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Transpiration-Inhibiting Abscisic Acid Analogs

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Abstract. Synthetic analogs of abscisic acid (ABA) and their inhibiting effect on transpiration rates of detached barley leaves are presented. By systematically varying the carbon skeleton of ABA, the influence of structural changes on biological activity was investigated. The results show that a properly substituted cyclohexane unit and a six-carbon side chain seem to be indispensable for high ABA-like activity, whereas the oxidation state of the terminal carbon atom in the side chain appears to be less essential. Thus, synthetic compounds have been created that exhibit biological activities comparable both in type and strength with ABA itself. On the basis of molecular models, a hypothesis of the geometric arrangement of essential substructural units is proposed.

Many regions worldwide, which are among the most productive agricultural areas, increasingly depend on artificial irrigation. Thus, the price of water will play an even more decisive role in the future of agricultural production with regard to profitability (Wittwer 1981). Therefore, the task of agrochemical research is to search for active compounds which regulate the water consumption of crop plants (Bergmann 1977, Davies et al. 1986, Fenton et al. 1982, Jones 1981, Jung and Rademacher 1983).

One possible solution to this problem is to use abscisic acid (ABA) to reduce the transpiration of the plants and thus decrease water consumption during cultivation (Addicott 1983, Mansfield et al. 1978, Quarrie 1984). This is greatly restricted by the high price and the complexity of the conventional synthesis of ABA (Meyer et al. 1976). Readily obtainable synthetic substances with ABA-like action would therefore be of considerable practical importance.

ABA-like compounds (Fig. 1), which have very high anti-transpiratory activity, have already been reported (Bliesener et al. 1985, Dörffling 1985, Grossmann and Jung 1984, Jung and Grossmann 1985, Rademacher et al. 1987). In addition, these compounds have been found to protect crop plants from chilling and freezing stress (Flores et al. 1988, Schmidt et al. 1988). Compared with ABA, these substances are easier to prepare.

The compounds listed in Fig. 1 differ structurally from ABA in three respects. The keto function in the six-membered ring is acetalized, the carboxylic acid radical is replaced by an acetal group, and the *trans*-double bond is replaced by a triple bond. The fact that the activity is similar to that of ABA shows that the structural differences have no detectable adverse effect on the biological activity of the compounds.

This fact becomes plausible if a three-dimensional structural comparison of the two compounds with ABA is made by means of computer modeling. It is found that replacement of the *trans*-double bond in ABA by the triple bond in the synthetic product has virtually no effect on the overall geometry of the molecules. That is the spatial position of the oxidized C-1 atom in the side chain, relative to the ring system, remains almost the same (see Fig. 2).

The aims of the present study were (1) to establish structure–activity relationships of synthetic analogs of ABA, characterizing the structural requirements for ABA-like activity, and (2) to find substances which would be more easily obtainable and more effective in reducing the water consumption than the compounds shown in Fig. 1.

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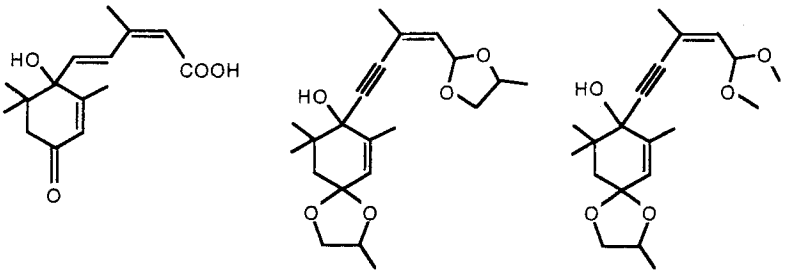


ABA LAB 144 143 LAB 173 711

Fig. 1. Chemical structures of ABA and synthetic analogs (see Bliesener et al. 1985).

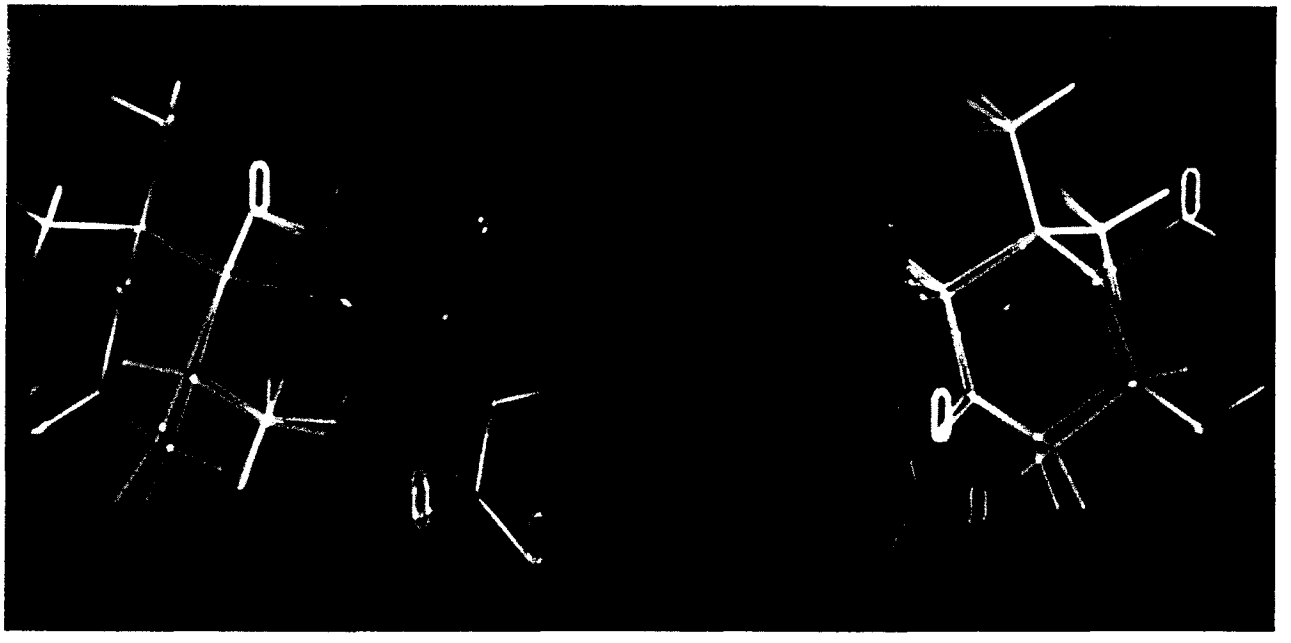
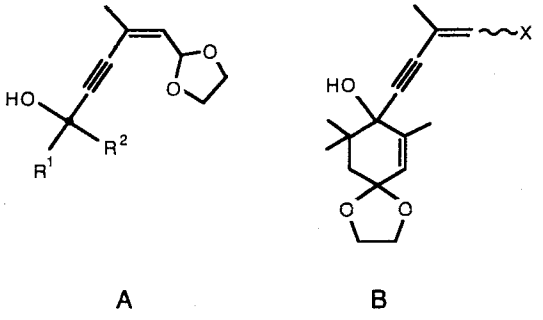


Fig. 2. Three-dimensional structural comparison of LAB 173 711 with ABA by means of computer modeling.



A B

Fig. 3. General structures of the synthesized compounds. The first type of variation—compared with LAB 173 711 (Fig. 2)—was made by replacing the cyclohexene alcohol moiety with other tertiary alcohols (Type A) leaving the side chain nearly unchanged. The second type consists of compounds with modified side chains (Type B).

Materials and Methods

Synthesis of the ABA Analogs

To cover as wide a range as possible of very different structures without losing sight of the basic structure of ABA or the ABA analogs found in Fig. 1, we concentrated our work on the synthesis of the readily obtainable enynes *A* and *B* (Fig. 3).

In contrast to the complicated synthesis of the ABA type with a diene side chain, the preparation of these compounds can be carried out in a simple manner by reacting suitable ketones with acetylenes and a base (Fig. 4, showing the synthesis of LAB 221 218). The acetylenes were obtained from commercially available *cis*- or *trans*-enynols by conventional methods.

n-Butyllithium (n-BuLi) was used as the base. Other, cheaper bases, such as potassium hydroxide, can also be used. However, *n*-BuLi was the most suitable for our laboratory synthesis, since these experiments were all reproducible and gave good yields (generally >80%) and byproducts occurred only rarely. Furthermore, the experimental procedure was very simple: *n*-BuLi was

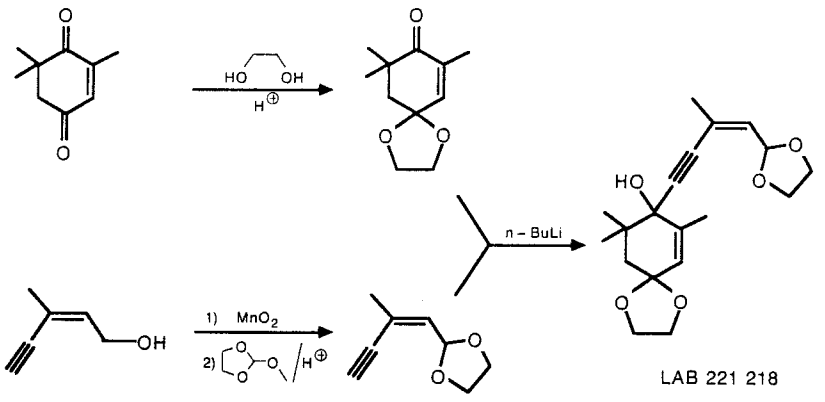


Fig. 4. Typical synthesis of the ABA analogs. As an example, the synthesis of the biologically most active compound LAB 221 218 is shown. Reaction of a ketone with an anionic acetylene leads to tertiary alcohols, normally in high yields.

dissolved in tetrahydrofuran (THF), the acetylene derivative was added dropwise at −40°C, the mixture was stirred for 15 min, a solution of the ketone in THF was added dropwise, then slowly warmed to room temperature, allowed to react from 10 min to 2 h, the product was then hydrolyzed, and finally extraction with ether was performed. The products were generally obtained in sufficient purity (>90%). If necessary, purification was carried out by chromatography (silica gel, cyclohexane/ethyl acetate mixtures), distillation (bulb tube), or, in individual cases, by recrystallization. Chiral compounds were all obtained in the form of racemates. The resulting diastereomers were not separated. Regarding the double bond in the enyne system of the side chain, all respective compounds contained at least 85% of the Z-isomer. With regard to the other possible isomerisms, no attempt was made to determine the configuration of the components and their proportions.

Transpiration Test with Detached Barley Leaves

Spring barley (*Hordeum vulgare* L. cv. Union) was grown under greenhouse conditions (Jung and Grossmann 1985). Primary leaves were removed and the cut ends immersed in aqueous test solutions in plastic vials as described by Jung and Grossmann (1985). For the preparation of the test solutions, the compounds (10% formulated in a mixture containing 80% of cyclohexanone and 20% of Emulphor EL) were diluted with distilled water to give a final concentration of 10^{-4} mol L^{-1}. After a 24-h incubation under standardized conditions in a growth chamber (14 h/day, 250 μmol m^{-2} s^{-1}, 400–700 nm; fluorescent lamps, radium HLRV, 1000 W; at 25°C), the water loss per vial was determined by weighing samples and was expressed as a percent with reference to the control. The mean of three replicates was determined. The individual standard errors were less than 10%.

Presentation of the Results

In Figs. 5–8, the various types of compounds synthesized are listed and at the same time classified according to their action in the biological test. The anti-transpiratory activity of the compounds shown increases from top to bottom—corresponding to the percentage scale of water consumption.

Results and Discussion

Figure 5 includes compounds in which the keto group of ABA in the six-membered ring was replaced by a methylene group. For comparison of activities, ABA is also included. In the set of derivatives shown, it is clear that high activity (<60% water consumption relative to the control) is achieved only when two geminal methyl substituents are present in the ring in the α-position with respect to the acetylene side chain. Other sub-

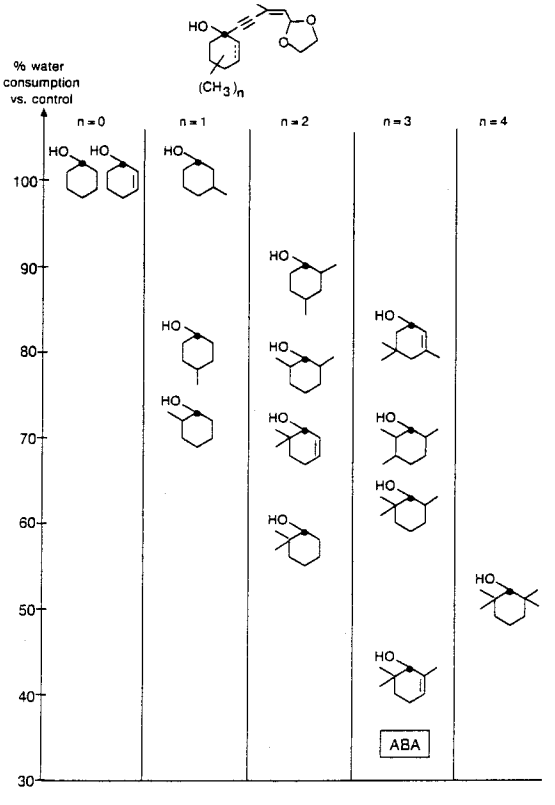


Fig. 5. Influence of ABA and analogs on transpiration of detached barley leaves. The activity of ABA and analogs with unchanged side chain is shown. The cyclohexane (ene) ring bears 0–4 methyl substituents in different positions. Diastereometric mixtures were not separated, the stereochemical structure of diastereomeric mixtures and their composition was not determined.

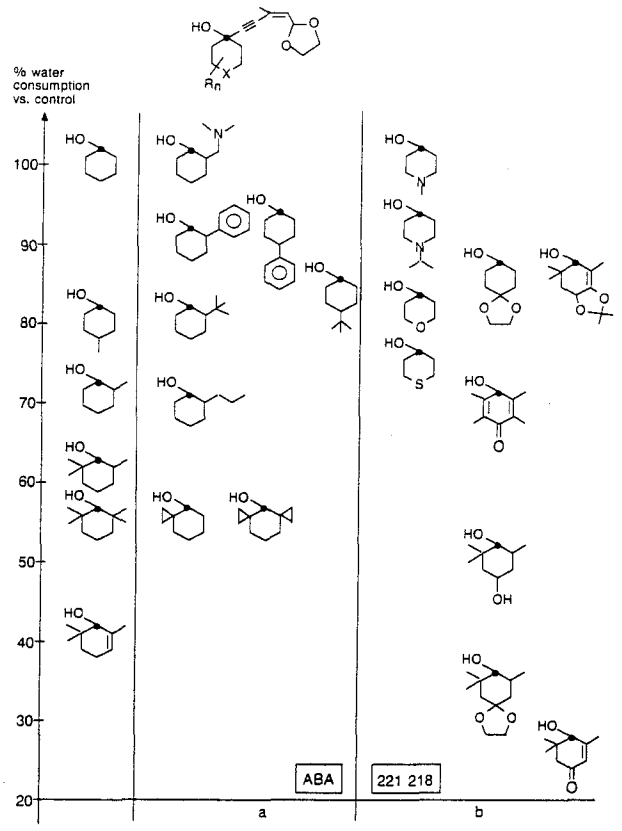


Fig. 6. Influence of ABA and analogs with (**a**) different alkyl and aryl substituents and (**b**) heteroatoms in or attached to the ring on transpiration of detached barley leaves. For comparison the results of structurally similar compounds of Figure 5 are listed in the first column.

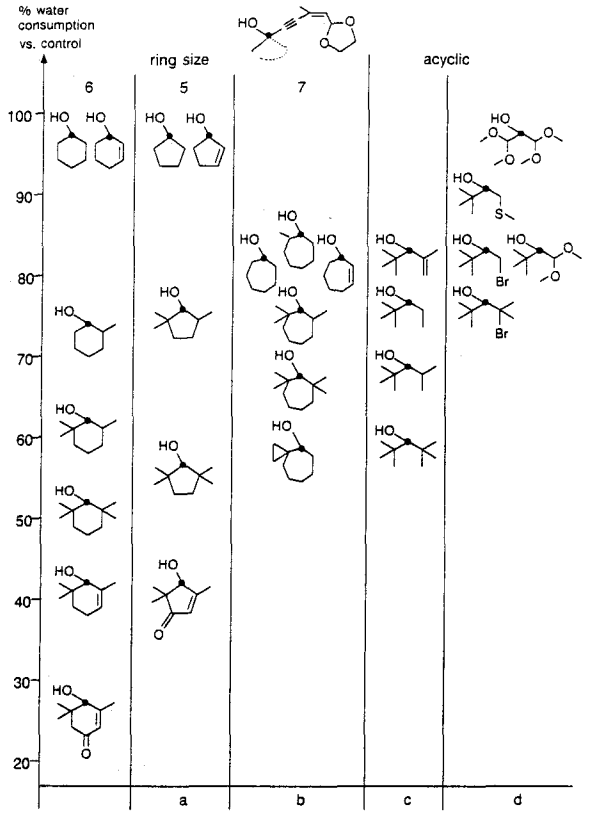


Fig. 7. Influence of ABA analogs with five- and seven-membered carbocyclic alcohols (**a, b**) and acyclic tertiary alcohols with and without heterosubstituents (**c, d**) on transpiration of detached leaves. For comparison the six-membered carbocyclic analogs are shown in the first column.

stitution patterns led to a decrease in activity or, as in the case of the unsubstituted cyclohexane or cyclohexene derivative, to a loss of activity.

It should also be noted that the compounds in Fig. 5 are actually obtained as diastereomer mixtures of different compositions in cases where *cis/trans*-stereo-isomerism of the methyl groups relative to the acetylene side chain is possible. For this reason, no conclusions can be drawn at present beyond those stated above.

Figure 6a shows that replacement of *one* methyl group in the α-position by larger substituents (*n*-propyl, tert-butyl, or phenyl) tended to have an adverse effect. In contrast, the activity did not change significantly if the geminal methyl groups in the α-position were cyclized to form a spiro-fused three-membered ring.

Figure 6b shows the effect of various groups in position 4 relative to the acetylene side chain. The loss of activity after replacement of the keto or ketal group by the methylene group is evident. Surprisingly, the activity increased again if the methylene group was relaced by NR, O, or S in the corresponding piperidines, pyran, or thiopyran.

Figure 7 shows compounds which, instead of the six-membered ring, contain correspondingly substituted five- (Fig. 7a) and seven-membered (Fig. 7b) rings. The optimum compounds all contained the six-membered ring. In the tetramethyl derivatives, the trend of declining activity from the six-membered ring through the five-membered ring to the seven-membered ring is apparent.

All open-chain analogs (Fig. 7c) were also substantially less active than the six-membered rings, in this case replacement of methyl groups by bromine, methoxy, or thiomethyl radicals (Fig. 7d) led to a further decrease in activity.

Finally, Fig. 8 contains results for compounds in which the side chain was varied while the optimally substituted six-membered ring was kept constant. The optimum structures here, in addition to the Z-dienecarboxylic acid chain of ABA itself, are the Z-enynealdehyde and the corresponding (methyl)ethylene glycol acetal, the dimethyl acetal, and the Z-enynecarbinol.

It is known that only the Z-isomer of ABA possesses activity, whereas the E-isomer is substantially inactive (e.g., Kriedemann et al. 1972). Hence, it is surprising that the E- and Z-isomers of the enynealdehydes were almost identical in activity. In the case of the enynecarbinol also, the "wrong" E-isomer still has considerable activity. It is not clear whether this is due to isomerization of the aldehyde or carbinol in the course of testing. For ABA itself, the barrier for such isomerization reactions might be higher. In comparison to the Z-carbinol itself, the Z-carbinol methyl ether showed a considerable decrease in activity.

In the neopentyl glycol acetal, which was relatively difficult to hydrolyze, the activity was virtually lost. This is particularly

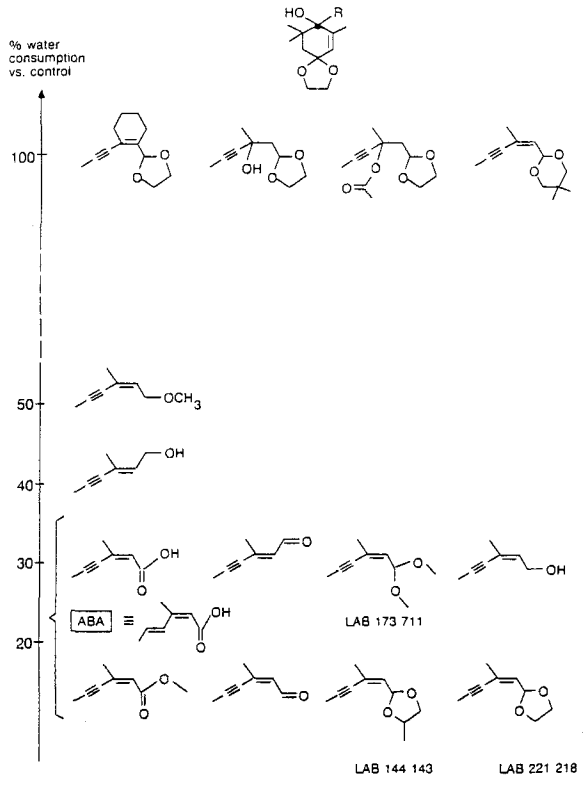


Fig. 8. Influence of ABA and analogs with modified side chains compared to ABA on transpiration of detached barley leaves. Aldehydes, simple acetales, acids, and esters are in the same range of activity as ABA.

impressive on comparing the highly active five-membered ring acetals.

The formal addition of water or acetic acid at the double bond of the enyne system also led to inactive derivatives, as did the incorporation of a double bond into a cyclohexene ring.

On comparison of our results with earlier work on structure–activity relationships of ABA-like compounds (e.g., Milborrow 1974, Orton and Mansfield 1974, Walton 1983), three basic comments can be made.

First, our work on ABA analogs with modified side chains confirms earlier results obtained with similar molecules. In all our active compounds the terminal carbon atoms of the side chain are present in an oxidized form [CH_2OH, CHO, $CH(OR)_2$], which allows further oxidation. Two compounds, which are in a form difficult to hydrolyze or oxidize, are significantly less active. Namely, the methyl ether and the neopentyl acetal possess little or no activity (Fig. 8).

Second, the geminal methyl substituents in the α-position, with respect to the acetylene side chain, make a very major contribution to the activity. This is demonstrated by the comparatively high activity of the otherwise unsubstituted 2,2-dimethylcyclohexanol derivative in Figure 5. Conversely, the lack of these two methyl groups leads to an extremely sharp decrease in activity, as reported for the methyl ester of 6′,6′-didemethyl-ABA (Fig. 9) (Nagano et al. 1980). In comparison with ABA, this derivative exhibited little activity (about 5%) in a rice seedling test.

Finally, a new aspect is evident from the observation that

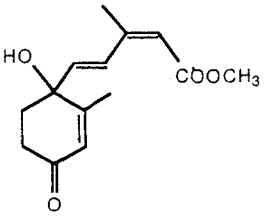


Fig. 9. Chemical structure of methyl 6′,6′- didemethylabscisate (see Nagano et al. 1980).

replacement of the methylene group in position 4 to the side chain by hetero atoms leads to compounds with higher activity (Fig. 6b). Here, suitably modified (SO, SO_2, NR) and α-methylated derivatives could give highly active compounds.

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