Testosterone, Epitestosterone and Androstenedione in the Pollen of Scotch Pine *P. silvestris L.*

Some data exist about the finding of estrogens in pollen[1,2] and it was presumed that pollen contains androgens, but experiments to find androgenic activity in corn pollen were without success[3]. Containing studies on the pollen of some pine species[4], we tried to find and estimate testosterone, epitestosterone and androstenedione in the pollen of Scotch pine *P. silvestris* L.

Isolation and quantitative determination was carried out by 4 methods combined from the 3 different procedures for extraction and purification of extracts, and 2 procedures for separation of the steroids mentioned and their quantitative or semiquantitative determinations. Two procedures for extraction were of recent date, one for the determination of testosterone in plasma[5] and the second one for the determination of testosterone and androstenedione in human urine[6] extended for epitestosterone. The third procedure was that of BUTENANDT and JACOBI[7] for isolation of α-follicle hormone from the press cake of palm kernels. The paper chromatographic separation of ketosteroids and the quantitative determination of testosterone, epitestosterone and androstenedione were made by the method of TAJIĆ[6]. The thin layer chromatography for separation of ketosteroids was carried out on Silicagel HF 254 in 3 solvent systems and the aforesaid steroids were evaluated under UV-lamp 254.

In Table I are shown methods in performance, in Table II the results obtained. None of these steroids could be determined by method 1, whereas such low values as those obtained by method 2 could not be counted in considering the colorimetric determination. The highest values were obtained by using method 3. The means of the 3 determinations were for testosterone 0.8, epitestosterone 1.1 and androstenedione 5.9 μg/10 g pollen. When the hydrolysis with β-glucuronidase was omitted in method 3a and the suspension of pollen was hydrolyzed only with hydrochloric acid, the values were less than half of the former, probably indicating presence of steroid glucuronides in the material.

By the semiquantitative method 4 the values obtained agreed well with those of method 3. In the solvent systems used, the zones of testosterone and epitestosterone overlapped and the values shown in Table II are the sum of both of them. Moreover this method served as the qualitative proof, which at the same time gave an

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Table I. The flow sheet of methods used for the determinations of testosterone, epitestosterone and androstenedione

Method 1	Method 2	Method 3	Method 4
pollen	suspension of pollen	suspension of pollen	suspension of pollen
extraction with methanol	extraction with ether-ethanol	extraction with ether	extraction with ether
hydrolysis of dry residue with methanolic KOH	hydrolysis with β-glucuronidase	hydrolysis with β-glucuronidase	hydrolysis with β-glucuronidase
at pH 5.5 extraction with ether	extraction with ether-ethanol	extraction with ether	extraction with ether
	acid hydrolysis	acid hydrolysis	acid hydrolysis
	extraction with ether-ethanol	extraction with ether	extraction with ether
	dry residue of the combined extracts treated with 70% methanol at -15°C and *n*-hexane		dry residue of the combined extracts treated with 70% methanol at -15°C
separation ketonic from nonketonic steroids by means of Girard T	separation ketonic from nonketonic steroids by means of Girard T	separation ketonic from nonketonic steroids by means of Girard T	separation ketonic from nonketonic steroids by means of Girard T
paper chromatography of ketonic steroids	paper chromatography of ketonic steroids	paper chromatography of ketonic steroids	thin layer chromatography on Silicagel HF 254 nonacetylated steroids in chloroform/acetone 9:1 benzene/ether/acetic acid 70:30:1 acetylated steroids in benzene/ethyl acetate 8:2
elution of testosterone, epitestosterone and androstenedione	elution of testosterone, epitestosterone and androstenedione	elution of testosterone, epitestosterone and androstenedione	semiquantitative determination under lamp UV 254
spectrophotometric determination at 565, 600 and 635 nm	spectrophotometric determination at 565, 600 and 635 nm	spectrophotometric determination at 565, 600 and 635 nm	

Table II. Micrograms of testosterone, epitestosterone and androstenedione found in 10 g pollen of *P. silvestris* L.

Methods	Testosterone Individual values	Means	Epitestosterone Individual values	Means	Androstenedione Individual values	Means
1	Not detected		Not detected		Not detected	
2	Not detected		0.06		0.03	
3	0.7		0.7		6.6	
	0.6	0.8	1.5	1.1	4.4	5.9
	1.1		1.1		6.7	
3a[a]	0.3		0.3		0.6	
	0.3	0.3	0.3	0.4	0.2	0.8
	0.3		0.6		1.6	
4[b]	Testosterone + Epitestosterone $\frac{2-3}{3}$				Androstenedione $\frac{3-4}{5}$	

[a] The hydrolysis with β-glucuronidase was omitted. [b] The zones of testosterone and epitestosterone overlapped.

identification of the substances determined. In the 3 different solvent systems the Rf-values of free and acetylated reference steroids covered those of testosterone, epitestosterone and androstenedione isolated from pollen. On this base testosterone, epitestosterone and androstenedione are present in the pollen of Scotch pine in amounts from 0.8 to 5.9 μg/10 g pollen.

Zusammenfassung. Im Pollen von *Pinus silvestris* L. wurden die androgenen Steroide Testosteron, epi-Testosteron und 4-Androsten-3,7-dion nach vier verschiedenen Methoden bestimmt.

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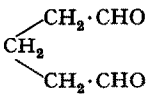
Interaction of Glutaraldehyde with Some Micro-organisms

Glutaraldehyde is a 5-carbon dialdehyde which is active against viruses, fungi and bacterial spores and



vegetative cells. Its antimicrobial activity is enhanced at alkaline pH[1-5], and it is used as a chemical sterilizer[6-8]. The mode of action of glutaraldehyde is unknown, but interactions between the dialdehyde and proteins have been described[9,10], and it has been suggested[5] that glutaraldehyde reacts with amino groups present in the bacterial cell.

Recently, it was found in this laboratory[11] that under alkaline, but not under acid, conditions, glutaraldehyde produced a red colouration with whole cells and penicillin-induced spheroplasts of, and cell envelopes isolated from, *Escherichia coli* NCTC 9001. Since this suggested possible binding sites for glutaraldehyde with components of bacterial cells, the effects of alkaline glutaraldehyde (glutaraldehyde 0.2% + sodium bicarbonate 0.3%) on various bacteria were investigated.

Interaction of glutaraldehyde with some micro-organisms

Organism	Strain of organism	Culture or preparation[a]	Colour after exposure to alkaline glutaraldehyde[b]
E. coli	NCTC 9001	NB 2, 18 h, 37 °C	Red
		Penicillin-spheroplasts	Red
		Cell walls	Red
		Cytoplasmic constituents	Yellow
Klebsiella aerogenes	NCTC 8172	NB 2, 18 h, 37 °C	Red
Serratia marcescens	NCTC 8706	NB 2, 18 h, 30 °C	Red
Proteus vulgaris	Laboratory	NB 2, 18 h, 37 °C	Red
Micrococcus lysodeikticus	NCTC 2605	NB, 18 h, 37 °C	None
Staphylococcus aureus	NCTC 6571	NB, 18 h, 37 °C	Slight yellow
Bacillus subtilis	NCTC 8236	NB, 18 h, 37 °C	Yellow
B. subtilis (fattened)	NCTC 8236	GB, 10 subcultures for 24 h, 37 °C	Light yellow
B. subtilis spores	NCTC 8236	Penicillin assay medium 7 days, 37 °C	Light orange/yellow (24 h only)
B. polymyxa	NCTC 10343	NBY, 18 h, 37 °C	Light orange/yellow
B. megaterium	NCTC 6005	NB, 18 h, 37 °C	Yellow
		Lysozyme-protoplasts	None detectable
		Cytoplasmic constituents	Yellow
Saccharomyces carlsbergensis	NCYC 74	NBG, 36 h, 37 °C	None
Penicillium chrysogenum	Laboratory	S, 7 days, 25 °C	None

[a] NB, nutrient broth (Oxoid); NB 2, nutrient broth no. 2 (Oxoid); GB, nutrient broth + 3% glycerol; NBY, nutrient broth + 1% yeast extract; NBG, nutrient broth + 1% glucose; S, saboraud liquid medium (oxoid). [b] Exposure for 2 h at 37 °C to 0.2% glutaraldehyde + 0.3% sodium bicarbonate.