COMMUNICATIONS

Cis,Trans-α-Ionylideneacetic Acid

A Bioactive Analog of Abscisic Acid

Cis,trans-α-ionylideneacetic acid [*cis,trans*-5-(2,6,6,-trimethyl - 2 - cyclohexen - 1 - yl) - 3 - methyl - 2,4-pentadienoic acid] is a potent plant bioactive substance approaching abscisic acid (ABA) in activity. This acid was prepared via a Reformatsky reaction of α-ionone (I) with ethyl bromoacetate (II). The initial condensation product was the β-hydroxy ester (III) and this was dehydrated to give two isomeric α-ionylideneacetates (V_a, VI_a) and a third isomer (IV_a). The isomers were separated by vacuum distillation and after alkaline hydrolysis, the *cis,trans* and *trans,trans*-α-ionylideneacetic acids (V_b, VI_b) were crystallized. The bioactivity of *cis,trans*-α-ionylideneacetic acid (V_b) relative to abscisic acid was determined by the inhibition of 3-indoleacetic acid (IAA) induced *Avena* straight growth and by the acceleration of abscission in cotton explants.

A study of a chemical structure-biological activity relationship was undertaken as an approach toward understanding the mode of action of the plant hormone, abscisic acid (VII) (Addicott and Lyon, 1969). The analog of abscisic acid which lacks both ring oxygens was prepared by repeating the Reformatsky reaction between α-ionone (I) and ethyl bromoacetate (II) (Sobotka *et al.*, 1945; Suga, 1958; Zawasza and Kuczynski, 1964). It was necessary to dehydrate the initial condensation product (III) as stated by Young *et al.* (1944) and separate and characterize the isomers produced. Two analogs, with the same carbon skeleton as abscisic acid, were obtained by alkaline hydrolysis and tested in two different bioassays. This reaction scheme is shown in Figure 1 along with the structure of abscisic acid (VII) for comparison.

EXPERIMENTAL

The Reformatsky reaction was carried out as described by Karrer *et al.* (1949, 1932) as follows.

One hundred grams of ionone alpha white, (I), (Dodge and Olcott, 110 ml) was mixed with 110 g of dry ethyl bromoacetate (II) (Eastman Organic, 73 ml) and dissolved in 250 ml of dry, thiophene free benzene in a 1-l. round bottom flask. The flask was fitted with a wide bore bulb type reflux condenser and the mixture heated to reflux with a heating mantle set at 60 volts. As soon as reflux was established, the mantle voltage was reduced to 30 volts and 10 g of granular zinc was poured down the condenser. A vigorous reaction soon commenced. As soon as the reaction subsided to the initial level of reflux, another 10 g of granular zinc was added. This sequence was repeated until a total of 50 g of zinc had been added. After the final addition, any zinc clinging to the walls of the condenser was rinsed in with a little dry benzene, the heating mantle voltage increased to 40 volts to maintain a gentle reflux, and a calcium chloride drying tube was fitted to the top of the condenser. The mixture was then refluxed an additional 2 hr to complete the reaction.

At the end of this period the dark brown reaction mixture was cooled and 500 ml of 10% acetic acid was added. The flask was tightly stoppered and then shaken vigorously on a wrist action shaker for 1 hr to hydrolyze the zinc complex. The benzene layer was then separated from the acetic acid washings and rinsed with water. This initial product was the β-hydroxy ester (III) as others have shown (Young *et al.*, 1944). This was confirmed by the appearance of a single major peak upon gas chromatographic analysis of the crude product isolated by stripping off the benzene and by the observation of a strong hydroxyl absorption in the characteristic infrared region. This compound (III) was not usually isolated, but was dehydrated in the original benzene solution by placing it in a 1-l. flask containing 50 g of potassium bisulfate, and fitted with a Dean-Stark trap and reflux condenser. The mixture was refluxed until no further water separated. The benzene solution was filtered from the potassium bisulfate, and the benzene solvent was stripped off with a rotary film evaporator under vacuum. The product was then distilled under vacuum and the fraction boiling between 130–140° C at 0.7–0.8 mm was collected. The total yield was 120 g (89%).

This product had three components in about equal amounts,

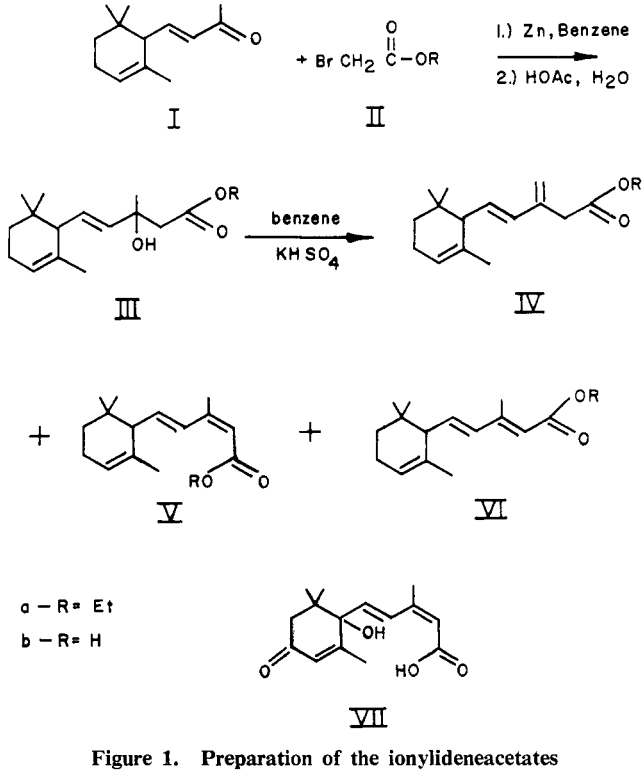

Figure 1. Preparation of the ionylideneacetates

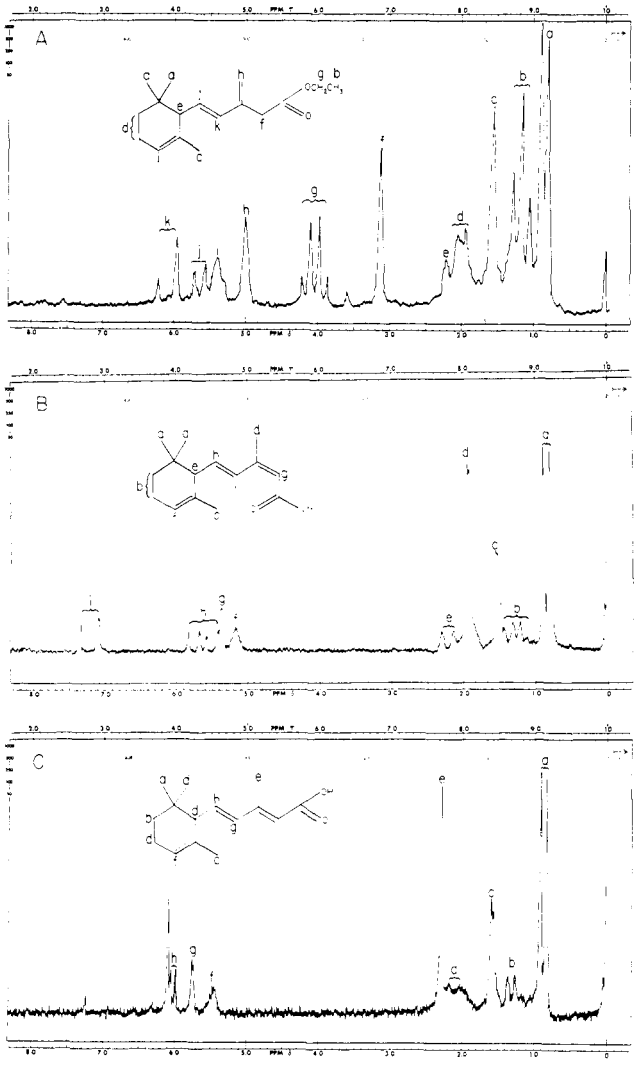

Figure 2. Nmr spectra of IV_a (A), V_b (B), and VI_b (C)

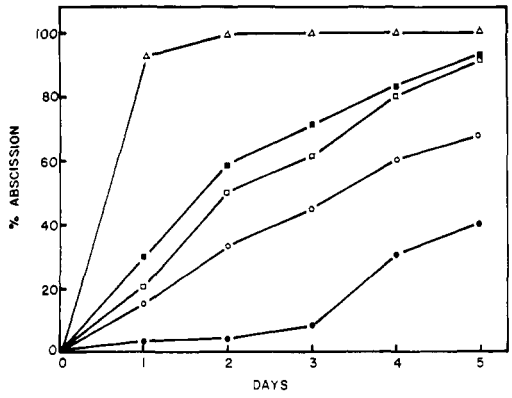

Figure 3. The rate of abscission of petiole stumps in the cotton explant test

△—△ 0.05 μg abscisic acid (ABA) (VII), applied per zone
■—■ 0.05 μg *cis,trans*-α-ionylideneacetic acid (V_b) per zone
□—□ 0.005 μg ABA (VII) per zone
○—○ 0.025 μg *cis,trans*-α-ionylideneacetic acid per zone
●—● Control

as shown by gas chromatographic analysis. This mixture was separated by distillation through a Nester-Faust auto-annular spinning band distillation column under vacuum. The lowest boiling fraction could not be obtained pure because of its thermal instability, but the nuclear magnetic resonance spectrum, Figure 2A, of an enriched fraction [nmr (neat) δ 0.80 (s, 3), 0.88 (s, 3), 1.15 (t, 3, J = 7Hz), 1.55 (s, 3), 2.00 (m, 4), 2.21 (s, 1), 3.10 (s, 2), 4.04 (m, 2), 5.08 (s, 2), 5.37 (s, 1), 5.60 (m, 1, J = 9Hz), 6.08 (d, 1, J = 15Hz)] showed that it had the unconjugated structure indicated (IV). The presence of the well resolved methyl peaks at δ = 0.80 and 0.88 shows that the ring double bond is in the same position as in α-ionone (Varian nmr spectra catalog V.2 #616). The triplet at δ = 1.15 and the quartet at δ = 4.04 are due to the ethyl group. The third ring methyl group comes at δ = 1.55, compared to δ = 1.57 in α-ionone. The sharp singlet at δ = 3.10 is due to the methylene adjacent to the carboxylic acid function and the sharp singlet at δ = 5.08 is due to the olefinic methylene. The side chain olefinic protons are represented by a partially obscured quartet at δ = 5.60 and a doublet at 6.08. The ring olefinic proton comes at δ = 5.37.

The other two products were obtained in gas chromatographically homogeneous states by taking small cuts from the distillation head and analyzing them. The product corresponding to the middle peak in the gas chromatogram (g.c.) of the crude mixture boiled at 94–5° C 0.05 mm; n_D^{25} = 1.5155. The product corresponding to the last peak boiled at 102–3° C 0.06 mm; n_D^{25} = 1.5157.

The α-ionylideneacetic acids were obtained from these latter two esters by hydrolysis with alcoholic potassium hydroxide. The acid from the middle g.c. peak (V_b) crystallized slowly on standing, but was easily recrystallized from acetonitrile using 4 ml of solvent per g of acid. This acid was collected as fine white needles melting at 88–88.5° C. A sample for analysis was prepared by vacuum sublimation. Anal. Calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46; Found: C, 76.96; H, 9.48. The nmr spectrum of this acid in carbon tetrachloride, Figure 2B, [δ 0.81 (s, 3), 0.89 (s, 3), 1.27 (m, 4, J = 6Hz), 1.54 (d, 3, J = 1.5Hz), 1.94 (d, 3, J = 1Hz) 2.22 (d, 1, J = 10Hz), 5.17 (s, broad, 1), 5.36 (s, 1), 5.62 (m, 1. J = 9Hz, J = 15Hz), 7.22 (d, 1, J = 15 Hz)] showed it to be *cis,trans*-α-ionylideneacetic acid. The gem dimethyls at δ = 0.81 and δ = 0.89 are well resolved, as is characteristic of the α system. The third ring methyl comes at δ = 1.54, and the side chain methyl appears at δ = 1.94. The ring methylenes come at δ = 1.27 and around δ = 2.0, which is obscured by the side chain methyl peak. A nice doublet at δ = 2.22 is due to the tertiary hydrogen at the ring-side chain junction. The ring olefinic proton comes at δ = 5.17 and the side chain olefinic proton adjacent to the carboxylic acid group appears at δ = 5.36. The large difference of the chemical shifts for the remaining two side chain olefinic protons provides the best information for the *cis,trans* structural assignment (Δ δ = 1.60, as compared to Δ δ = 1.69 for ABA) (Roberts *et al.*, 1968). The olefinic proton nearest the ring is represented by a quartet at δ = 5.62 and the next one by a doublet at δ = 7.22.

The acid from the last g.c. peak after two recrystallizations from 2 ml of acetone per g, melted at 101–3° C (Roberts *et al.*, 1968). The nmr spectrum of this acid in deuterochloroform showed it to be *trans,trans*-α-ionylideneacetic acid (Figure 2C) [δ 0.83 (s, 3), 0.91 (s, 3), 1.32 (m, 4, J = 6Hz), 1.58 (d, 3, J = 1.5Hz), 2.12 (d, 1, J = 9Hz), 2.30 (d, 3, J = 0.5Hz), 5.48 (m, 1), 5.78 (d, 1, J = 1Hz), 6.04 (d, 1, J = 4Hz), 6.10 (s, 1)]. The interpretation of this spectra is similar to that of the *cis,trans* isomer except on two points. First, the side chain methyl is at a lower field since it is now *cis* to the carboxylic acid group (compare δ = 2.30 to 1.94 for Vb); and second, the two side chain olefinic protons nearest

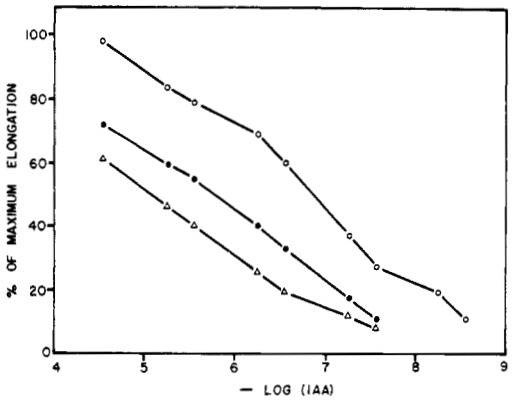

Figure 4. The inhibition of IAA stimulated *Avena* coleoptile elongation

The percent of maximum elongation is plotted so that the results of many bioassays could be averaged. Each point has a standard deviation of less than ±5%. ○—○ shows the variation of elongation with IAA concentration (molar) alone. ●—● shows the variation with IAA concentration and 0.1 mg/liter abscisic acid (VII) (3.79×10^{-7}M). △—△ shows the variation with IAA concentration and 0.1 mg/liter *cis, trans*-α-ionylideneacetic acid (V_b) (4.28×10^{-7}M)

the ring now have chemical shifts very close to one another [$\Delta\ \delta$ 0.06 compared to $\Delta\ \delta = 0.07$ for *trans,trans* ABA (Roberts *et al.*, 1968)]. *Anal.* Calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46: Found: C, 77.07; H, 9.56.

Biological Activity. One of these isomers, *cis,trans*-α-ionylideneacetic acid, is an analog of abscisic acid (Ohkuma *et al.*, 1965; Roberts *et al.*, 1968) which lacks both ring oxygens. When its bioactivity was investigated it proved to be a very potent plant bioactive substance. In the cotton explant bioassay (Ohkuma *et al.*, 1965) less than 10^{-10} moles of *cis,trans*-α-ionylidene-acetic acid per abscission zone gave a significant increase in abscission rate, and this is not more than one order of magnitude greater than the amount of natural abscisic acid required to give a comparable effect (Figure 3). In another bioassay system, the inhibition of auxin-stimulated *Avena* coleoptile elongation, *cis,trans*-α-ionylideneacetic acid was slightly more effective than abscisic acid (Figure 4), but the molecular ratio is only about 9:10. In contrast, *trans,trans*-α-ionylideneacetic acid is moderately active in the cotton explant test, and completely inactive in the *Avena* test. *Trans,trans*-β-ionylideneacetic acid [*trans,trans*-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-methyl-2,4-pentadienoic acid] (Phillips, 1956) was also tested with explants and is completely inactive.

Since it is unlikely that a precursor should have a greater activity, these data suggest that the ring oxygen functions of abscisic acid are relatively unimportant to its *Avena* activity and that the carbon-carbon double bond system is of considerable importance.

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