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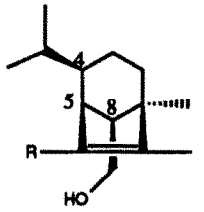
Novel Compound Stimulates Amylase Induction in Barley Seeds

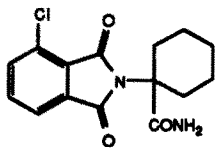
Bum-Tae Kim[a], Tadao Asami*[b], Chang-Hoh Soh[b], Noboru Murofushi[a] and Shigeo Yoshida[b]
[a]Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo113, Japan
[b]The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako 351-01, Japan

Abstract Active helminthosporic acid analogs were found to induce the production of amylase in barley aleurone layers. The carbon skeleton of the active compound was different from that of helminthosporic acid, and it required both hydroxy and carboxy groups for its activity.

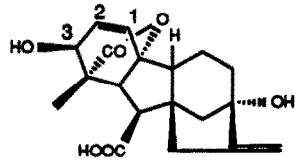
Gibberellins promote the production of amylase in aleurone layers of barley. This response has been extensively studied because it is tightly correlated with seed germination, and provides a good model for the action of plant growth regulators. Helminthosporol (**1**) and its oxidation product helminthosporic acid (**2**)[1-3], and the synthetic compound AC-94377 (**3**)[4] promote the production of amylase in aleurone layers of barley at the same activity level, although they are far less active than gibberellins. Briggs suggested that the biological activity of helminthosporol and helminthosporic acid stemed from the similarity of their molecular structures to the C/ D ring system of gibberellin (GA_3)[5]. Comparison of the molecular model of GA_3 with that of helminthosporic acid indicates that the C/D ring system of the gibberellin overlaps the ring skeleton of **2** with the resultant carboxy group in both molecules positioned in the same spatial orientation. On the basis of this consideration, Turner et al.[6] synthesized several analogs of helminthosporol and polycyclic acids resembling GA_3 in the hope of improving their biological activities. The carboxy group was analyzed for potentially significant biological activities through the bioassay for growth promotion in rice seedlings, and the induction of amylase in barley aleurone layer. However, no remarkable change in the activities of the synthesized compounds was noted, and most of the analogs exhibited either the same or weaker activities than that of helminthosporic acid.



1: R = CHO, Helminthosporol
2: R = COOH, Helminthosporic acid



3: AC94377



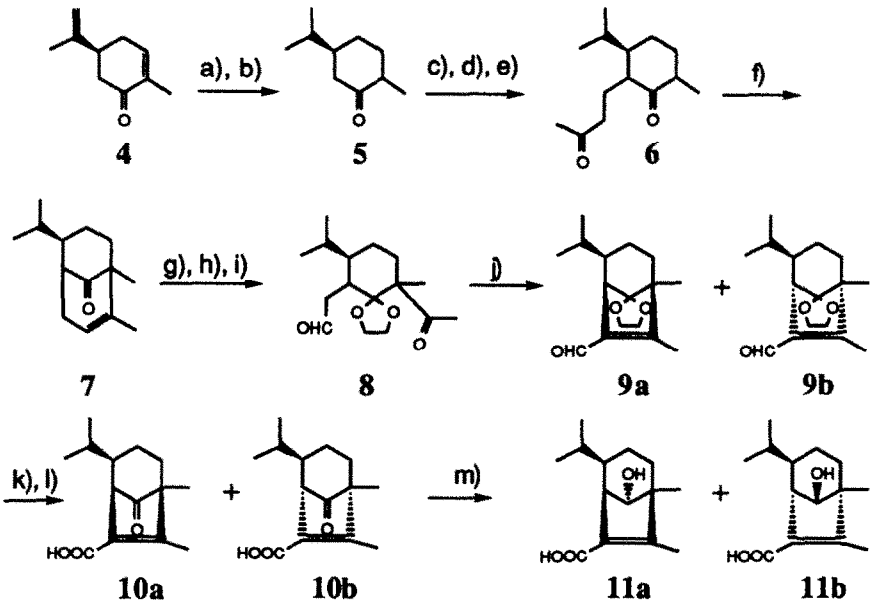
GA_3

Helminthosporic acid has a hydroxymethyl group at the C-8 position, but its biological function at the C-8 position has not been studied so far. In view of the importance of 3β-hydroxyl group in GA_3 for activity exhibition[7], we propose that the hydroxyl group in hydroxymethyl group at the C-8 position might similarly play an important role. To determine if the functional group at the C-8 position participates in a biologically significant function, we synthesized helminthosporic acid derivatives modified at the C-8 position as outlined below. An active novel compound was found to be far more

active than helminthosporic acid. This is the first report of an artificial compound without a gibberellane skeleton exhibits gibberellin-like activity at such a high level in the amylase induction test.

Synthesis of derivatives of bicyclo[3.2.1]oct-6-ene-8-ol

The diketone (**6**) was prepared from the (5*S*)-2-methyl-5-(1'-methylethyl)-cyclohexan-1-one (**5**), which was prepared by the reduction of (5*S*)-2-methyl-5-(1'-methylethenyl)-cyclohex-2-en-1-one (**4**, *S*-Carvone), by the same procedure of Corey and Nozoe (about 20% total yield through 5 steps from S-Carvone)[8]. The cyclization of **6** with a treament of tin tetrachloride (anhydrous) as a Lewis acid provided the desired bicyclo[3.3.1]non-7-en-9-one (**7**). The keto-aldehyde (**8**) was afforded by the protection of the

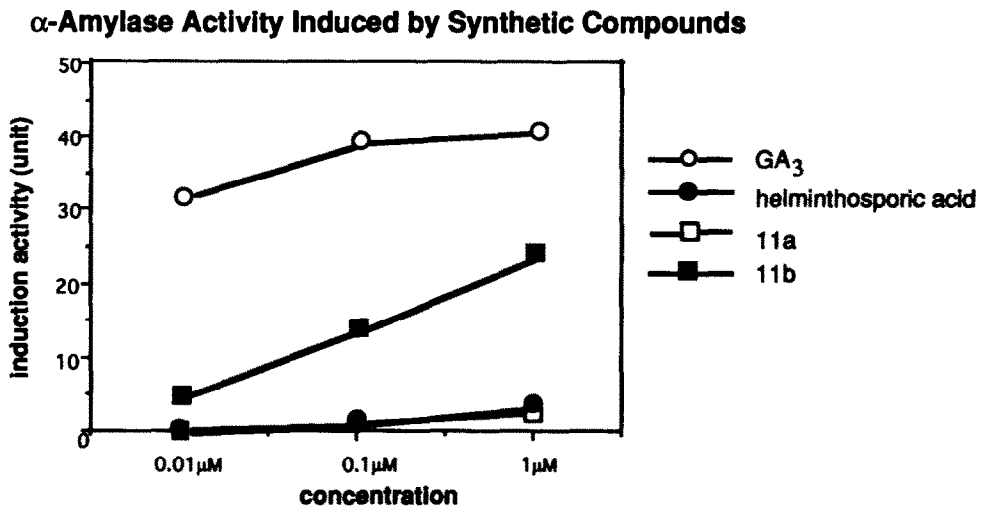


a) Li, NH_3, b) Pd/C, H_2 c) $HC(OEt)_3$, NaOMe d) Et_3N, MVK e) K_2CO_3, H2O f) $BF_3(OEt)_2$
g) ethyleneglycol, p-TsOH h) OsO_4 i) $Pb(OAc)_2$ j) NaOH k) NaOCl l) H_2O-THF, H_2SO_4 m) $NaBH_4$

ketone of 8-position and a subsequent hydroxylation of **7** with osmium tetroxide followed by the oxidation of the diol with lead tetraacetate (about 80% total yield through 3 steps from the compound **7**). After these steps, the two diastereomers of **8** derived from the bicyclo[3.3.1]non-7-en-9-one (**7**) were separated each other by column chromatography (silica gel, eluent; ethyl acetate:n-hexane=1:5) and subjected to the following reactions respectively. Intramolecular condensation of **8** with a catalytic amount of sodium hydroxide in ethanol led the keto-aldehyde to α, β-unsaturated aldehydes (**9a** and **9b**) separately (about 50% yield). Oxidation of the derived aldehyde groups with sodium chlorite and subsequent deprotection reaction with 10% aqueous sulfuric acid gave the 8-one compounds (**10a** and **10b**) in good yields, and a reduction of **10a** and **10b** with sodium borohydride gave a highly

stereocontrolled hydroxyl group with an axial conforamation on the cyclohexane ring (about 70% yield). This hydroxyl orientation of the reduction was simlar to the result of the stereoselectivity of the hydrogen transfer in the bicyclic ketone system[9], such as bicyclo[3.2.1]oct-6-en-8-one or norbornen-7-one. The stereochemistry of the two isomers (**11a-11b**) derived from the two isomers of the bicyclo[3.3.1]non-7-en-9-ones was determined by comparing the coupling constant of the bridgehead proton of helminthosporol with the coupling constant of the bridgehead proton at C-5 of the synthetic compounds recorded by a high resolution ^{1}H-NMR. Compound **11a** was given as an oil; ^{1}H-NMR (500 MHz; $CDCl_3$) δ: 0.818 (3H, d, J=6.03 Hz), 0.842 (1H, m), 0.984 (3H, s), 1.011 (3H, d, J=6.34 Hz), 1.106 (1H, m), 1.263 (2H, m), 1.481 (1H, m), 1.628 (1H, m), 2.083 (3H, s), 3.080 (1H, d, J=5.57Hz), 3.714 (1H, d, J=5.57 Hz) ; MS m/z (rel. int.): 238 (M^+, 63), 220 (78), 205 (39), 177 (100), 138 (91), 91 (68), and **11b** was also given as an oil; ^{1}H-NMR (500 MHz; $CDCl_3$) δ: 0.886 (3H, d, J=6.41 Hz), 0.973 (3H, s), 1.025 (3H, d, J=6.67 Hz), 1.03 (1H, m), 1.177 (1H, m), 1.415 (1H, m), 1.551 (1H, m), 1.657 (1H, m), 2.09 (1H, m), 2.078 (3H, s), 3.104 (1H, dd, J=2.70, 5.00 Hz), 3.683 (1H, d, J=5.38 Hz) ; MS m/z (rel. int.): 238 (M^+, 25), 220 (53), 205 (18), 177 (100), 138 (54), 91 (55).

Amylase induction activity in barley aleurone layer



The bioassay was carried out following the method of Momotani *et al*[10]. Endosperm pieces were prepared by cutting the seed of barley (*Hordeum vulgare L. var. Hexastichon*) transversely 4mm from the proximal end of the seed. Two pieces were added to each vial and sterilized, and were incubated in the solution freshly prepared with 2 ml of 1 mM acetate buffer (pH=5.1) with 20 mM $CaCl_2$, 25 mg of chloramphenicol, and known ammount of chemicals and GA_3 for 48 hr at 25 °C. After the incubation, the seeds were homogenized in the incubation solution, and 3ml of 1mM acetate buffer was added, and then the suspension was centrifuged on 2000 g for 10 min. The supernatant (enzyme solution) was allowed to

react with starch solution for 12 min at 30 °C. The activity was determined by measuring the reducing sugar content at an absorbancy of 620 nm .

The result obtained from the barley aleurone amylase bioassay showed that **11b** had the highest activity, and **11a** was as active as helminthosporic acid. Intermediates culled from the synthetic scheme were less active than helminthosporic acid. When the carboxyl group of **11b** was converted to methyl ester by treatment with diazomethane, the activity was extinguished. On the basis of these findings, we assume that the hydroxyl group and the carboxyl group of these synthetic analogs are essential for amylase induction. The hydroxy group and the carboxy group of **11b** are perceivably are able to take up suitable spatial positions with regard to an interaction between **11b** and a receptor(s). In our test concentration at 1 μM, AC-94377 showed no activity, while helminthosporol and helminthosporic acid were evaluated at about one-twentieth that of GA_3. While gibberellins and synthetic compounds with the gibberellane skeleton showed activity[11], exceptinally compound **11b** without the gibberellane skeleton showed the similar activity. Therefore, comparison of the spatial arrangement of the essential functional groups of this compound with that of gibberellins will further help our understanding of the structural requirements for amylase induction activity, and will be useful in designing new and highly active compounds.

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