SYNTHESIS AND ACTIVITY OF NEW ABSCISIC ACID ANALOGUES POSSESSING HETERO FIVE-MEMBERED RING IN THEIR MOLECULES

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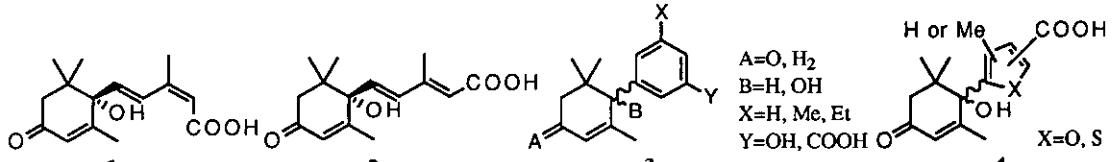
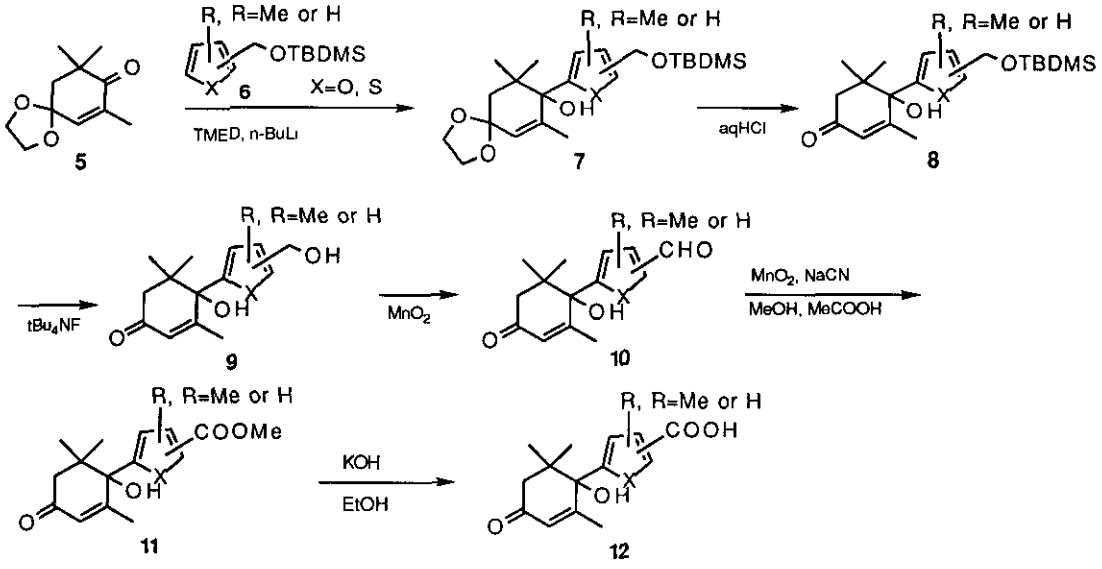
Abstract-The conjugated dienyl side chain of plant hormone abscisic acid was substituted with five-membered hetero-rings having a carboxyl group on their 2-position. Some of the synthesized compounds showed a remarkable activity similar to ABA in inhibiting cress seed germination and α-amylase induction by gibberellin in half-cut barley seed assays.

Abscisic acid (ABA, **1**) is a plant hormone regulating many physiological processes in plants. However, it has a serious drawback for its application as a plant growth regulator because its side chain (2*Z*, 4*E*-3-methyl-2,4-pentadienoic acid moiety) is readily isomerized to the biologically inactive 2*E* isomer (**2**) by light. Creating highly active ABA analogs with a fixed structure may provide new compounds as useful plant growth regulators, and may lead to an understanding of the mechanism of functions of ABA. Previously synthesized analogs did not have ABA-like activities.[1] Recently, we reported that replacement of the side chain of ABA with a phenyl group involving the proper functional groups resulted in light-stable racemic ABA analogues (**3**). Some of these



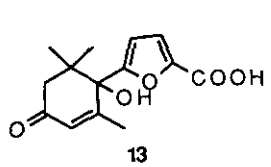
newly synthesized compounds exhibited biological activities equivalent to 1/3 to 1/10 that of ABA.[2] Through studies on the structure-activity relationships of **3**, we found that the combination of the functional groups (A=O, B=OH and Y= COOH) was essential for the activity exhibition. The methyl

group was the best functional group at the position X for the highest activity and could be replaced with a hydrogen without significant reduction in its activities. On the basis of this result, we thought that a compound possessing the essential functional groups in the same spatial arrangement like in the ABA should have ABA-like activity. Many compounds were nominated and their structural similarities to ABA and **3** were evaluated by comparing the computer-calculated structures. Consequently, the ABA analogues (**4**) having hetero five-membered ring were thus selected as the target compounds, for which we anticipated interesting biological activity because of the different chemical character of the hetero ring from the phenyl one.



The tested products were prepared as shown in the scheme above. All the products were purified by column chromatography (silica gel), and characterized by spectroscopic methods (^{1}H-nmr and ms). First, the synthesis of the key intermediate carbinol (**7**) was carried out from the previously reported keto ketal (**5**)[3] by the coupling reaction with lithiated furan or thiophene (**6**) which were readily prepared by reduction of the corresponding furan or thiophene 2-carboxaldehyde with sodium borohydride and subsequent protection of the generated hydroxymethyl group with t-butyldimethylsilyl group. Treatment of **7** with aqueous hydrochloric acid readily afforded the keto alcohol (**8**). After desilylation with tetrabutylammonium fluoride, the alcohol (**9**) was converted to the methyl ester (**11**) by two-step oxidation using manganese(II) dioxide. Finally, hydrolysis of the methyl ester (**11**) with KOH-EtOH gave the corresponding target compounds.

We determined the ABA-like activity of the synthesized compounds (**13-22**) in two assay systems, *i.e.* (i) inhibition of cress seed germination and (ii) inhibition of α-amylase induction with treatment of gibberellic acid (GA). We selected these assays as typical representatives of *in vitro* and *in vivo* tests for ABA-like activities respectively because the induction of α-amylase is thought to be the initial step of germination after imbibition. The germination test following Taylor *et al.*[4]was carried out using duplicates of 25 seeds for each concentration of the analogs. The inhibitory activity of α-amylase was measured by the procedure of Yomo.[5] The activity of the isolated enzyme tested was only a sample representative of the total enzymes secreted by the barley seeds, since some of the enzymes were lost during extraction.



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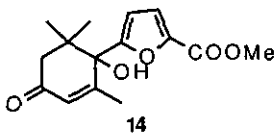

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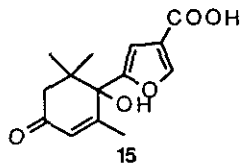

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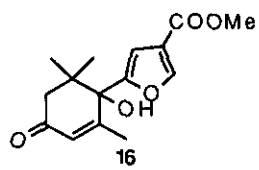

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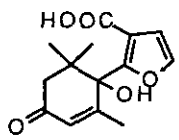

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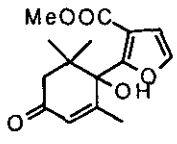

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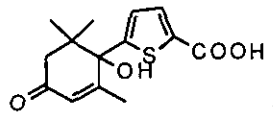

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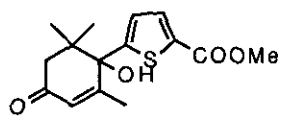

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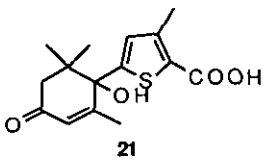

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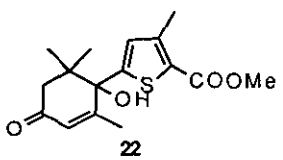

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The results of the bioassays are listed in Tables I (inhibition of cress seed germination) and II (inhibition of GA-induced α-amylase activity). The activity is expressed as pI_{50} value which indicates the negative logarithm of the concentration (M) of a compound for 50 % inhibition.

In the germination test, furan derivatives (**13**, **14**, **17** and **18**) showed inhibitory activity while thiophene derivatives (**19**, **20**, **21** and **22**) did not. The methyl ester (**14**) was 20 times more active than the free acid (**13**). In the α-amylase induction test, **13** showed activity but **14** showed no activity. As for the thiophene compounds, the free acid (**19**) showed the inhibitory activity but the methyl ester (**20**) did not. Generally, ester derivatives of ABA and ABA analogs were more active than their free acid in the germination test while in the α-amylase induction test an opposite result was recorded. The difference of the compounds in the ability to permeate seeds and in the

hydrolyzability of the esters may perhaps explain the results observed in **13, 14, 15, 16, 17** and **18**. The methyl group in the side chain of ABA has a significant role in activity exhibition[6] therefore we introduced a methyl group into the hetero ring. However, the 3-methylthiophene derivative possessed no ABA-like activity. In the α-amylase induction test, **21** completely lost its activity with the introduction of methyl group. We think that the geometrical relationship between the methyl group and the carboxyl group of **21** is just like that of inactive 2*E* isomer (**2**), which may throw some light as to why **21** does not show the activity. ABA showed the inhibitory activity against both tests at almost same concentration, but free acids of synthetic analogs showed some differences in their inhibitory activity from abscisic acid. For example, the inhibitory activity of cress seed germination of **13** and **17** is about 40-times less active than that of α-amylase induction. This implies that **13** and **17** are more specific inhibitors against α-amylase induction system than ABA and will be of great use in studying the mechanism of seed germination.

Table I Inhibitory Activity of Cress Seed Germination

Compounds	pI_{50}
13	2.8
14	4.2
15	<3
16	3.6
17	3.7
18	4.3
19	<3
20	<3
21	<3
22	<3
ABA	5.9

Table II Inhibitory Activity of α-Amylase Induction

Compounds	pI_{50}
13	4.5
14	<3
15	3.4
16	<3
17	5.3
18	<3
19	3.1
20	<3
21	<3
22	<3
ABA	5.8

The results obtained from the assays which were designed to identify the ABA-like activity indicated that some of the newly designed compounds did possess such activity, but they were less active than ABA. However, it is noteworthy that the hetero ring-substituted ABA analogs also exhibit the ABA-like activity and have a different manner of activity emergence from ABA. Consideration of the spatial arrangement of the essential functional groups as conducted in this research will be of great assistance in designing new compounds with ABA-like activity.

EXPERIMENTAL

The typical procedure for the synthesis of ABA analogs is described below. Other types of ABA analogs were synthesized using similar synthetic procedures.

2-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)-5-tert-butyldimethylsilyloxymethylfuran (**8**, R=H, X=O). A solution of *n*-buthyllithium in *n*-hexane (14 ml, 22 mmol) was added dropwise to a stirred solution of 3-*tert*-butyldimethylsilyloxymethylfuran (4.56 g, 20 mmol) and tetramethylethylenediamine (2.32 g, 20 mmol) in dry THF (50 ml)below -50 °C under N_2 flow. The mixture was then stirred for 10 min at -78 °C, and to this was added the keto ketal (**5**) (3.92 g, 20 mmol) in dry THF (20 ml). After the addition, the mixture was allowed to warm to room temperature, stirred for 30 min. and the poured into ice-cooled water. After addition of conc. HCl (10 ml), the mixture was stirredat room temperature for 30 min, and extracted with ether. The organic solution was washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo* . The residue was roughly chromatographed over silica gel (*n*-hexane: EtOAc=4: 1) to give 5.02 g of yellow oil, which was subjected to the next reaction without further purification after confirming the production of **8** (R=H, X=O) by GC-ms.

2-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)-5-hydroxymethylfuran(**9**, R=H, X=O). Tetrabutylammonium fluoride hydrate (2.83 g, 11 mmol) was added to a stirred solution of yellow oil (3.80 g) obtained above in THF (50 ml) at room temperature. After stirring for 2 h, the mixture was poured into 0.5N HCl solution and extracted with ethyl acetate. The organic solution was washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo* . The residue was chromatographed over silica gel (*n*-hexane: EtOAc=2: 1) to give 1.77 g of **9** (R=H, X=O). mp 78-80 °C; ^{1}H-nmr ($CDCl_3$) δ: 0.91 (3H, s), 1.16 (3H, s), 1.92 (3H, s), 2.20 (1H, d, *J*=16 Hz), 2.56 (1H, d, *J*=16 Hz), 4.59 (1H, s), 6.18 (1H, s), 6.28 (1H, d, *J*=3.4 Hz), 6.89 (1H, d, *J*=3.4 Hz). HR-EIms: Found: 250.1210 (M^+), Calcd for $C_{14}H_{18}O_4$: 250.1205.

5-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)furan-2-carboxaldehyde (**10**, R=H, X=O). Active MnO_2 (15 g) was added to a stirred solution of **9** (1.50 g, 5.7 mmol) in acetone (20 ml) and the mixture was stirred for 1 h. The suspension was filtered and residual MnO_2 was washed with

MeOH, then the organic solution was concentrated *in vacuo* . The residue was passed through the short column over silica gel (*n*-hexane: EtOAc=2: 1) to give 1.39 g (93.3 %) of **10** (R=H, X=O). mp 100-101 °C; ^{1}H-nmr ($CDCl_3$) δ: 0.92 (3H, s), 1.26 (3H, s), 1.91 (3H, s), 2.27 (1H, d, *J*=16 Hz), 2.60 (1H, d, *J*=16 Hz), 6.07 (1H, s), 6.51 (1H, d, *J*=3.6 Hz), 7.24 (1H, d, *J*=3.6 Hz), 9.61 (1H, s). HR-EIms: Found: 248.1091 (M^+), Calcd for $C_{14}H_{16}O_4$: 248.1049.

Methyl 5-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)furan-2-carboxylate (**11**, R=H, X=O, =**14**). To a solution of **10** (0.88 g, 3.3 mmol) in MeOH (30 ml) was added sequentially MnO_2 (4.8 g, 52.8 mmol), NaCN (0.39 g, 7.92 mmol) and acetic acid (0.20 g, 3.3 mmol). The reaction mixture was stirred at room temperature for 4 h. The suspension was filtered and residual MnO_2 was washed with MeOH, then the organic solution was concentrated *in vacuo*. The residue was partitioned between ether and water and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed over silica gel (*n*-hexane: EtOAc=3: 1) to give 0.86 g (88.6 %) of **11** (R=H, X=O); mp 111-113 °C; ^{1}H-nmr($CDCl_3$) δ: 0.92 (3H, s), 1.17 (3H, s), 1.92 (3H, s), 2.27 (1H, d, *J*=17 Hz), 2.63 (1H, d, *J*=17 Hz), 3.87 (3H, s), 6.04 (1H, s), 6.41 (1H, d, *J*=3.3 Hz), 7.16 (1H, d, *J*=3.3 Hz). HR-EIms: Found: 278.1128 (M^+), Calcd for $C_{15}H_{18}O_5$: 278.1154.

5-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)furan-2-carboxylic acid (**12**, R=H, X=O, =**13**). A solution of **11** (90 mg, 0.31 mmol) in MeOH(10 ml) was added to a solution of KOH (35 mg, 0.62 mmol) in MeOH (1 ml). The mixture was stirred under refluxing for 30 min, then it was diluted with 1N HCl (2ml) and extracted with CH_2Cl_2. The organic solution was washed with sat. NaCl solution, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed over silica gel (*n*-hexane: EtOAc=2: 1) to give crude **12** (R=H, X=O), which was recrystallized from ether to give 65 mg (74.8 %) of **12** (R=H, X=O); mp >300 °C; ^{1}H-nmr ($CDCl_3$) δ: 0.92 (3H, s), 1.18 (3H, s), 1.93 (3H, s), 2.29 (1H, d, *J*=17 Hz), 2.60 (1H, d, *J*=17 Hz), 6.07 (1H, s), 6.42 (1H, d, *J*=3.6 Hz), 7.27 (1H, d, *J*=3.6 Hz). HR-EIms: Found: 264.0972 (M^+), Calcd for $C_{14}H_{16}O_5$: 264.0998.

5-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl) furan-3-carboxylic acid (**15**); mp 118-120 °C; ^{1}H-nmr ($CDCl_3$) δ: 0.95 (3H, s), 1.23 (3H, s), 1.93 (3H, s), 2.27 (1H, d, *J*=17 Hz), 2.62 (1H, d, *J*=17 Hz), 6.08 (1H, s), 6.92 (1H, d, *J*=3.9 Hz), 7.77 (1H, d, *J*=3.9 Hz). HR-EIms: Found: 264.0989 (M^+), Calcd for $C_{14}H_{16}O_5$: 264.0998.

Methyl 5-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)furan-3-carboxylate (**16**); oil; ^{1}H-nmr ($CDCl_3$) δ: 0.93 (3H, s), 1.22 (3H, s), 1.92 (3H, s), 2.24 (1H, d, *J*=17 Hz), 2.60 (1H, d, *J*=17 Hz), 3.88 (3H, s), 6.04 (1H, s), 6.90 (1H, d, *J*=3.9 Hz), 7.68 (1H, d, *J*=3.9 Hz). HR-EIms: Found: 278.1125 (M^+), Calcd for $C_{15}H_{18}O_5$: 278.1154.

2-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)furan-3-carboxylic acid (**17**); mp 111-113 °C; ^{1}H-nmr ($CDCl_3$) δ: 0.95 (3H, s), 1.23 (3H, s), 1.93 (3H, s), 2.27 (1H, d, *J*=17 Hz), 2.62 (1H, d, *J*=17 Hz), 6.08 (1H, s), 6.92 (1H, d, *J*=3.9 Hz), 7.77 (1H, d, *J*=3.9 Hz). HR-EIms: Found: 264.1013 (M^+), Calcd for $C_{14}H_{16}O_5$: 264.0998.

Methyl 2-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)furan-3-carboxylate (**18**); oil; ^{1}H-nmr ($CDCl_3$) δ: 0.93 (3H, s), 1.22 (3H, s), 1.92 (3H, s), 2.24 (1H, d, *J*=17 Hz), 2.60 (1H, d, *J*=17 Hz), 3.88 (3H, s), 6.04 (1H, s), 6.90 (1H, d, *J*=3.9 Hz), 7.68 (1H, d, *J*=3.9 Hz). HR-EIms: Found: 278.1136 (M^+), Calcd for $C_{15}H_{18}O_5$: 278.1154.

5-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)thiophene-2-carboxylic acid (**19**); mp 180-181.5 °C; ^{1}H-nmr ($CDCl_3$) δ: 0.95 (3H, s), 1.23 (3H, s), 1.93 (3H, s), 2.27 (1H, d, *J*=17 Hz), 2.62 (1H, d, *J*=17 Hz), 6.08 (1H, s), 6.92 (1H, d, *J*=3.9 Hz), 7.77 (1H, d, *J*=3.9 Hz). HR-EIms: Found: 280.0766 (M^+), Calcd for $C_{14}H_{16}O_4S$: 280.0769.

Methyl 5-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)thiophene-2-carboxylate (**20**); mp 120-121 °C; ^{1}H-nmr ($CDCl_3$) δ: 0.93 (3H, s), 1.22 (3H, s), 1.92 (3H, s), 2.24 (1H, d, *J*=17 Hz), 2.60 (1H, d, *J*=17 Hz), 3.88 (3H, s), 6.04 (1H, s), 6.90 (1H, d, *J*=3.9 Hz), 7.68 (1H, d, *J*=3.9 Hz). HR-EIms: Found: 294.0923 (M^+), Calcd for $C_{15}H_{18}O_4S$: 294.0926.

5-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)-3-methylthiophene-2-carboxylic acid (**21**); mp 250-253 °C; ^{1}H-nmr ($CDCl_3$) δ: 0.94 (3H, s), 1.20 (3H, s), 1.91 (3H, s), 2.21 (1H, d, *J*=17 Hz), 2.48(3H, s), 2.63 (1H, d, *J*=17 Hz), 6.02 (1H, s), 6.71 (1H, s). HR-EIms: Found: 294.0923 (M^+), Calcd for $C_{15}H_{18}O_4S$: 294.0925.

Methyl 5-(1-Hydroxy-4-oxo-2, 6, 6-trimethyl-2-cyclohexen-1-yl)-3-methylthiophene-2-carboxylate(**22**); mp 150-152 °C; ^{1}H-nmr ($CDCl_3$) δ: 0.94 (3H, s), 1.20 (3H, s), 1.91 (3H, s), 2.21 (1H, d, *J*=17 Hz), 2.48(3H, s), 2.63 (1H, d, *J*=17 Hz), 6.02 (1H, s), 6.71 (1H, s). HR-EIms: Found: 308.1053 (M^+), Calcd for $C_{16}H_{20}O_4S$: 308.1082.

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