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INSECT PHEROMONES AND THEIR ANALOGS.

IV. THE SYNTHESIS OF THE SEX ATTRACTANT OF THE HONEY BEE *Apis mellifera*

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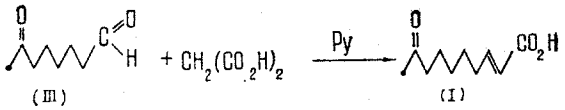
A new and convenient method for the synthesis of 9-oxodec-2E-enoic acid (I) from the readily accessible octa-2E,7-dienyl acetate is proposed. From the latter compound by a series of transformations (oxidation of the terminal double bond to a ketone, saponification, hydrogenation, and oxidation with Corey's reagent) 7-oxooctanal is obtained, and this is condensed by the Knoevenagel reaction with malonic acid to give compound (I) with a yield of 69%.

The synthesis of the pheromone of the honey bee, 9-oxodec-2E-enoic acid (I), has been considered in a number of papers [1-5]. In the majority of cases, as the initial compounds for obtaining the acid (I) difficultly accessible compounds are used or the syntheses are characterized by a large number of stages.

We have proposed a new approach to the synthesis of the acid (I) which is based on the use as the key compound the readily accessible octa-2E,7-dienyl acetate (II). The latter was obtained by the telomerization of butadiene with acetic acid under the action of low-valence phosphine complexes of palladium [6].

In particular, the synthesis of the acid (I) is effected by the Knoevenagel condensation of malonic acid with 7-oxooctanal (III) in pyridine [7]. Under these conditions, the yield of compound (I) is not less than 90%.



(III) (I)

To obtain the aldehyde (III), the acetate (II) was first converted into 7-oxooct-2E-enyl acetate (IV) by a literature method [8], and the hydrolysis of the latter with 5% NaOH in methanol led to the alcohol (V) in quantitative yield. Hydrogenation of the unsaturated compound (V) over 5% Pd/C in ethyl acetate gave the oxo alcohol (VI) with a yield of ∿100%. Compound (VI) was readily oxidized by the Corey reagent at room temperature into compound (III) with a yield of 80% [9].

It must be mentioned that in the reduction of the acetate (IV) with 5% Pd/C hydrogenolysis of the acetate group takes place, leading with high yield to 2-oxooct-6E-ene (VII). Thus, we have proposed a simple and convenient route for the synthesis of the attractant of the honey bee from octa-2,7-dienyl acetate and malonic acid (see Scheme 1).

EXPERIMENTAL

We used octa-2E,7-dienyl acetate with a purity of 98%. The compounds obtained were analyzed on a Khrom-41 chromatograph with a 1.2-m column containing 15% of SE-30. PMR spectra were recorded on a Tesla 480 BS instrument in CCl_4 solution (with HMDS as internal standard). IR spectra were taken on a UR-20 instrument (film) and mass spectra on a MKh-1306 instrument with an energy of the ionizing electrons of 70 eV at a chamber temperature of 200°C.

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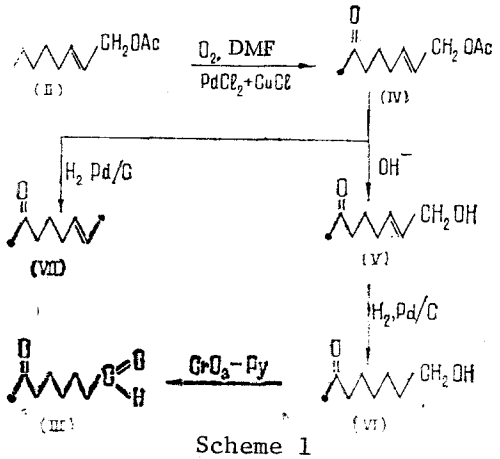

Scheme 1

7-Oxooct-2E-enyl Acetate (IV). A mixture 1.5 g of $PdCl_2$ (0.0085 mole) and 8.5 g of CuCl (0.085 mole) in aqueous dimethylformamide (50 ml, H_2O, 5 ml) was stirred in an atmosphere of oxygen for 1 h. Then 14.2 g (0.085 mole) of the octadienyl acetate (II) was added dropwise and the mixture was stirred for 5 h with the passage of a current of oxygen. The reaction mixture was treated with 10% HCl until the salts of the transition metals had dissolved and it was then extracted with CH_2Cl_2 (3 × 100 ml). The combined extracts were dried over $MgSO_4$. After distillation, 14 g (90%) of the acetate (IV) was obtained, with bp 140-143°C (2 mm), n_D^{20} 1.4532. IR spectrum (ν, cm^{-1}): 978, 3015 (trans- —CH=CH—), 1715 (—C—), 1740 (CH_3—C—O).

PMR spectrum (δ, ppm): 1.57 (2H, $-CH_2-$); 1.96 (3H, CH_3 in CH_3COO); 2.05 (3H, CH_3C—, 2H, $-CH_2-C=$); 2.37 (2H, $-CH_2CO-$); 4.40 (2H, $-CH_2O-$); 5.59 (2H, —HC=); M^+ 184.

7-Oxooct-2E-en-1-ol (V). A solution of 9.2 g (0.05 mole) of the acetate (IV) in alkaline methanol (2 g of NaOH in 30 ml of CH_3OH) was stirred at 30°C for 7 h. The resulting precipitate was filtered off, and the methanol was evaporated off. Distillation of the residue yielded 6.8 g (96%) of the alcohol (V) with bp 120-122°C (2 mm), n_D^{20} 1.4642. IR spectrum (ν, cm^{-1}): 980 (—CH=CH—), 1715 (—C—), 3450 ($-CH_2OH$). PMR spectrum (δ, ppm): 1.6 (2H, $-CH_2-$); 2.0 (3H, CH_3-); 2.35 (2H, $-CH_2CO-$), 3.70 (2H, $-CH_2O-$), 5.40 (2H, —CH=); M^+ 142.

7-Oxooctan-1-ol (VI). A solution of 7.1 g (0.05 mole) of the alcohol (V) in 50 ml of ethyl acetate was hydrogenated in the presence of 0.3 g of 5% Pd/C for 8 h. After separation from the catalyst and elimination of the solvent, 6.8 g (94%) of the oxo alcohol (VI) was isolated with bp 105-108°C (2 mm), n_D^{20} 1.4595. IR spectrum (ν, cm^{-1}): 1715 (—C—), 3400 (CH_2OH). PMR spectrum (δ, ppm): 1.58 (8H, $-CH_2-$); 1.98 (3H, CH_3-); 2.25 (2H, $-CH_2CO-$); M^+ 144.

7-Oxooctanal (III). The oxidation of 5.8 g (0.04 mole) of compound (VI) with the CrO_3—Py—HCl complex (10.5 g, 0.05 mole) in CH_2Cl_2 [7] yielded 4.8 g (85%) of the aldehyde (III) with bp 70-72°C (3 mm), n_D^{20} 1.4698, which corresponds to information in the literature [5].

9-Oxodec-2E-enoic Acid (I). A solution of 4.2 g (0.03 mole) of the aldehyde (III) and 3.1 g (0.03 mole) of malonic acid in 3 ml of absolute pyridine was stirred at 20°C for 10 h. Then the temperature of the reaction mixture was raised to 90°C and stirring was continued for another 2 h. The mixture obtained was treated with 2 ml of 2 N H_2SO_4 and extracted with ether (3 × 50 ml), and the combined extracts were dried over $MgSO_4$. After the solvent had been driven off and the residue had been distilled in vacuum, 3.8 g (69%) of the acid (I) was obtained, with physicochemical constants agreeing completely with those of the material obtained by previous workers [2, 3].

2-Oxooct-6E-ene (VII). The hydrogenation of 18 g (0.1 mole) of the acetate (IV) in 100 ml of MeOH in the presence of 1 g of 5% Pd/C at 20°C for 20 h led to 11.5 g (91%) of the

ketone (VII) with bp 70-72°C (2 mm), n_D^{20} 1.4340. IR spectrum (ν, cm^{-1}): 975, 3025 (trans-—HC=CH—), 1715 (—CO—). PMR spectrum (δ, ppm): 1.25 (2H, —CH_2—); 1.57 (3H, CH_3CH=); 2.0 (3H, CH_3CO—); 2.30 (4H, —CH_2CO—, CH_2CH=); 5.30 (2H, —CH=CH—); M^+ 126.

SUMMARY

A new route for the synthesis of the attractant of the honey bee *Apis mellifera* using readily accessible reactants has been developed.

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AN INVESTIGATION OF THE VENOM OF RENARD'S VIPER *Vipera ursini renardi*. III. ISOLATION OF PHOSPHOLIPASES A_2

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Two phospholipases A_2 with molecular weights of 15,600 and 13,200 and pI values of 7.57 and 6.73, respectively, have been isolated from viper venom in the pure state with the aid of gel filtration on Sephadex G-75 followed by ion-exchange chromatography on CM-cellulose and SE-Sephadex C-25.

Phospholipase A_2 (phosphatide acylhydrolase, E. C. 3.1.1.4) is a component part of many snake venoms and plays a fundamental role in the mechanism of their action [1, 2]. The majority of the biological effects of the enzyme are probably due to the hydrolysis of membrane phospholipids, the degree of degradation of which determines the beginning of lysis of the cells [3]. The phospholipase A_2 from the venoms of different snakes differ from one another in their capacity for attacking a membrane substrate [4]. Thus, the enzyme from the venom of the Palestine viper *Vipera palestinae*, in contrast to the phospholipase A_2 from the venom of the cobra *N. naja*, does not penetrate into the structure of a substrate organized in a membrane. We have previously [5, 6] isolated pure phospholipase A_2 from the venom of Central Asian cobra *Naja oxiana* Eichwald and have studied its hemolytic action. The aim of the present investigation was to isolate pure phospholipases A_2 from the venom of the viper *V. ursini renardi* Ch.

The initial phospholipase A_2 fraction (fraction III) was obtained by the gel filtration of the whole viper venom on Sephadex G-75 [7]. It was subjected to further separation by chromatography on CM-cellulose (Fig. 1). On application to the column of ion-exchange resin, the part of fraction III denoted by the letter A and having no phospholipase activity was not adsorbed and issued from the column with the equilibrating 0.05 M ammonium acetate buffer (pH 4.7). The superimposition of a gradient of ammonium acetate buffer from 0.05 M (pH 4.7) to 0.5 M (pH 6.7) permitted separation into three components, designated in the order of their emergence from the column III-1, III-2, and III-3, and the addition of 0.01 M caustic soda solution to the column desorbed component III-4. They all possessed phospholipase A_2 activity. The initial fraction III contained mainly protein-peptide components (98%). On chromatography on CM-cellulose, the bulk of the proteins was distributed in the following way: III-1, 17.66%; III-2, 49.1%; III-3, 22.76%; and III-4, 4.4%; 6.08%

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