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# Preparation of a growth-promoting substance, lepidimoic acid, from okra pectic polysaccharide

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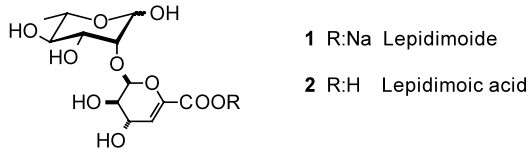
**Abstract**—Lepidimoic acid, a 1,2-*cis*-linked disaccharide, is an interesting plant growth-promoting substance and has been synthesized from D-glucose and L-rhamnose by Kosemura et al., 1993. Fry et al., 1993, in view of the biosynthetic origin, suggested that it could be produced by the cleavage of a pectic polysaccharide such as rhamnogalacturonan. In this study lepidimoic acid was prepared from okra (*Hibiscus esculentus* L.) pectic polysaccharide in a passable yield by sequential decomposition reactions. The plant growth-promoting activity of the prepared lepidimoic acid was confirmed. 

Lepidimoide **1** was first isolated as a potent allelopathic substance, which was exuded from germinating cress (*Lepidium sativum* L.) seeds into the environment and stimulated the growth of other plant species. The absolute configuration was confirmed in Figure 1 by spectra analyses and total synthesis.[1–3] This compound is an interesting unsaturated disaccharide. Lepidimoide has been identified in the seed exudates of various plant species.[4] Moreover, it has exhibited multiple functions in the growth and development of plants: Lepidimoide promotes chlorophyll accumulation in sunflower and cucumber cotyledons, leaf development, flowering and seed production in *Arabidopsis thaliana* as well as shoot growth in seedlings of various plant species, and inhibits the loss of total chlorophyll in *Avena* leaf segments and the formation of abscission in bean petiole explants.[5–8] On the basis of this information, lepidimoide appears to be a new phytohormone-like growth substance. The structure–activity relationship study showed that lepidimoic acid **2** had as high as the growth-promoting activity of lepidimoide **1**, suggesting that sodium salt is not the structural requirement for plant growth-promoting activity.[9] In some experiments, lepidimoide was also used to rule out the effect of $Na^+$.[7] Kosemura et al., 1993 synthesized lepidimoide from D-glucose and L-rhamnose with a complicated 22-step chemical reaction.[2] On the other hand, Fry et al., 1993, in view of the biosynthetic origin of lepidimoide, suggested that it could be produced by the cleavage of a pectic polysaccharide such as rhamnogalacturonan.[10] Against this background, we undertook to prepare lepidimoic acid with the cleavage of a pectic polysaccharide. Okra mucilage F was extracted from immature okra fruits and identified as rhamnogalacturonan by Tomoda et al.[11] They reported that it is a glycoprotein (the molecular weight is about 1,700,000) and also a rhamnogalacturonan which has a hexasaccharide repeating unit **3**. We assumed it would be available as the starting material of lepidimoic acid.




**1** R:Na Lepidimoide

**2** R:H Lepidimoic acid

**Figure 1.** Absolute configuration of lepidimoide **1** and lepidimoic acid **2**.

We started to prepare lepidimoic acid from this okra mucilage by the route of Figure 2. The starting substance, okra mucilage, was prepared as follows. Immature okra fruit (4.5 kg) was homogenized and extracted with water. After filtration, MeOH was added, and the precipitate was collected. It was dissolved in water and this treatment was repeated two times. The precipitate was dried in vacuo and okra mucilage (50 g) was

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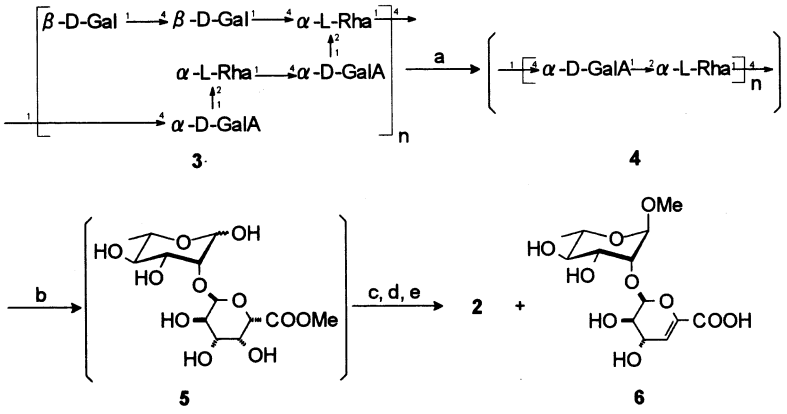


**Figure 2.** Acid degradation process for **2**. *Reagents and conditions*: (a) TFA aq.; (b) HCl in MeOH; (c) NaOMe in MeOH; (d) $H_2O$; (e) separation.

obtained as a brown block. The obtained mucilage was a glycoprotein that was a perfect Ca salt and showed molecular weight of about 10,000,000.[12] The component sugars were rhamnose, galacturonic acid, galactose, glucose and glucuronic acid in the ratio of 1:1.83:2.49:0.6:0.17.[13] We presumed the obtained mucilage included a large amount of rhamnogalacturonan **3**. We intend to prepare lepidimoic acid from this rhamnogalacturonan **3**. The starting material is an enormous glycoprotein and the purification is difficult. Therefore, we chose the way fundamentally without purification of these intermediates. This rhamnogalacturonan **3** has four types of bonds and their degradation speeds are different in an acid medium. The uronide bond is strongly resistant to acid degradation.[14] Okra mucilage was partially hydrolyzed with 0.1 mol/L trifluoroacetic acid under reflux for 4 h and purified with DEAE–cellulose chromatography. The obtained residue **4** was reacted with 0.1 mol/L hydrogen chloride in MeOH under reflux for 1 h. After evaporation of hydrogen chloride and the solvent, the residue **5** was reacted with 1 mol/L sodium methoxide in MeOH at room temperature for 10 h and the obtained α,β-unsaturated carboxylic ester was hydrolyzed by adding water.[15] After acidification, the obtained residue was purified by DEAE–cellulose chromatography, centrifugal partition chromatography and HPLC. From 50 g of okra mucilage, 180 mg (0.36% from **3**) of compound **2** and 400 mg (0.8% from **3**) of compound **6** were obtained and assigned by spectral analyses.[16] These chemical structures confirmed that compound **2** was lepidimoic acid and compound **6** was lepidimoic acid methyl glycoside which was obtained for the first time by us.[3] Thus, we successfully produced lepidimoic acid from pectic polysaccharide following Fry's hypothesis.[10]

On the other hand, β-eliminative depolymerization of carbohydrates containing uronic acid residues had been reported (Fig. 3).[17]

We supposed this process might be applied to effective preparation of lepidimoic acid from okra mucilage.

The partially hydrolyzed rhamnogaracturonan **4** was acetylated with $Ac_2O$, AcONa under reflux for 1.5 h and ethyl acetate was added and washed with brine. After evaporation of the solvent, the residue was dissolved in a mix-solvent (9:1) of MeOH and benzene and esterified with $TMSCHN_2$. The obtained **7** underwent β-eliminative depolymerization with 1 mol/L sodium methoxide in MeOH at room temperature for 10 h and was saponified by adding water. After acidification, the reaction mixture was purified by the same method previously described. From 20 g of okra mucilage, 580 mg (2.90% from **3**) of lepidimoic acid **2** was obtained. We succeeded in preparing again lepidimoic acid **2** in a good yield without producing the undesired methyl glycoside **6** (Fig. 4).

These activities in Figure 5 were measured by means of the *Celosia argentea* elongation test.[18] Lepidimoic acid **2** obtained from okra mucilage showed as high growth-promoting activity as that of synthetic lepidimoide.[2] This is the same result of the structure–activity relationship study.[9] However, methyl glycoside **6** does not have

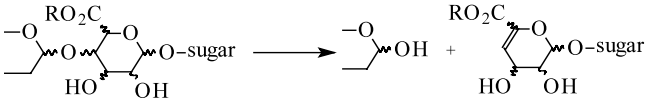


**Figure 3.** β-Eliminative depolymerization model.

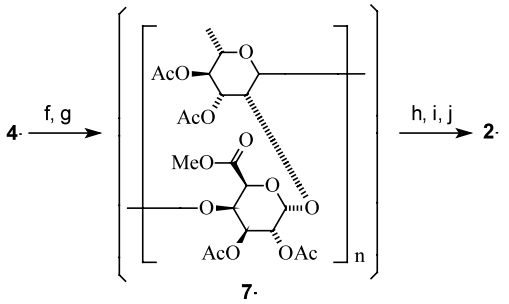


**Figure 4.** β-Eliminative depolymerization process for **2**. *Reagents and conditions*: (f) $Ac_2O$, AcONa; (g) $TMSCHN_2$; (h) NaOMe; (i) $H_2O$; (j) separation.

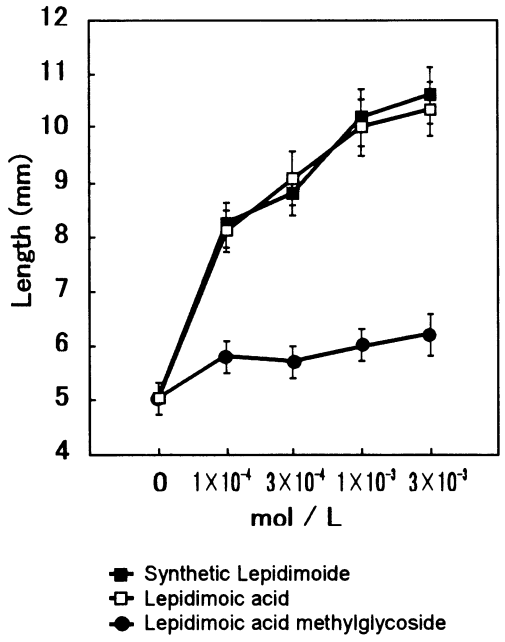


**Figure 5.** Plot of the activities of synthetic lepidimoide, lepidimoic acid and lepidimoic acid methyl glycoside on hypocotyl growth of cockscomb. Each value is the average of 10 measurements; bars indicated s.c.

the activity. It is very interesting to note that the anomeric hydroxyl group is very important for hypocotyl growth of cockscomb.

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   Lepidimoic acid **2**; 2-*O*-(4-deoxy-β-L-threo-hex-4-enopyranuronosyl)-L-rhamnopyranose.
   Lepidimoic acid methylglycoside **6**; 2-*O*-(4-deoxy-β-L-threo-hex-4-enopyranuronosyl)-β-L-rhamnopyranose methylglycoside.
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13. The ratio of component sugars was measured with GLC of the sample which was hydrolyzed with 1.5 mol/L-HCl at 100°C for 5 h and reacted with TMS–HT.
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16. Optical rotation was measured by a Horiba SEPA-200 Polarimeter. IR spectra were recorded on a Jasco FT/IR-410 spectrometer. $^1$H NMR spectra were measured with a Varian Mercury 400vx (400 MHz) spectrometer in $D_2O$ at rt. Mass spectra (MALDI-TOF-MS) were recorded on a KRATOS KOMPACT MALDI II using a fine cobalt powder as a matrix.
   Compound **2** as a hygroscopic white powder: $[\alpha]_D^{21}$ +57.1° (*c* 0.028, $D_2O$); IR: 1635 cm$^{-1}$; $^1$H NMR ($D_2O$) 5.89 (d, 1H, $J_{10,9}$ 3.0 Hz, H-10), 5.08 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 5.05 (d, 1H, $J_{7,8}$ 2.4 Hz, H-7), 4.23 (dd, 1H, $J_{9,8}$ 7.6 Hz, H-9), 3.96 (dd, 1H, $J_{2,3}$ 3.6 Hz, H-2), 3.73–3.65 (3H, H-3, 5, 8), 3.21 (dd, 1H, $J_{4,3}$ 9.6, $J_{4,5}$ 9.6 Hz, H-4), 1.10 (d, 3H, $J_{6,5}$ 6.4 Hz, H-6); MALDI-TOF-MS calcd for $C_{12}H_{18}O_{10}$ *m*/*z*: 322. Found *m*/*z*:345 [M+Na]$^+$. Anal. calcd for $C_{12}H_{18}O_{10}\cdot 2H_2O$: C, 40.23; H, 6.19. Found: C, 40.20; H, 6.07.
   Compound **6** as a white powder: $[\alpha]_D^{21}$ +108.3° (*c* 0.030, $D_2O$); IR: 1635 cm$^{-1}$; $^1$H NMR ($D_2O$) 5.94 (d, 1H, $J_{10,9}$ 3.2 Hz, H-10), 5.03 (d, 1H, $J_{7,8}$ 2.0 Hz, H-7), 4.65 (1H, H-1), 4.22 (dd, 1H, $J_{9,8}$ 7.6 Hz, H-9), 3.93 (dd, 1H, $J_{2,3}$ 3.6 Hz, $J_{2,1}$ 1.6 Hz, H-2), 3.65 (dd, 1H, H-8), 3.57 (dd, 1H, $J_{3,4}$ 9.6 Hz, H-3), 3.49 (dd, 1H, $J_{5,4}$ 9.6, $J_{5,6}$ 6.0 Hz, H-5), 3.21 (4H, H-4, $OCH_3$), 1.1 (d, 3H, H-6); MALDI-TOF-MS calcd for $C_{13}H_{20}O_{10}$ *m*/*z*: 336. Found *m*/*z*: 359 [M+Na]$^+$, 375 [M+K]$^+$. Anal. calcd for $C_{13}H_{20}O_{10}\cdot H_2O$: C, 44.07; H, 6.27. Found: C, 43.80; H, 5.97.
   Compound **6** sodium salt: $^1$H NMR ($D_2O$) 5.64 (d, 1H, $J_{10,9}$ 3.2 Hz, H-10), 4.98 (d, 1H, $J_{7,8}$ 2.4 Hz, H-7), 4.69 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1), 4.18 (dd, 1H, $J_{9,8}$ 6.8 Hz, H-9), 4.02 (dd, 1H, $J_{2,3}$ 3.6 Hz, H-2), 3.64 (dd, 1H, H-8), 3.61 (dq, 1H, $J_{3,4}$ 9.6 Hz, H-3), 3.53 (dq, 1H, $J_{5,4}$ 9.6, $J_{5,6}$ 6.0 Hz, H-5), 3.24 (dd, 1H, H-4), 3.24 (s, 3H, H-$OCH_3$), 1.13 (d, 3H, H-6). The configuration of **6** was determined by the $^1$N NMR of compound **6** sodium salt. The coupling constant ($J$ = 1.4 Hz) between H-1 and H-2 shows that the anomer of methyl glycoside **6** is β-bonding. Also, we think this is reasonable because of the reaction mechanism.
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