These results, which are at odds with those of Schmidt et al.[1], are of importance for developing the correct strategy for disrupting mating behavior. As Schmidt et al. point out[1], there would be distinct advantages from the technical and commercial viewpoints if there was wide latitude in the range of blends required to disrupt mating. The fact that this is not so implies that a precise blend (specifically a 96:4 ratio of (E:Z)-11-TDAL) should be used to disrupt mating to ensure maximum effectiveness.

It must be remembered, however, that disruption of any of the many steps in the mating procedure of the spruce budworm could result in mating failure. Orientation of males to females is only one such step; other steps may be affected differently by other blends.

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7 Analyses of the data were done with the help of Dr J. Régnière of the Great Lakes Forest Research Centre, Sault Ste. Marie, Ont. whom I would also like to thank.

The secret of truffles: A steroidal pheromone?

R. Claus, H.O. Hoppen and H. Karg

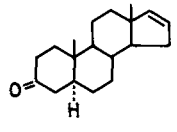
Lehrstuhl für Physiologie der Fortpflanzung und Laktation, Technische Universität München, D-8050 Freising-Weihenstephan (Federal Republic of Germany), and Institut für biochemische Endokrinologie, Medizinische Hochschule, D-2400 Lübeck (Federal Republic of Germany), 25 March 1981

Summary. The steroid 5α-androst-16-en-3α-ol has a pronounced musk-like scent. It is a major constituent of the pheromone of the boar. It occurs also in axillary sweat of men but is devoid of androgenic activity. The presence of this steroid has been demonstrated in truffles *(Tuber melanosporum)* both by radioimmunoassay and by gas chromatography-mass spectrometry in quantities of 40-60 ng/g fresh material. This offers an explanation for the ability of pigs to detect truffles growing as deep as 1 m under ground.

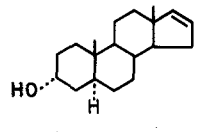
Some C_{19}-Δ16 steroids which have no androgenic activity exhibit a very peculiar smell[1-3] (fig.). The compounds are synthesized in the testes of the boar, are transfered to the salivary glands from which they are secreted during the premating behaviour. This scent, emanating from the saliva foam, is smelt by the sow and prompts her standing reflex. Thus Δ16-steroids, mainly 5α-androst-16-en-3α-ol are male sex pheromones in the pig[3,4]. Δ16-Steroids have also been detected in humans: 5α-androst-16-en-3-one and 5α-androst-16-en-3α-ol are synthesized by the testes and secreted with the axillary sweat in men[5-7]. 5α-Androst-16-en-3α-ol was found in the urine of women[8,9]. A possible pheromonal action of 5α-androst-16-en-3α-ol might be concluded from the studies of Kirk-Smith et al.[10]; judging the sexual attractiveness of photographs of normally dressed women the volunteers gave higher grades while smelling 5α-androst-16-en-3α-ol. The occurrence of Δ16-steroids is not confined to the animal kingdom. Celery and parsnip contain about 8 ng/g plant of 5α-androst-16-en-3-one[11]. Being aware of the ability of pigs to pinpoint the location of truffles (Tuber melanosporum), growing as deep as 1 m under ground, we extended our search for Δ16-steroids to this valuable fungus.

In a pilot study 1 g of canned truffles (from the Périgord, France) were homogenized in 3 ml of water and extracted with methylene chloride/ethylacetate (1/1). Aliquots of this crude extract were analyzed with our RIA system for 5α-androst-16-en-3-one, which cross-reacts with 5α-androst-16-en-3α-ol to 8%[12]. Further aliquots were subjected to TLC (silica gel, benzene/acetone = 85/15) in parallel to tritiated standards. After elution and subsequent radioimmunological measurement only 5α-androst-16-en-3α-ol but not 5α-androst-16-en-3-one was detected in a concentration (corrected for losses on TLC) which is in good agreement to the value of 25 ng/g measured in the crude extract (table).

For a 2nd study 4 g of fresh white truffles (Italian origin, purchased in a gourmet restaurant) and 4 g of black truffles from the Périgord (kindly provided by Mr Flourey) were



5α-androst-16-en-3-one (urine smell).



5α-androst-16-en-3α-ol (musk smell).

Odoriferous Δ16-steroids.

5α-androst-16-en-3α-ol in truffles (ng/g): comparative measurements by RIA and GC-MS

Sample	RIA*	GC-MS
Canned truffles	26.3	-
Fresh black truffles	59.0	42.1
Fresh white truffles	61.6	58.6

* Corrected for cross reactivity. All values corrected for procedural losses.

homogenized and extracted. Measurement by RIA was carried out after isolation of the 5α-androst-16-en-3α-ol fraction on silica-gel (see table). The remaining extract was also purified by TLC after addition of ^{3}H-5α-androst-16-en-3α-ol as an internal standard. TMS derivatives were prepared and analyzed by GC-mass spectrometry as described elsewhere[13] (LKB 2091 instrument 1.5 m GC glass column with 1% OV 3 on chromosorb WHP) modified by a temperature program of 150–200 °C (3 °C/min). The identity with authentic 5α-androst-16-en-3α-ol was confirmed by gas-chromatographic retention time and mass spectrometric behaviour[3]. These data establish the presence of 5α-androst-16-en-3α-ol in truffles. The organoleptic examination of thin layer chromatograms of truffleextracts indicates that the fungus contains at least 1 additional musk compound of similar polarity, the flavour of which has a more herbal quality. Attempts for identification are in progress. It is remarkable that the concentration of 5α-androst-16-en-3α-ol in truffles surpasses its level in boar plasma 2-fold[14]. The biological role of this boar sex pheromone might explain the efficient interest of pigs in search of this delicacy.

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Enhancing effect of heparin on aprotinin activity

T. Shikimi

Department of Pharmacology, Shimane Medical University, Enya, Izumo, Shimane 693 (Japan), 30 December 1980

Summary. Preincubation of heparin with aprotinin enhanced the inhibitory effect of aprotinin on the esterolytic activity of trypsin, but did not change its effect on the proteolytic activity of trypsin or on the esterolytic and proteolytic activities of chymotrypsin.

Aprotinin is used in the therapy of various pathological states such as acute pancreatitis, owing to its inhibitory effects on trypsin, chymotrypsin, plasmin and kallikrein[1]. It is widely distributed in ruminant tissues, especially bovine tissues[1], and in previous work[2,3] we confirmed that it is present in mast cells of bovine tissues. Heparin is a main component of mast cell granules[4] and the existence of a proteinase such as a trypsin-like or chymotrypsin-like enzyme in the cells has also been reported[5–8]. This paper describes the effect of heparin on the inhibition of trypsin and chymotrypsin by aprotinin.

Materials and methods. A commercial sample of Trasylol [10,000 kallikrein inhibitor units (KIU) per ml; Bayer AG, FRG], was used as aprotinin, and the following reagents were used: tosyl-L-arginine methyl ester hydrochloride (TAME), and benzoyl-L-tyrosine ethyl ester (BTEE) (Protein Research Foundation, Japan); casein according to Hammarsten (Wako Pure Chemicals, Japan); trypsin (EC 3.4.21.4) and α-chymotrypsin (EC 3.4.21.1) – both 3 times crystallized – (Miles Laboratories, USA); diphenylcarbamyl chloride (DPCC)-treated trypsin – type XI – and tosyl-L-lysine chloromethyl ketone (TLCK)-treated α-chymotrypsin – type VII – (Sigma USA); Novoheparin 130 IU/mg (Novo Industry, Denmark).

Aprotinin was preincubated with various amounts of heparin at 37 °C for 6, 24 and 48 h, and then the mixture was incubated with enzyme and its substrate. The inhibitory effects of aprotinin on the esterolytic activities of trypsin and α-chymotrypsin were determined by the method of Simlot and Feeney[9]. In this case, the preincubation mixture was incubated with trypsin (5 μg/ml) or α-chymotrypsin (15 μg/ml) at 37 °C for 5 min and then the substrate (TAME and BTEE, respectively) was added. For determination of the inhibitory effects of aprotinin on the caseinolytic activities of trypsin and α-chymotrypsin, the preincubation mixture of heparin and aprotinin was incubated with DPCC-treated trypsin (5 μg/ml) or TLCK-treated α-chymotrypsin (5 μg/ml) and casein (0.3%, w/w), prepared by the method of Kunitz[10], at 37 °C for 10 min. The protein was precipitated with trichloroacetic acid (final concentration 3%, w/w), and the mixture was centrifuged. Aliquots of the clear supernatant (2 ml) were mixed with 2.5 ml of 1 N sodium hydroxide, 1 ml of 20% (w/w) sodium carbonate and 0.5 ml of phenol reagent. The mixture was allowed to stand at room temperature for 10 min, and then the amount of amino groups liberated from casein was determined by measuring the absorption at 650 nm.

Results and discussion. To exclude the possible effect of mutual contamination of the commercial trypsin and α-chymotrypsin, the synthetic substrates TAME and BTEE were used for determination of the esterolytic activities of trypsin and α-chymotrypsin, respectively, and in measurement of the caseinolytic activities of these enzymes, DPCC-treated trypsin and TLCK-treated α-chymotrypsin, respectively, were used. In the present study, linear inhibition of the esterolytic activity of trypsin was observed with up to 5 KIU/ml of aprotinin and of that of α-chymotrypsin with up to 10 KIU/ml of aprotinin; linear inhibition of the caseinolytic activity of both enzymes was observed with up