Structures Related to Abscisic Acid and Their Effect on Plant Growth

Ivan C. Popoff,* Roy M. Sachs,[1] and John B. Gibbs

Eight lower homologs of abscisic acid were prepared and tested as growth inhibitors on barerooted bean seedlings. The data suggest that a skeleton of citrylideneacetic acid, rather than of ionylideneacetic acid, is sufficient for growth inhibition caused by structures related to abscisic acid.

The effects of Abscisin-II on plants (Ohkuma *et al.*, 1963), its identification as abscisic acid (ABA, I-a) by Ohkuma *et al.* (1965), and its synthesis by Cornforth *et al.* (1965) led to numerous investigations which were reviewed by Addicott and Lyon in 1969. Recent studies on related structures (Asmudson *et al.*, 1968; Sondheimer and Walton, 1970; Orinati and Kamashita, 1970a–d) indicate that, if an appreciable activity of I-a is to be retained, its polyfunctional structure can be simplified only to a certain limited extent. Dealing primarily with the unsaturation of ABA-s molecule, with its oxygen-containing functions, and with the cis–trans isomerism, these studies suggest that a skeleton of α- or β-2-*cis*-ionylideneacetic acid (I-b and II-a) may be a prerequisite for the plant response caused by I-a. The primary objective of the work reported in this paper was to study the effect of some additional ABA-skeleton modifications on growth inhibition of barerooted bean seedlings.

Of particular interest to us were compounds of the general structure I containing a trimethylated cyclohexene ring attached to an acrylic moiety (R=C=C—COOR′′′) rather than to the pentadienoic chain of I-a; furthermore the open-chain structures III were intriguing since they are isomeric to I and could be considered as natural precursors of I. The compounds I-c, II-c, and III-c, containing the pentadienoic acid moiety of I-a, were prepared and tested primarily for comparison with the shorter-chain products. After we completed our study, the activities of the α,β-cis and trans acids of I-c on rice seedlings (Orinati and Yamashita, 1970a), α,β-cis and trans acids of II-c on bean embryonic axes (Sondheimer and Walton, 1970), and the α,β-cis and trans acids of III-c on rice seedlings (Orinati and Yamashita, 1970d) were published. Furthermore Orinati and Yamashita (1970b) had included in their rice seedlings test the methyl esters homologous to our ethyl esters I-f and I-g, and Mousseron-Canet *et al.* (1970) disclosed also recently the growth effect of the free trans acid of I-g on coleoptiles. These publications now make possible a correlation of the test systems of these three distinguished teams with our tests.

PRODUCTS

The structures of the twelve compounds studied in this work and the three reference products (I-a, I-b, and II-a) are shown under the general formulas I–III (see Table I). The α- and β-cyclocitrylideneacetic acids I-d and II-b, respectively, and ethyl α-cyclocitrylideneacetate (I-e) were prepared as described by Royals (1947). The configuration of the side chain of II-b and I-d was known to be trans and was confirmed by our nmr analyses. The ethyl acetate I-e was obtained in 50% yield from the known open chain ester III-a (Royals, 1947) which in turn resulted (60% yield) from a Wittig-type reaction of citral with triethyl phosphonoacetate as described for III-c, rather than by the Reformatsky reaction of citral utilized by Royals. The physical properties of I-e and III-a, however, were in agreement with the data given for these compounds in the literature. The cis–trans isomers ratio for the α,β-unsaturation of our III-a was not determined but, as for I-c, II-c, I-h, III-b, and III-c, on the basis of previous studies (Roberts *et al.*, 1968; Popoff *et al.*, 1969), we assumed that the trans isomer is predominant if not exclusive. Thus, the trans isomer was assumed to be predominant also for I-e. The other two known structures I-c and II-c were also prepared by the Wittig-type reaction described for III-c rather than by the Reformatsky method used for their syntheses (Young *et al.*, 1944). Their physical properties were in agreement with the published data and the trans configuration of their γ,δ unsaturation was the same as for the side-chain unsaturation of α- and β-ionone used as starting materials.

The preparation of the novel compounds I-f, I-g, I-h, I-i, III-b, and III-c is described in the Syntheses section. As indicated by the structures listed in Table I, with the exception of II-b and I-d, the products tested did not represent the pure cis or trans isomers relative to the unsaturation in α,β position to the carboxylic group. Their elemental analyses and infrared spectra, however, confirmed the expected empirical formulas and functional groups.

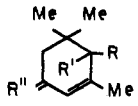
TEST PROCEDURE

Black Valentine bean seedlings (*Phaseolus vulgaris* L.) grown in sand were placed barerooted in 1 l. of continuously aerated 0.5-Hoagland's nutrient solution. When their first trifoliate leaf was 50% expanded, they were selected for uniformity and treated with 1 ml of a 10^{-1} *M* acetone solution of the test compound; thus the concentration of the compound in the test was 10^{-4} *M*. The tests were conducted at controlled temperature (26 ± 2°C) with a daily photoperiod of 16 hr (*ca.* 1300 ft-candles at plant top level). The effect in the stem elongation between the cotyledonary node and terminal meristem was measured on 4–6 replicas for each compound after 4-day exposure; in some cases also a 3-day exposure test was carried out. The average activity found is reported in Table I in terms of percent of growth inhibition relative to (RS)-abscisic acid (I-a). The actual average growth inhibition by (RS)-ABA was 64 and 83% in the 3-day and 4-day test, respectively.

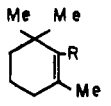
The 4-day test with I-h is considered unreliable due to irregularities observed during the experiment with this compound.

Pennwalt Corporation, King of Prussia, Pa. 19406
[1] Plant Sciences Laboratories, Department of the Army, Fort Detrick, Frederick, Md. 21701. Present address: Department of Environmental Horticulture, Agricultural Experiment Station, University of California, Davis, California 95616.

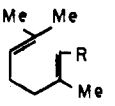
Table I. Structures and Growth Inhibition Data



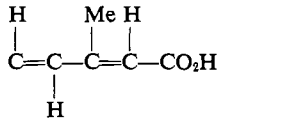
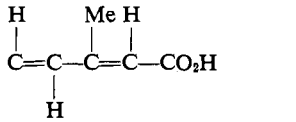
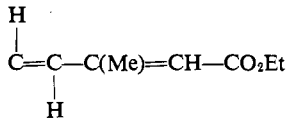
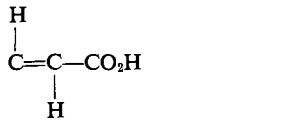
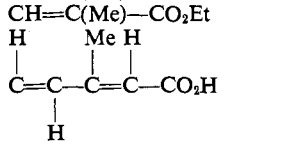
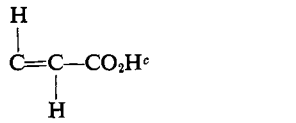
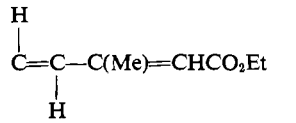
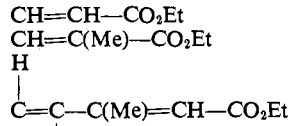
I



II



III

No.	R	R′	R″	% Activity relative to (RS)-ABA(I-a) exposure days 3	4
I-a		OH	O	100	100
I-b		H	H_2	*a*	*a*
I-c		H	H_2	...	2
I-d		H	H_2	48	89
I-e	CH=CH—CO_2Et	H	H_2	0	66
I-f	CH=CH—CO_2Et	H	O	56	13[b]
I-g	CH=CH—CO_2Et	OH	O	...	48
I-h	CH=C(Me)—CO_2Et	H	H_2	48	53
I-i	CH=C(Me)—CO_2Et	H	O	63	61
II-a				*a*	*a*
II-b		...	...	93	100
II-c		...	...	...	27
III-a	CH=CH—CO_2Et	...	...	0	55
III-b	CH=C(Me)—CO_2Et	...	...	56	78
III-c		...	...	...	18

[a] Product was not tested; its structure was used as reference in this paper. [b] Data not reliable due to anomalies in the 4-day test of I-f. [c] Product contaminated with 10–15% of citral.

DISCUSSION OF RESULTS

The data of the two tests indicate that the growth inhibition is enhanced by longer exposure. Thus, it seems that the uptake of the agent is relatively slow even by the bareroot system or the uptake and translocation are in competition with a possible deactivation of the product in the plant. Since our products represent predominantly the α,β-trans isomers for reasons mentioned above, the very low relative activity of the α- and β-ionylideneacetates I-c and II-c is in agreement with the data recently reported for the respective free trans acids by Orinati and Yamashita (1970a) and by Sondheimer and Walton (1970). The small effect of III-c observed by us confirms the lack of activity on rice seedlings reported for the α,β-trans acid corresponding to III-c (Orinati and Yamashita, 1970d). The activity of I-g (and I-f) on bean seedlings is in better agreement with the wheat coleoptile test of the trans acid of I-g carried out by Mousseron-Canet *et al.* (1970), than with the negative rice seedlings results for the methyl esters corresponding to I-f and I-g (Orinati and Yamashita, 1970b).

The relatively high activity exhibited by the nine structures (see Table I) indicates strongly that an ionylideneacetic acid skeleton is not absolutely necessary for growth inhibition by ABA-related products. A lower homolog of ionylideneacetic acid, *i.e.*, cyclocitrylideneacetic acid (I-d and II-b), appears to be almost as active as I-a on bean seedlings. This raises the question of possible enzymatic degradation of ABA-s pentadienoic acid chain (I-a) to the acrylic acid moiety of I-d as part of ABA-s mechanism of action. The relatively low growth inhibition caused by I-c, II-c, and III-c suggests that this degradation may be slow and possibly competing with a simultaneous deactivation by attack on the remaining unsaturation or on other sites of the molecules. Considering the generally observed specificity of enzyme reactions, the difference in activity of the cis and trans acids of I-c and II-c observed by Orinati and Yamashita (1970a) and Sondheimer and Walton (1970), respectively, would support this postulated degradation theory.

The difference in hydrophilic character and therefore in rate of uptake of the free trans acid I-d and its ethyl ester I-e (augmented by a possible small contamination of I-e with its α,β-cis isomer) is probably responsible for the somewhat lower activity of I-e; the latter caused about the same inhibition as the ethyl esters I-f to I-i, III-a, and III-b. A comparison of the activity of the acetates I-e and I-h with the data for their ring oxidation products I-g and I-i, respectively, is in agreement with the results observed for I-b by Asmudson *et al.* (1968); thus it appears that the oxo and hydroxyl groups in the rings of I-a, I-f, I-g, and I-i are not necessary for growth inhibition properties of I.

It should be noted that two of the three open-chain products (III) tested were active. They are possible natural precursors for the ring structures I and II. The shorter-chain structures of the citrylidenes III-a and III-b were three to four times more active on bean seedlings than the pseudoionylidene III-c, probably because of the trans configuration of the α,β unsaturation of III-c, although the cis isomer of the free acid was also reported to be inactive on rice seedlings (Orinati and Yamashita, 1970d).

SYNTHESES

Ethyl 3-(4-Oxo-2,6,6-trimethyl-2-cyclohexen-1-yl)acrylate (I-f). To a stirred refluxing solution of 50 g (0.225 mol) of ethyl α-cyclocitrylideneacetate (I-e) in 200 ml of *tert*-butyl alcohol was added slowly 530 ml of *tert*-butyl chromate reagent which was prepared as described by Roberts *et al.* (1968). The mixture was refluxed for 5 hr and then poured into 500 ml of water. The excess chromic acid was destroyed with methanol and oxalic acid and the product was taken up in ether (2 × 300 ml). The ether layer was washed with 10% sodium bicarbonate solution, dried over magnesium sulfate, and evaporated to provide a residue which, on distillation, gave 15 g (28%) of the desired product: bp 132–136°C/0.4 mm, $n^{25}D$ 1.5020. *Anal.* Calcd for $C_{14}H_{20}O_3$ (I-f): C, 71.16; H, 8.53. Found: C, 70.94; H, 8.89.

Ethyl 3-(1-Hydroxy-4-oxo-2,6,6-trimethyl-2-cyclohexen-1-yl)acrylate (I-g). To a stirred refluxing solution of 25 g (0.113 mol) of ethyl α-cyclocitrylideneacetate (I-e) in 100 ml of *tert*-butyl alcohol was slowly added 265 ml of *tert*-butyl chromate reagent which was prepared as described by Roberts *et al.* (1968). The mixture was refluxed for 24 hr before 500 ml of water was added; the excess chromate was decomposed with methanol and oxalic acid. After cooling, the aqueous solution was extracted with chloroform (3 × 200 ml). The chloroform extract, after being exhaustively washed with 10% sodium bicarbonate solution and concentrated *in vacuo*, provided 13.5 g of a crude product. Filtration of the benzene solution of this product through a column of Woeln's basic Grade I alumina, and removal of the benzene by evaporation gave a gummy residue which solidified slowly upon addition of 23 ml of hexane. Recrystallization from hexane gave 4 g (14%) of the white solid product (mp 85–86°C). *Anal.* Calcd for $C_{14}H_{20}O_4$ (I-g): C, 66.64; H, 7.99. Found: C, 66.68; H, 8.01.

Ethyl 2-Methyl-3-(2,6,6-trimethyl-2-cyclohexen-1-yl)acrylate or Ethyl 2-(α-Cyclocitrylidene)propionate (I-h). A mixture of 50 g of sulfuric acid and 375 g of 85% phosphoric acid was cooled to 5°C and stirred vigorously while 60 g (0.25 mol) of ethyl 2-citrylidenepropionate (III-b) was added dropwise during 15 min. The cooling was removed and the temperature allowed to rise to 25°C during 30 min. The dark, reddish-brown reaction mixture was poured into 500 ml of water and the yellow oily product was extracted with ether (2 × 300 ml). The ether layer was washed with water, dried over magnesium sulfate, and evaporated. Distillation of the residue provided 40 g (66%) of the desired product: bp 118–120°C/8 mm, $n^{25}D$ 1.4865. *Anal.* Calcd for $C_{15}H_{24}O_2$ (I-h): C, 76.22; H, 10.24. Found: C, 76.13; H, 10.38.

Ethyl 2-Methyl-3-(4-oxo-2,6,6-trimethyl-2-cyclohexen-1-yl)-acrylate (I-i). To a stirred refluxing solution of 45 g (0.19 mol) of ethyl 2-(α-cyclocitrylidene) propionate (I-h) in 200 ml of *tert*-butyl alcohol was added slowly 530 ml of *tert*-butyl chromate reagent (Roberts *et al.*, 1968). The mixture was refluxed for 5 hr and then poured into 500 ml of water. The excess of chromic acid was destroyed with methanol and oxalic acid; the product was extracted with ether (2 × 300 ml). The ether layer was washed with a 10% aqueous sodium bicarbonate solution, dried over magnesium sulfate, and evaporated to provide a residue which, on distillation, gave 12.5 g (26%) of the desired product: bp 117–120°C/0.45 mm, $n^{25}D$ 1.4980. *Anal.* Calcd for $C_{15}H_{22}O_3$ (I-i): C, 71.97; H, 8.86. Found: C, 72.20; H, 9.23.

Ethyl 2,5,9-Trimethyl-2,4,8-decatrienoate or Ethyl 2-Citrylidenepropionate (III-b). To a stirred solution of 7.2 g of sodium hydride (0.3 mol) in 200 ml of ether was added dropwise 71.4 g (0.3 mol) of triethyl α-phosphonopropionate (Tömöközi, 1966) at room temperature. The addition lasted 40 min. The mixture was stirred for an additional 30 min to allow complete evolution of hydrogen; 45 g (0.3 mol) of citral was then added to the stirred solution over a period of 30 min. Stirring was continued at room temperature for an additional 1 hr. The reaction mixture was filtered and the ether filtrate was evaporated to provide a residue which, on distillation, afforded 60 g (85%) of product: bp 120–125°C/8 mm, $n^{25}D$ 1.5015. *Anal.* Calcd for $C_{15}H_{24}O_2$ (III-b): C, 76.22; H, 10.24. Found: C, 77.00; H, 10.48. Based on its infrared spectrum and C analysis, the product was contaminated with 10–15% of citral.

Ethyl 3,7,11-Trimethyl-2,4,6,10-dodecatetraenoate or Ethyl Pseudoionylideneacetate (III-c) (bp 136–140°C/0.25 mm, $n^{25}D$ 1.5380) was prepared in 17% yield from pseudoionone and triethyl phosphonoacetate by the method described above (III-b). *Anal.* Calcd for $C_{17}H_{26}O_2$ (III-c): C, 77.82; H, 9.99. Found: C, 77.96; H, 9.97.

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