PROTEINS OF THE SEEDS OF Ricinis communis.

I. A STUDY OF THE WATER-SOLUBLE PROTEINS OF THE SEEDS AND MEAL OF Ricinis communis

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The electrophoretic spectrum of the water-soluble proteins of the seeds and meal of the castor-oil plant has been studied. A correlation has been found between a decrease in the relative amount of electrophoretic components with R_f 0.04 and 0.11 and the hemagglutinating activity of the meal.

Among oil crops, Ricinus communis L. (the castor-oil plant) occupies a special position. The seeds of the plant contain about 50% of oil and 18% of protein and are an important industrial raw material [1].

The meal remaining after the extraction of the oil contains 42% of protein but its use for fodder purposes without preliminary hot moist treatment (toasting) is impossible because of the presence of toxic components [2-4]. The degree of detoxification of the meal is determined by the erythrocyte-agglutinating action [5].

The aim of the present investigation was to study the change in the water-soluble proteins during the toasting process.

Protein extracts were obtained from seeds and meal defatted with cooled ether provided by an oil-extracting factory.

Hemagglutinating activity was determined by a standard method [6]. Changes in the protein spectra were studied by electrophoresis in polyacrylamide gel (PAAG) in the presence of Triton X-100.

Figure 1 shows a sketch of electrophoretograms of the water-soluble proteins of the seeds of the castor-oil plant and also of the first and second toastings of the meal.

In a control sample (seeds) we detected five main bands (Fig. 1a). After the first toasting, according to the results of densitometry (Fig. 1b) the relative amounts of the components with R_f 0.04 and 0.11 had fallen 3- and 4-fold, respectively. In the second toasting (Fig. 1c), the component with R_f 0.04 was absent and the amount of the component with R_f 0.11 had fallen 8-fold. The relative amounts of the other three components had changed only slightly. Comparative results on the determination of the hemagglutinating activities of extracts of the seeds and meal of the castor-oil plant are given below.

Sample	Hemagglutingating titer
Seeds	1:512
Toasting I, obtained after the solvent had been distilled off	1:16
Toasting II, obtained after the hot moist treatment	Not detected

Thus, the experimental results obtained permit the conclusion that there is a correlation between the decrease in the relative amount of the electrophoretic components with R_f 0.04 and 0.11 and the hemagglutinating activity of castor-oil plant meal.

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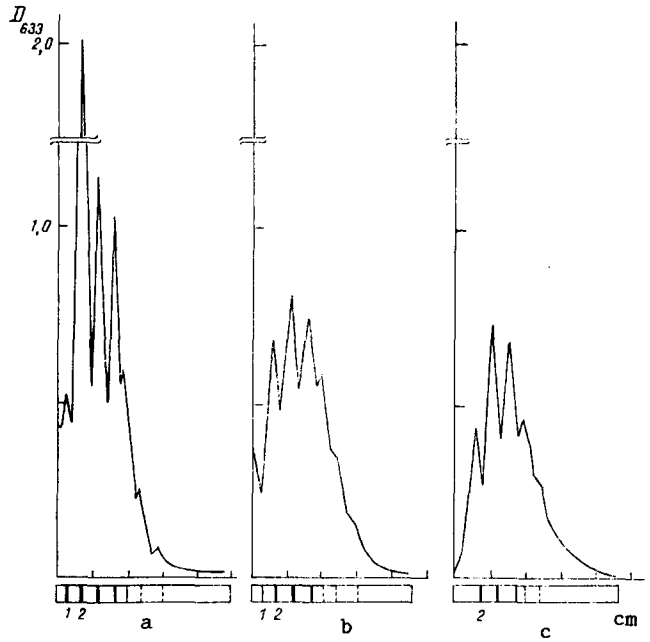

Fig. 1. Sketch of electrophoretograms and densitometry curves for the water-soluble proteins of the castor-oil plant: a) seeds; b) toasting I; c) toasting II; 1) R_f 0.04; 2) R_f 0.11.

EXPERIMENTAL

The extraction of the proteins was carried out at a flour:water ratio of 1:10 and a meal: water ratio of 1:2. The mixtures were centrifuged at 1500 g for 10 min, the supernatants were separated off. Triton X-100 was added to a concentration of 1%, and the solutions obtained were added to the wells of a separating gel (50 µl each).

Electrophoresis was performed in a AVGE-1 instrument by the method of Ornstein and Davis [7] in 7.5% PAAG in the presence of 1% Triton X-100. The separating buffer was 0.375 M Tris-HCl buffer, pH 8.9, containing 1% of Triton X-100. The electrode buffer was a 0.005 M Tris-glycine buffer in 0.1% Triton X-100. Voltage 250 V, current strength 30 mA, plate 10 × 10 × 0.1 cm, time of electrophoresis 2 h, length of travel of the leading dye 64 mm.

Fixing and Staining of the Gels. The protein bands were fixed with 10% TCA for an hour. Then the plates were washed with a 7% solution of acetic acid in 10% ethanol (solution 1) for an hour three times. They were stained with a 1% solution of Amido Black 10B, and the unbound dye was washed out with solution 1, changed every 30 min, until it had been eliminated from the gel completely.

The densitometry of the gel was performed in an LKB Ultrascan instrument (Sweden). The rate of scanning the gel was 1 mm/sec.

Hemaggluting activity was determined from the agglutination of rabbit-blood erythrocytes in 50-µl micro-test tubes [6]. As the titer we took the maximum dilution of the solution at which agglutination of the erythrocytes was still observed visually.

SUMMARY

The fall in hemagglutinating activity of castor oil plant meal from the first and second toastings is connected with a decrease in the amount of protin components with R_f 0.04 and 0.11 in them.

The electrophoresis of the water-soluble proteins can be used to evaluate the quality of castor-oil plant meal.

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INSECT PHEROMONES AND THEIR ANALOGS.

XV. THE SYNTHESIS OF 9-OXODEC-2E-ENOIC ACID – A PHEROMONE OF THE HONEYBEE *Apis melliferana*

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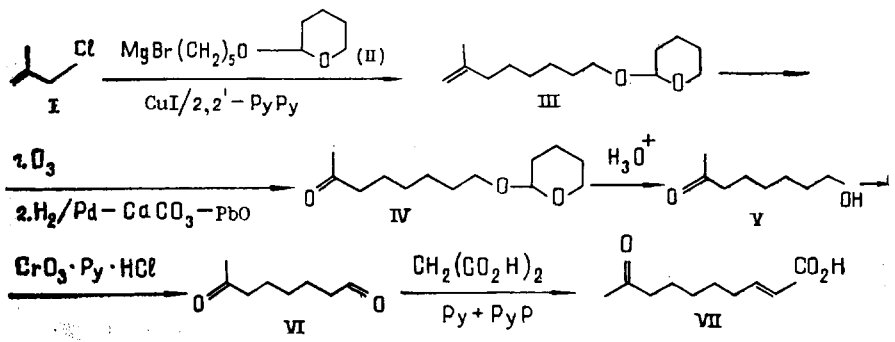
A new effective route to the synthesis of 7-oxooctanal has been found which is based on the ozonolysis of the tetrahydropyran-2-yl ether of 7-methyloct-7-en-1-ol – the product of the coupling of the readily available methallyl chloride and the Grignard reagent from the corresponding ether of 5-bromopentan-1-ol. From the 7-oxooctanal has been obtained 9-oxodec-2E-enoic acid – a pheromone of the honeybee *Apis melliferana*.

9-Oxodec-2E-enoic acid fulfills important functions in the vital activity of the honeybee *Apis melliferana* [1, 2]. A number of methods of synthesizing this compound are known [1, 3]. The introduction of the keto group has been effected by the $PdCl_2$-catalyzed oxidation of a vinyl group [1, 4], by the cleavage of 1-methylcyclopentanone or of thiophene derivatives [1], and by the action of methylmagnesium iodide on 7-hydroxyheptanal followed by oxidation with pyridinium chlorochromate [5, 6].



We have found a new route to the synthesis of the key synthon – 7-oxooctanal (VI), starting from the readily available methallyl chloride (I). The coupling of the latter with 5-(tetrahydropyran-2-yloxy)pentylmagnesium bromide (II) gives a high yield of the tetrahydropyran-2-yl (THPL) ether of 7-methyloct-7-en-ol (III), the ozonolysis of which leads to the 2-THPL ether (VIII) of 8-hydroxyoctan-2-one (V). Hydrolysis of the ether (IV) followed by oxidation of the hydroxy ketone (V) gives the desired keto aldehyde (VI) with an overall yield of 53% calculated on the bromo alcohol derivative (I). Condensation of the keto aldehyde (VI) with malonic acid under standard conditions [1, 4-6] leads to 9-oxide-2E-enoic acid (VII).

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