ISOLATION AND IDENTIFICATION OF A COMPONENT OF THE SEX PHEROMONE OF *Agriotis ponticus*

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The composition and structures of the sex attractants of three species of click beetles belonging to the genus *Agriotis* have been described: the steppe click beetle, *A. gurgistanus*, the striped click beetle, (*A. lineatus*), and Kuban click beetle (*A. litigiosus*) [1]. The sex attractants of these species are represented by esters of geraniol with n-butyric and isovaleric acids and of nerol with isovaleric acid and by trans,trans-farnesyl acetate.

The smooth clock beetle (*A. ponticus* Step. (Coleoptera: Elateridae)) is distinguished by a high destructiveness on tomatoes, pepper, and potatoes growing in reclaimed lands in the flood plains of the rivers Dnestr and Prut [2]. The sex attractant of this species is unknown and we have therefore begun its study in order to use this substance for the fight against this pest.

An extract of sex pheromone was prepared by steeping in methylene chloride at 5-10°C the dissected out last segments of the abdomens of 300 females caught in nature in a light trap. When the crude extract was chromatographed on an analytical plate with silica gel in benzene in the presence of authentic samples of geranyl isobutyrate and franesyl acetate and the substances were revealed with a 0.5% solution of morin in ethanol followed by observation of the plates in UV light, it was found that the standard substances were located above a brightly fluorescing spot with R_f 0.4. For purification, the crude extract was chromatographed on a 10 × 20 cm preparative plate under the conditions described above. After the plate had been treated with morin and viewed in UV light, a zone 5 cm wide located above the fluorescing band was taken off and the silica gel was washed with ether.

When the purified extract was subjected to gas–liquid chromatography on a column containing OV-101, an intense peak was observed with a retention time close to that for the esters of geraniol and nerol with acids containing four and five carbon atoms and a second peak corresponding in its retention time to farnesyl acetate.

Capillary gas–liquid chromatography of the acetate fraction (glass column, 40 m, internal diameter 0.2 mm, SE-30) showed the presence of a peak with a retention time of 28.09 min. The mass spectrum of this peak had a molecular ion of 224 and a characteristic peak with m/z 71 corresponding to the acid residue (butyryl or isobutyryl), and coincided almost completely with the mass spectrum of authentic samples of geranyl and neryl n-butyrates and geranyl isobutyrate. The retention times of synthetic neryl and geranyl n-butyrates under these conditions were 27.3 and 27.95 min, respectively.

Thus, it may be assumed that the substance with a retention time of 28.09 min was the ester of geraniol with butyric or isobutyric acid. The assignment to a definite type of compound was made on the basis of retentiom times. When geranyl isobutyrate, geranyl n-butyrate, neryl isobutyrate, and neryl n-butyrate were chromatographed (10% of OV-101 on Chromosorb W-AW, 1.8 m, internal diameter 2 mm, 160°C), their retention times were 9.17, 11.31, 8.26, and 10.29 min, respectively. Under these conditions the component of the sex pheromone had a retention time of 11.30 min, and when they were chromatographed together it coincided completely with geranyl n-butyrate.

To identify the peak corresponding to farnesyl acetate, the acetate fraction was chromatographed through a capillary column (glass column, 50 m, OV-101, internal diameter 0.2 mm).

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Under these conditions the retention time of the assumed farnesyl acetate was 27.7 min, and that of a synthetic sample 27.9 min. However, the mass spectrum of this peak differed sharply from that of trans,trans-farnesyl acetate and probably corresponded to the ester of a high-molecular-weight carboxylic acid with methyl alcohol.

On the basis of the results obtained, the component of the sex pheromone of the smooth click beetle has been assigned the structure of geranyl n-butyrate.

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PREPARATION AND PROPERTIES OF A NEW STRUCTURAL ANALOG OF HUMAN INSULIN — ASPARAGINE ^{B30}-INSULIN

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In connection with the study of the laws of the structural-functional organization of the insulin molecule, we have obtained a previously unknown analog of human insulin differing from the natural hormone by the replacement of the L-threonine residue in the B^{30} position by and L-asparagine residue.

The preparation of the asparagine-B^{30}-insulin (I, R — de-Thr^{B30}-(human insulin)) was performed by an enzymatic-chemical method using a scheme proposed for the enzymatic transamination of porcine insulin (II, R — de-Ala^{B30}-(porcine insulin)) with the chemical splitting out of the protective groups.

The trypsin-catalyzed transamination [1] of porcine insulin (II) takes place on the reaction of the latter with the tert-butyl ester of L-asparagine (III) in an aqueous organic medium (water—dimethylformamide) at 24°C and pH 6.3. Under these conditions, the tryptic transamination reaction proceeded only at the Lys^{B29} residue, and the undesirable side reaction of the Arg^{B22} residue did not occur.

I. R—Asn—OH; III. H—Asn—OBu^t;
II. R—Ala—OH; IV. R—Asn—OBu^t.

The following stage was the chemical demasking of the ester derivative of insulin formed (IV), ensuring the complete elimination of the C-protective groups from Asn^{B30} residues. Compound (IV) was first purified by ion-exchange chromatography on DEAE-Sephadex A-25. Demasking was carried out by treating compound (IV) with trifluoroacetic acid at 20°C in the presence of anisole as protector. The asparagine^{B30}-(human insulin) (I) obtained was isolated from the reaction mixture with the aid of gel filtration on Sephadex G-25f. The course and degree of purification were monitored by means of thin-layer chromatography (TLC) on silica gel, electrophoresis on cellulose, and disk electrophoresis in polyacrylamide gel. After the lyophilization of the eluate, asparagine^{B30}-(human insulin) (I) was obtained in the analytically pure form.

Asparagine^{B30}-(human insulin) (I). R_f 0.46 (C_5H_5N—C_4H_9OH—CH_3CO_2H—H_2O, (10:15:3:12)), 0.40 (iso-C_3H_7OH—25% NH_4OH, (7:4)), 0.88 (C_5H_5N—CH_3COCH_3—H_2O, (1:1:2)), 0.93 (iso-C_3H_7OH—25% NH_4OH—H_2O, (7:4:6)) (TLC on Silufol UV-254 plates, spots revealed with the Pauly reagent [2]). Electrophoretic mobilities: 1.50 (electrophoresis on Whatman No. 1 paper, pH 1.9, 450 V, 7

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