Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 3, pp. 275-278, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00014-3

Synthesis and Biological Activities of New Fluorinated Abscisic Acid

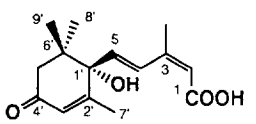
Bum Tae Kim[a]*, Yong Ki Min[a], Tadao Asami[c], No Kyun Park[a], In Howa Jeong[b], Kwang Yun Cho[a] and Shigeo Yoshida[c]

[a]Korea Research Institute of Chemical Technology, P.O.Box 107, Yusong, Taejon 305-606, Korea
[b]Department of Chemistry, Yonsei University, Wonju, Kangwondo 222-701, Korea
[c]The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako 351-01, Japan

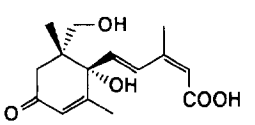
Abstract : A fluorinated analog of abscisic acid(ABA), which was prepared from 6-trifluoromethyl-2,6-dimethyl-2-cyclohexen-1,4-dione, showed potent inhibitory activities such as amylase induction in barley seeds and germination in cress seeds.

Abscisic acid (ABA, **1**), a widely distributed plant hormone in higher plants, is involved in the regulation of significant physiological processes such as abscission of leaves, stomatal closure and seed germination and dormancy[1]. Moreover, it is noted as an anti-stress agent for cold and drought[2]. However, it has two major drawbacks for application as a plant growth regulator; 1) its side chain (the 2-*Z*-4-*E*-3-methyl-2,4-pentadienoic acid moiety) is readily isomerized to a biologically inactive 2-*E* isomer by light[3] and 2) it is easily converted to phaseic acid (**3**)[4] *via* 8'-hydroxyabscisic acid (**2**) by enzymatic oxidation in plants with concomitant loss of biological activity[5]. Intensive works[6] were done in an attempt to overcome the sensitivity of the side chain of ABA to light by designing compounds possessing ABA-like activities, but few compounds were reported to have ABA-like activity with level approaching that of ABA. As for the drawback of oxidative metabolism, only 8'- or 9'-methoxyabscisic acids have been reported as antimetabolic analogs which had more potent activities than ABA in some biological tests[7]. Thus, designing highly active ABA analogs which block the metabolism of ABA is shown to be an attractive way of finding highly active ABA analogs.

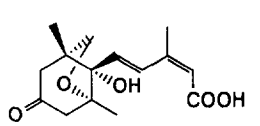
Recently, fluorinated analogs of biologically active molecules have been important tools for studying receptors and active sites of enzymes[8]. Replacing hydrogen with fluorine results in a slight change in the size or shape, but greatly affects the electronic nature of the molecule due to the strong electronegativity of fluorine. On the basis of the above reasons, fluorine trisubstituted ABA at C-8', 8',8',8'-trifluoroabscisic acid (**4**), was selected as a target molecule. We hope this compound shows high ABA-like activities and might help us to understand the function of ABA and its receptors.



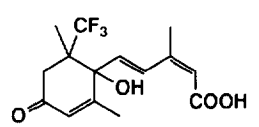
Abscisic acid(ABA, 1)



2



3

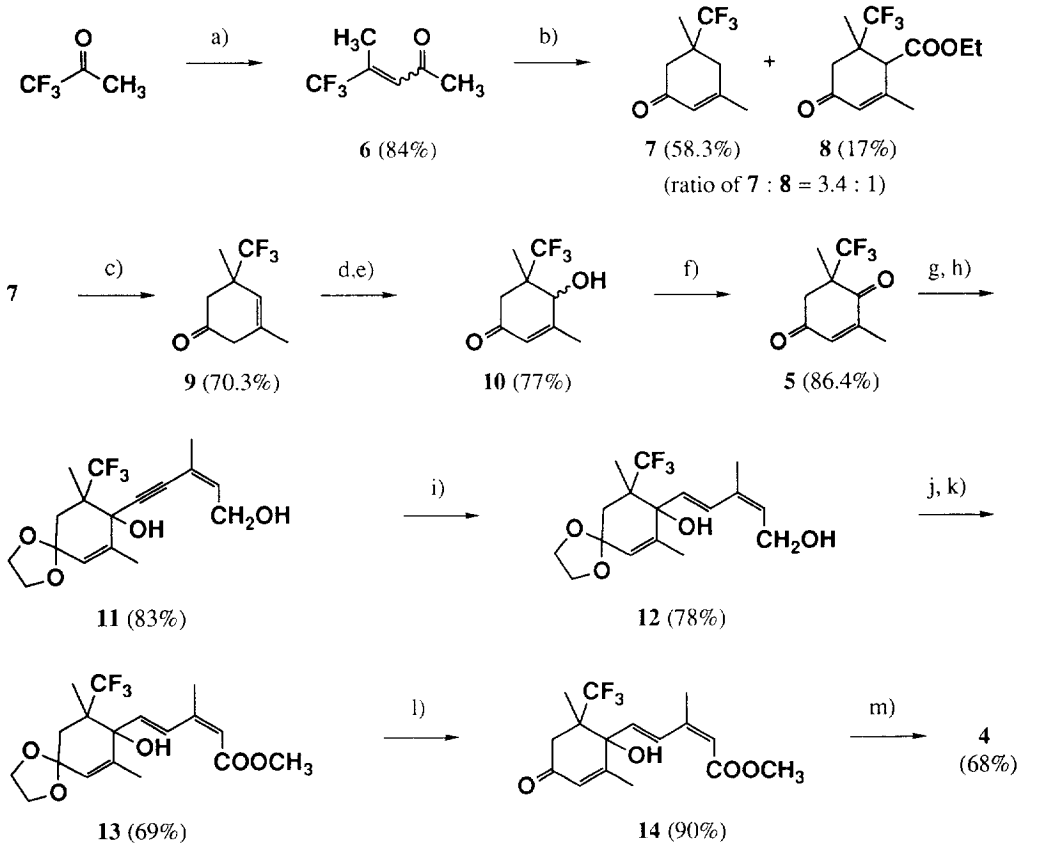


4

In this paper, we wish to describe the synthesis of the fluorinated ABA analog (**4**) as racemic form from trifluoroacetone and its biological activities.

Synthesis of trifluorinated abscisic acid (**4**)

Fluorinated 4-oxoisophorone (**5**), a key intermediate in the synthesis of **4**, can be synthesized from fluorinated mestyl oxide (**6**). Reaction of trifluoroacetone with acetylmethylenetriphenylphosphorane afforded a *E* and *Z* mixture (7 : 1 ratio) of unsaturated ketone (**6**) in high yield. Michael addition of **6** to ethyl acetoacetate and subsequent cyclization with sodium ethoxide gave a mixture of 3,5-dimethyl-5-trifluoromethyl-2-cyclohexen-1-one (**7**) and 4-ethoxycarbonyl-3,5-dimethyl-5-trifluoromethyl-2-cyclohexen-1-one (**8**). Treatment of **7** with methylmagnesium bromide in the presence of ferric chloride resulted in the formation of double bond migrated isomer **9**[9]. Epoxidation of **9** with *m*-chloroperbenzoic acid and continuous



a) $Ph_3P{=}CHCOCH_3$, CH_2Cl_2, b) ethyl acetoacetate, EtONa, EtOH, c) CH_3MgBr, $FeCl_3$, ether, d) *m*-CPBA, $CHCl_3$, e) NaOH, H_2O, f) CrO_3, H_2SO_4, g) $HO(CH_2)_2OH$, TsOH, toluene, h) (Z)-3-methyl-2-penten-3-yn-1-ol, 2eq. of n-BuLi, THF, -78°C, i) Red-Al, THF, j) MnO_2, acetone, k) NaCN, MnO_2, AcOH, methanol, l) HCl, acetone, m) NaOH, methanol.

treatment with sodium hydroxide provided 4-hydroxy-3,5-dimethyl-5-trifluoromethyl-2-cyclohexen-1-one (**10**) as a diastereomeric mixture. Jones oxidation of **10** produced dione (**5**) in high yield. For introduction of the side chain, our attention turned to the functionalization of **5** toward the fluorinated ABA analog **4**. After the protection of **5** with ethylene glycol, using a catalytic amount of TsOH, alkylation with dianionic (*Z*)-3-methyl-2-penten-3-yn-1-ol which was generated by the addition of 2eq. of n-BuLi at -78°C provided alcohol (**11**)[10] as a racemic mixture[11]. Reduction of the alcohol (**11**) with Red-Al in THF afforded dienol (**12**)[12]. The sequential oxidation with manganese dioxide, then Corey oxidation of **12** produced ester (**13**) through the corresponding aldehyde[13]. Finally, deprotection with HCl in acetone, and hydrolysis with NaOH, converted the ester (**13**) to a racemic mixture of 8',8',8'-trifluoroabscisic acid (**4**).

Compound **4** was given as a solid; mp: 183°C, ^{1}H-NMR (200MHz; $CDCl_3$+CD_3OD) δ : 1.27(s, 3H), 1.97(s, 3H), 2.04(s, 3H), 2.55(d, 1H, *J*=17.6Hz), 2.81(d, 1H, *J*=17.8Hz), 5.80(s, 1H), 5.98(s, 1H), 6.07(d, 1H, *J*=16.1Hz), 7.79(d, 1H, *J*=16.1Hz); ^{19}F-NMR (200MHz; $CFCl_3$+CD_3OD) δ : -67.526ppm(s, 3F): MS *m/z* (rel. int.): 318(M^+, 8), 301(80), 259(9), 221(100), 208(43), 190(85), 162(52), 134(76), 111(100); HR-MS *m/z*: 318.1065 (M^+, calcd. for $C_{15}H_{17}F_3O_4$, 318.2098).

Biological Activities of trifluorinated abscisic acid (**4**)

The fluorinated ABA analog (**4**) exhibited potent inhibitory activity equivalent to that of ABA in barley (*Hordeum vulgare L. var. Hexastichon*) seeds amylase induction assay[14] and in cress (*Lepideum sativum*) seed germination test[15]. The pI_{50} values of these compounds obtained from two bioassays are shown in **Table I.**

Table I. pI_{50} Values of Compound **4** and ABA for Inhibition Activities Toward Barley Seeds Amylase Induction and Cress Seeds Germination.

Compd.	Inhibitory Activities (pI_{50} Values[a])	
	Amylase Induction[b]	Germination[c]
4	7.00	5.96
ABA	6.30	6.01

a: pI_{50} value indicates the negative logarithm of the concentration (M) of compounds for 50% inhibition.

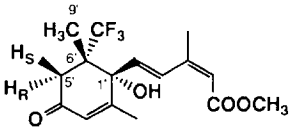
b: Amylase induction assay was conducted under the following condition: Ten embryo-less barley half seeds were incubated in 4ml of buffer (2mM acetate and 2μM $CaCl_2$), with GA_3 (100nM) + ABA or compound **4** (10nM, 30nM, 100nM, 0.3μM, 1μM, 3μM, 10μM) for 48h at 25°C. The activity of amylase secreted under these condition was measured by the procedure of Yomo[14].

c: The bioassay was conducted under the following condition: Cress seeds were purchased locally. The germination test following Taylor et al.[15] was carried out on duplicates of 25 seed for each concentration of the sample. The test seeds were placed on Toyo No. 2 filterpapers (5cm, Toyoroshi Co.) in plastic petri dishes (5cm i.d., Eizai Co., Japan) and kept in the dark at 25°C. The germination rate was counted 48 hr after sowing.

The replacement of the C'-6 methyl group by trifluoromethyl group did not affect the inhibitory activity of the germination, but it improved the inhibitory activity of the amylase induction. At present, it is still unclear that the effect of the trifluoromethyl group on this activity enhancement is due to antimetabolism or improvement of the binding affinity to receptor etc, but we think that the activity and the design concept of this compound will lead to new ABA analogs which will be a great use in studying the mechanism and binding site of ABA.

REFERENCES AND NOTES

1) (a) Addicott, F. T. *Abscisic Acid*; Addicott, F. T., Ed.; Praeger Publishers: New York, 1983. (b) Schopfer, P.; Plachy, C. *Plant Physiol.* **1984**, *76*, 155. (c) Karssen, C. M.; Brinkhorst-van der Swan, D. L. C. Breekland A. E.; Koornneef, M. *Planta* **1983**, *157*, 158. (d) Oishi, M. Y.; Bewley, J. D. *Plant Physiol.* **1990**, *94*, 592.
2) (a) Paton, D. M.; Dhawan, A. K.; Willing, R. R. *Plant Physiol.* **1980**, *66*, 254. (b) Li, Y.; Walton, D. C. *Plant Physiol.* **1987**, *85*, 910. (c) Gusta, L. V.; Fowler, D. B; Tyler, N. J. *Can. J. Bot.* **1982**, *60*, 301. (d) Chen, T. H. H.; Li, P. H.; Brenner, M. *Plant Physiol.* **1983**, *71*, 362.
3) Milborrow, B. V. *Journal of Experimental Botany* **1970**, *21*, 17.
4) Milborrow, B. V. *J. Chem. Soc., Chem. Commun.* **1969**, 966.
5) (a) Dashek, W. V.; Singh, B. N.; Walton, D. C. *Plant Physiol.* **1979**, *64*, 43. (b) Davis, L. A.; Lyon, J. L.; Addicott, F. T. *Planta* **1972**, *102*, 294.
6) Kim, B. T.; Asami, T.; Morita, K.; Soh, C. H.; Murofushi, N.; Yoshida, S. *Biosci. Biotech. Biochem.* **1992**, *56*, 624.
7) Todoroki, Y.; Hirai, N.; Koshimizu, K. *Biosci. Biotech. Biochem.* **1994**, *58*, 707.
8) Welch, J. T. *Tetrahedron* **1987**, *43*, 3123.
9) Kharasch, M. S.; Tawney, P. O. *J. Am. Chem. Soc.* **1941**, *63*, 2308.
10) Derguini, F.; Balogh-Nair, V.; Nakanishi, K. *Tetrahedron Lett.* **1979**, *51*, 4899.
11) In this coupling reaction, dianionic nucleophile may attack the carbonyl group only from the back side of the trifluoromethyl group to give only one racemic mixture. The result was shown by NOE difference spectra of compound **14** in $CDCl_3$ solution: Trifluoro ABA methyl ester **14** had strong NOE between C-9' protons and the $\mathbf{H_S}$ (upfield C-5' proton which is *cis* to the side chain), the irradiation of C-9' protons led to increase in the intensity of the signal for $\mathbf{H_S}$ while no such effect was observed in the case of $\mathbf{H_R}$ (downfield C-5' proton which is *trans* to the side chain). And signal for $\mathbf{H_R}$ shifted to the downfield owing to the anisotropic effect of trifluoromethyl group. These results implied that the alkylation reaction proceeded with high stereoselectivity because of the steric and/or electronic effects of trifluoromethyl group.



Compound **14**

12) Jones, T. K.; Denmark, S. E. *Org. Synth.* **1986**, *64*, 182.
13) Corey, E. J.; Gilman, N. W.; Ganem, B. E. *J. Am. Chem. Soc.* **1968**, *90*, 5616.
14) Yomo, H. *Jikken Seibutsugaku Kouza*; Katsumi, M.; Masuda, Y., Ed.; Maruzen: Tokyo, 1983; Vol. 15, pp. 173-181.
15) Taylor, H. F.; Burden, R. S. *Proc. R. Soc. London, Ser. B* **1972**, *180*, 317.

(*Received in Japan* 7 *November* 1994; *accepted* 4 *January* 1995)