Pa, 200 to 300° at 10°/min, ionizing voltage 22.5 eV. The sample was concd by evapn on the needle and introduced into the column. The samples were methylated with fresh CH_2N_2 soln and then silylated with a mixture of *N,O*-bis-(trimethylsilyl)-trifluorideacetamide–piperidin (1:1). Me-$(Me_3Si)_3$-PGE_1: GC-MS (probe) 22.5 eV, *m/z* (rel. int.) 659 $[M-Me]^+$ (6), 584 $[M]^+$ (5), 513 $[Me-(C_{16}–C_{20})]^+$ (4), 494 $[M-(Me_3Si)OH]^+$ (51), 423 $[M-(C_{16}-C_{20})-(Me_3Si)OH]^+$ (30), 411 $[M-(C_{15}–C_{20})]^+$ (100), 404 $[M-2(Me_3Si)OH]^+$ (6), 385 $[M-(C_{13}-C_{20})]^+$ (6), 351 $[M-(C_1-C_7)-(Me_3Si)OH]^+$ (10), 321 $[M-(C_{15}-C_{20})-(Me_3Si)OH]^+$ (8), 261 $[M-(C_1-C_7)-2(Me_3Si)OH]^+$ (4), 199 $[C_{13}–C_{20}]^+$ (15), 173 $[C_{15}–C_{20}]^+$ (27). $(Me_3Si)_4$-PGE_1: GC/MS (probe) 22.5 eV, *m/z* (rel. int.): 642 $[M]^+$ (2), 627 $[M-Me]^+$ (11), 571 $[M-(C_{16}–C_{20})]^+$ (3), 552 $[M-(Me_3Si)OH]^+$ (100), 481 $[M-(C_{16}–C_{20})-(Me_3Si)OH]^+$ (50), 469 $[M-(C_{15}–C_{20})]^+$ (79), 462 $[M-2(Me_3Si)OH]^+$ (75), 443 $[M-(C_{13}–C_{20})]^+$ (6), 441 $[M-(C_1–C_7)]^+$ (3), 379 $[M-(C_{15}–C_{20})-(Me_3Si)OH]^+$ (42), 353 $[M-(C_{13}–C_{20})-(Me_3Si)OH]^+$ (1), 351 $[M-(C_1–C_7)-(Me_3Si)OH]^+$ (17), 289 $[M-(C_1–C_5)-2(Me_3Si)OH]^+$ (2), 261 $[M-(C_1–C_7)-2(Me_3Si)OH]^+$ (6), 199 $[C_{13}–C_{20}]^+$ (18), 173 $[C_{15}–C_{20}]^+$ (56).

Me–$(Me_3Si)_3$-PGE_2: GC-MS (probe) 22.5 eV *m/z* (rel. int.): 582 $[M]^+$ (30), 567 $[M-Me]^+$ (8), 511 $[M-(C_{16}–C_{20})]^+$ (4), 492 $[M-(Me_3Si)OH]^+$ (59), 421 $[M-(C_{16}–C_{20})-(Me_3Si)OH]^+$ (30), 409 $[M-(C_{15}–C_{20})]^+$ (87), 402 $[M-2(Me_3Si)OH]^+$ (20), 383 $[M-(C_{13}–C_{20})]^+$ (16), 319 $[M-(C_{15}–C_{20})-(Me_3Si)OH]^+$ (20), 293 $[M-(C_{13}–C_{20})-(Me_3Si)OH]^+$ (10), 287 $[M-(C_1–C_5)-2(Me_3Si)\ OH]^+$ (6), 261 $[M-(C_1–C_7)-2(Me_3Si)OH]^+$ (10), 199 $[C_{13}–C_{20}]^+$ (11), 173 $[C_{15}–C_{20}]^+$ (100).

$(Me_3Si)_4$-PGE_2: GC-MS (probe) 22.5 eV, *m/z*, (rel. int.): 640 $[M]^+$ (10), 625 $[M-Me]^+$ (17), 569 $[M-(C_{16}–C_{20})]^+$ (5), 550 $[M-(Me_3Si)OH]^+$ (70), 479 $[M-(C_{16}–C_{20})-(Me_3Si)OH]^+$ (40), 467 $[M-(C_{15}–C_{20})]^+$ (39), 460 $[M-2(Me_3Si)OH]^+$ (100), 441 $[M-(C_{13}–C_{20})]^+$ (16), 441 $[M-(C_1–C_7)]^+$ (10), 377 $[M-(C_{15}–C_{20})-(Me_3Si)OH]^+$ (49), 351 $[M-(C_{13}–C_{20})-(Me_3Si)OH]^+$ (43), 351 $[M-(C_1–C_7)-(Me_3Si)OH]^+$ (13), 287 $[M-(C_1–C_5)-2(Me_3Si)OH]^+$ (22), 261 $[M-(C_1–C_7)-2(Me_3Si)OH]^+$ (16), 199 $[C_{13}–C_{20}]^+$ (24), 173 $[C_{15}–C_{20}]^+$ (50).

HPLC. Sample treated with bromphenacylbromide (5 mg/ml) in the presence of Et_3N (2.8 mg/ml) in dry MeCN at room temp. for 30 min. An aliquot of the reaction mixture was then analysed on a column of Ultraspere ODS 5 μ (25 cm × 4 mm) (Altex, U.S.A.) with MeOH–H_2O (4:1) at 1 ml/min and detection at 260 nm. ^1H NMR (200 MHz, TMS as int. standard): $PGF_{2\alpha}$: δ0.86 (3H, *m*, H-20), 1.26–1.66 (12H, *m*, H-3, H-4, H-7, H-17, H-18 and H-19), 2.12–2.30 (8H, *m*, H-2, H-8, H-10, H-12, and H-16), 3.90 (1H, *m*, H-10a), 4.05–4.13 (2H, *m*, H-15 and H-10b), 5.38–5.49 (4H, *m*, H-5, H-6, H-13 and H-14), 4.40 (3H, *br s*, OH).

Mps: uncorr.

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