SYNTHESIS AND BIOLOGICAL ACTIVITY OF RS-ABSCISIC ACID AND ITS ANALOGS

N. D. Livshits, Ch. Sh. Kadyrov, and V. I. Kefeli

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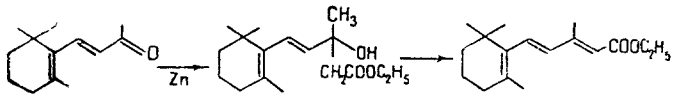
The aim of the present work was to determine the dependence of biological activity on the structure of abscisic acid and its analogs.

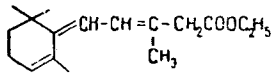
Abscisic acid – a natural growth regulator – was first isolated from the cotton plant [1], and its structure was confirmed by synthesis [2].

A three-stage method of obtaining abscisic acid is known [3]. We have repeated this synthesis of abscisic acid, simplifying some of its stages. As a result, we achieved a 24% yield of the intermediate 1-hydroxy-4-oxo-α-ionone (literature data 23% total yield consisting of a 19% yield of crystalline product and a 4% yield of the same product during the chromatography of the oily residue). We did not chromatograph the residue. The purity of the ketone was checked by thin-layer chromatography. The condensation of 1-hydroxy-4-oxo-α-ionone with ethoxycarbonylmethylenetriphenylphosphorane led to a mixture of isomeric esters of (±)-cis, trans- and (±)-trans,trans-abscisic acids. The triphenylphosphine oxide that deposited was separated from the mixture of isomeric esters of abscisic acid. Then, without separating the mixture of esters of abscisic acid from the triphenylphosphine oxide on a column, we saponified them. When the residue was treated with a mixture of ether and petroleum ether, abscisic acid was isolated with a yield of 34%. The physicochemical properties of this abscisic acid coincided with those published previously [3, 4]. A mixture with an authentic sample gave no depression of the melting point.

A possible biosynthetic precursor of RS-abscisic acid – ethyl β-ionylideneacetate – was obtained by the Wittig reaction from β-ionone and ethoxycarbonylmethylphosphonic acid. The reaction was performed with sodium and lithium hydrides in an atmosphere of nitrogen in dimethylformamide (Table 1). We also obtained ethyl β-ionylideneacetate by the Reformatsky reaction from β-ionone and ethyl bromoacetate.



However, in this reaction, in addition to the main product, a byproduct is formed – ethyl retro-β-ionylideneacetate,



which is difficult to separate and the formation of which is due to the rearrangement of the polyene system, as has been confirmed by later investigations [5]. We have studied the influence of various factors on the condensation of β-ionone with ethyl bromoacetate: reaction time, nature of the catalyst (Zn powder, Zn amalgam) and its amount, and also the presence of impurities affecting the yield of product. By saponifying ethyl β-ionylideneacetate we obtained the trans,trans (mp 112-114°C) and cis,trans isomers, the latter in the form of an amorphous product.

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TABLE 1

No.	Starting material	Reaction performed	Reaction product	Characteristics of the product: bp, °C (mm)	n_D^{20}	yield, %	ratio of cis,trans to trans, trans isomers, %
1	β-Ionone	Wittig with NaH and LiH	Ethyl β-ionylidene-acetate	143—145 (3)	1,5360	58	29:71
2				105 (0,07)	1,5332	55	48:52 [6]
3				141—150 (3)	1,5230	15,5	21:20
4		Reformatsky		168—170 (7)	1,5350	60	41:51
5				162,5—164,5 (6)	1,5310	48	—[7]
6			Dehydro-β-ionone	98—100 (2)	1,5508	50	—[10]
7	Ethyl β-ionyl-ideneacetate (1)	Allyl bromination with N-bromo-succinimide	Ethyl dehydro-β-ionylideneacetate	150—155 (6)	1,5590	49	30:38
8	The same (4)			148—150 (5)	1,5500	57	27:51
9	Methyl trans, trans-β-ionylidene-acetate		Methyl dehydro-β-ionylidene-acetate	112—114 (1,5)	—	73	—[8]

Note. The retention times of the products from reactions 1, 3, and 4 and from reactions 7 and 8 coincided. Stationary phase PEGA, 194.5°C, pressure 0.9 atm, rate 31 ml/min.

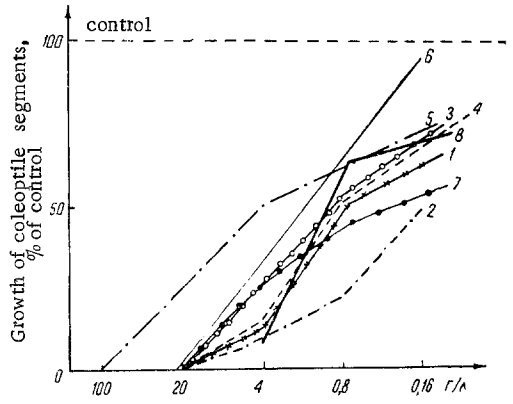

Fig. 1. Curves of the suppression of the growth of segments of wheat coleoptiles by α- and β-ionone derivatives: a) β-ionone; 2) dehydro-β-ionone; 3) dehydro-β-ionylideneacetic acid; 4) ethyl dehydro-β-ionylideneacetate; 5) trans,trans-β-ionylideneacetic acid; 6) ethyl β-ionylideneacetate; 7) α-ionone; 8) 1-hydroxy-4-oxo-α-ionone.

Other possible precursors of RS-abscisic acid are ethyl dehydro-β-ionylideneacetate and dehydro-β-ionone obtained by the allyl bromination with N-bromosuccinimide of ethyl β-ionylideneacetate and of β-ionone, respectively. HBr was split off to form the allyl double bond. Various substances of basic nature forming quaternary salts with HBr were used as dehydrobrominating agents. Thus, we used diethylaniline [9], dimethylaniline and pyridine (3 : 1) [10], quinoline, and sodium iodide in acetone. The reaction was carried out in methylene chloride or carbon tetrachloride.

The best yield of products for dehydro-β-ionone (50%) was obtained when the reaction was carried out in CCl_4 (treatment with dimethylaniline and pyridine in a ratio of 3 : 1) and for dehydro-β-ionylideneacetate (57%) in methylene chloride (treatment with quinoline) (see table).

By saponifying ethyl dehydro-β-ionylideneacetate we obtained dehydro-β-ionylideneacetic acid (mixture of isomers). The trans,trans isomer had mp 130-131°C [petroleum ether (40-60°C)] (literature data 130-131°C). All the products (abscisic acid, ethyl β-ionylideneacetate, trans,trans-β-ionylideneacetic acid, mixture of isomers of dehydro-β-ionylideneacetic acid and of ethyl dehydro-β-ionylideneacetate, α-ionone, and 1-hydroxy-4-oxo-α-ionone) were subjected to biological tests. The α-ionone and β-ionone intermediates in the synthesis of RS-abscisic acid were tested for the growth of segments of coleoptiles (Fig. 1). All the intermediates suppressed the growth of

the coleoptile segments by 30-50% in concentrations of 800-160 mg/liter while the racemate of abscisic acid itself suppressed growth by 50% in a concentration of 0.5 mg/liter. The racemic abscissic acid that we had synthesized also suppressed the growth of a wheat sprout (C_{50} 4 mg/liter) and the growth of wheat roots (C_{50} 10 mg/liter) and inhibited the germination of the seeds. Its biological activity was approximately equal to the activity of a sample of racemic abscisic acid obtained from Professor Cornforth (England).

EXPERIMENTAL

The α-ionone had bp 88-89°C (2 mm), n_D^{20} 1,4996, d_4^{20} 0.9330 (literature data: 79°C (0.8 mm), d_4^{20} 0.9363, n_D^{20} 1.4982); and the β-ionone bp 126°C (7 mm), n_D^{20} 1.5190 d_4^{20} 0.9467 (literature data 60.9°C (0.2 mm) n_D^{20} 1,5200, d_4^{20} 0.9477) [11].

Preparation of 1-Hydroxy-4-oxo-α-ionone. A butyl chromate mixture was prepared by the gradual addition of 37.5 g (0.37 mole) of chromic anhydride to 100 ml of carefully purified tert-butanol. Then 35 ml of dried acetic anhydride was added. The freshly prepared butyl chromate reagent was added dropwise to 12.5 g of α-ionone in 50 ml of tert-butanol at 25°C. Then the mixture was heated at 80-90°C for 6 h. After cooling, 200 ml of water, 13.5 g of oxalic acid, and 6 ml of methanol were added. The reaction mixture was extracted with chloroform and the extract was washed and dried. Distillation of the solvent yielded 9.89 g of a dark oil. On treatment with a mixture of ether and hexane (1 : 1) 3.5 g of 1-hydroxy-4-oxo-α-ionone was formed with mp 109-110°C (from toluene) (literature data 111-112°C). The purity of the product was checked by thin-layer chromatography, R_f 0.25 [ether–hexane (3 : 1) system]. The IR spectrum had absorption bands at 3482 cm^{-1} (OH group), 1639 cm^{-1} (C = O group of the nucleus), 1627 cm^{-1} (C = O of the side chain), and 995 cm^{-1} (deformation vibrations of a double bond).

Preparation of 5-(1-Hydroxy-4-oxo-2,6,6-trimethylcyclohex-2-enyl)-3-methylpenta-2,4-dienoic Acid. A mixture of 1.4 g (0.0062 mole) of 1-hydroxy-4-oxo-α-ionone and 2.64 g (0.0078 mole) of ethoxycarbonylmethylenetriphenylphosphorane was heated in an oil bath at 150-170°C for 45 min. The cooled mixture was treated with ether, whereupon 1.25 g of crystals of triphenylphosphine oxide deposited. The 2.8 g of oily residue was treated with 6.6 g (0.0165 mole) of NaOH in 47 ml of CH_3OH and 17 ml of H_2O, and the mixture was left at 24°C for 12 h. The methanol was eliminated in a rotary evaporator. The residue was diluted with water and extracted with ether. The aqueous solution was acidified with 10% HCl and extracted with ether. The ethereal extract was washed with water, dried with $MgSO_4$, and distilled. The residue, amounting to 1.1 g, was treated with a mixture of petroleum ether and diethyl ether, giving a white crystalline substance with mp 189-190°C (literature data: 190-191°C). The total yield of RS-abscisic acid, trans isomer, was 0.55 g, which amounts to 34% on the initial 1-hydroxy-4-oxo-α-ionone and 12.6% on the α-ionone.

The IR spectrum exhibited the following absorption bands, cm^{-1}: 3415 (OH group in the nucleus); 2791 (CH_3 in the nucleus); 1678 (C = O group in the ring); 1650 (C = O group of an acid); 1623 and 1600 (conjugated system of double bonds), 991 and 910 (deformation vibrations of a double bond); and 860 and 730 cm^{-1} (skeletal vibrations of a double bond).

Preparation of Ethyl β-Ionylideneacetate. Activated zinc was added in portions over 5 h to a mixture of 100 g of β-ionone (0.52 mole) in absolute benzene and 73 ml (0.065 mole) of $BrCH_2COOC_2H_5$. The reaction mixture was heated for another 3 h. After cooling, it was decomposed with 10% CH_3COOH and extracted with petroleum ether. The extract was washed with water, $NaHCO_3$, and water again, and was dried. The petroleum ether was distilled off, 20 g of calcined $KHSO_4$ in benzene was added to the residue and the mixture was heated in the oil bath at 148-150°C with the distillation of the solvent for 2 h. Then it was cooled and extracted with ether, and the extract was washed with sodium carbonate and with water to neutrality to litmus and was dried. The ethyl β-ionylideneacetate was distilled in vacuum. We also obtained this product by means of the Wittig reaction [6].

Preparation of Ethyl Dehydro-β-Ionylideneacetate. In a current of nitrogen and at 10°C, 12.4 g of N-bromosuccinimide was added in portions over 6 h to a mixture of 17.4 g (0.0064 mole) of ethyl β-ionylideneacetate, 62.5 ml (0.0662 mole) of methylene chloride, 3.5 g (0.063 mole) of CaO, and 5.5 g (0.065 mole) of $NaHCO_3$. Then 9 ml (0.064 mole) of quinoline was added to the mixture; this was filtered and then another 9 ml of quinoline was added and it was heated in the water bath for 1 h. The resulting product was decomposed with 3 N H_2SO_4 and extracted with petroleum ether. The extract was washed with 5% $NaHCO_3$ solution and with water, dried with $MgSO_4$, and distilled in vacuum.

The saponification of ethyl β-ionylideneacetate and dehydro-β-ionylideneacetate was carried out by the usual method.

SUMMARY

RS-Abscisic acid and some of its analogs have been obtained. The biological activity of these compounds has been studied in dependence on their structure.

It was found that precursors of RS-abscisic acid suppress the growth of segments of coleoptiles to a considerably smaller extent than the acid itself.

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