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A convenient synthesis of lepidimoide from okra mucilage and its growth-promoting activity in hypocotyls

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Abstract—The total synthesis of the unsaturated disaccharide, lepidimoide 4-deoxy-β-L-*threo*-hex-4-enopyranuronosyl-(1 → 2)-L-rhamnopyranose sodium salt, has been carried out from D-glucose and L-rhamnose (*Tetrahedron Lett.* 1993, *34*, 2653), but the process is very long and complicated. A method for more easily producing this compound and in large quantities is necessary for further research. We have succeeded in conveniently synthesizing lepidimoide from okra (*Hibiscus esculentus* L.) fruit mucilage. At the same time, the isomer (*epi*-lepidimoide) was obtained as a byproduct. The structure was determined as the 4-deoxy-β-L-*threo*-hex-4-enopyranuronosyl-(1 → 2)-6-deoxy-L-glucopyranose sodium salt by spectral analysis. We found that lepidimoide easily epimerized to *epi*-lepidimoide in alkaline media. Both lepidimoide and *epi*-lepidimoide exhibited the same high activity in the cockscomb hypocotyls elongation test.

Keywords: Lepidimoide; *epi*-Lepidimoide; Unsaturated disaccharide; Allelopathic substance; Okra mucilage; Growth promoter

1. Introduction

Lepidimoide is isolated from the exudate of germinated cress (*Lepidium sativum* L.) seeds and serves as a potent novel allelopathic substance that promotes the shoot growth of different plant species. This compound has been identified as **1** by spectral analyses and total synthesis.[1,2] Lepidimide is widespread in the exudate of germinated seeds of various plants species, and it is produced de novo in the kernels following water imbibition. It is also present in dry seeds and is released into the environment.[3] Additionally, it was found by HPLC analysis that lepidimoide is also included, not only in seeds, but also in growing rosette leaves, flower stalks, inflorescences, and roots of *A. thaliana*.[4] Lepidimoide promotes, not only shoot growth, but also leaf development, flowering, and seed production in arabidopsis and light-induced chlorophyll accumulation in cotyledons of sunflower seedlings.[5,6] Furthermore, lepidimoide inhibits the loss of total chlorophyll in excised *Avena* leaf segments during leaf senescence and delays abscission in bean petiole explants.[7,8] These results suggest that lepidimoide may be a natural hormone-like substance that controls various physiological developments in plants. Structure–activity relationship studies of lepidimoide by means of the cockscomb hypocotyls elongation test have indicated that the carboxylic acid free-type (lepidimoic acid) showed growth-promoting activity as high as that of the original lepidimoide (sodium salt type), suggesting that the sodium salt is not the structural factor for the activity. If the α,β-unsaturated carboxylic acid was hydrogenated or esterified with methanol, the growth-promoting activity became weak.[9] The methyl glycoside of lepidimoide also showed a weak activity.[10]

Lepidimoide was first synthesized from D-glucose and L-rhamnose in 22 steps by Kosemura et al., but the method is complicated and the yield is very low.[2] Therefore, it was deemed necessary to develop a simplified, large-scale production method for the synthesis of lepidimoide.

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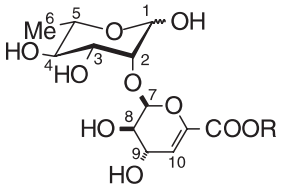


1 R = Na Lepidimoide

2 R = H Lepidimoic acid

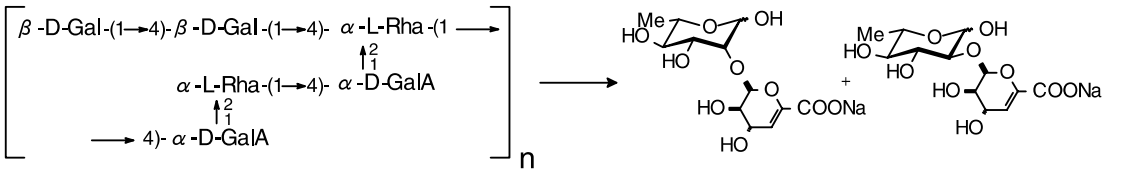
Fry et al., on the biosynthetic origin of lepidimoide, suggested that it is formed in seeds by the cleavage of pectic polysaccharides such as rhamnogalacturonan.[11] On the other hand, Tanaka et al. reported that out of 50 fungi and 8 bacteria, 29 fungi produced an oligosaccharide that has the same R_f value on thin-layer chromatography as lepidimoide from the polysaccharide of okra, and they confirmed that the fungal strain AHU9748 produced lepidimoide from okra polysaccharides.[12] Okra mucilage F, which was isolated from immature okra fruit (seeds removed),[13] is a very favorable pectic polysaccharide as the starting compound for the preparation of lepidimoide by a chemical procedure. It is known that the β-eliminative depolymerization of carbohydrates containing the parts of the uronic acid ester occurs in alkaline media to produce unsaturated sugars.[14] This degradation method would be applicable to the lepidimoic acid preparation. On the basis of this background information, we now disclose in detail the highly efficient preparation of lepidimoide from okra mucilage by sequential chemical degradation reactions.[10]

2. Results and discussion

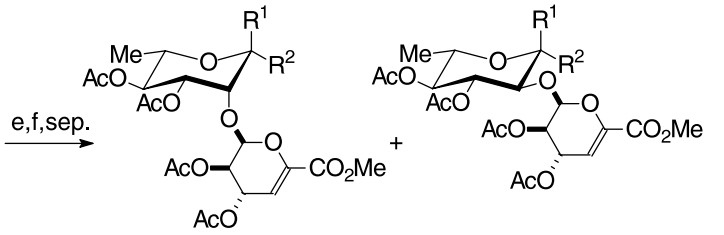
Tomoda et al. isolated pure okra mucilage, which is called okra mucilage F, from deseeded immature okra fruits using several purification procedures.[13] This compound was reported to be a glycoprotein with a molecular weight of nearly 1,700,000 and to have a hexasaccharide repeating unit. It is composed of rhamnose, galacturonic acid, and galactose in the ratio of 1:1:1. However, the yield of okra mucilage F from okra fruit was very low (0.18% of the deseeded immature fruits). We then tried to modify their isolation method for obtaining it in a better yield. Immature okra fruits (4.5 kg) including the seeds were homogenized and extracted with water. After filtration, methonal was added, and the precipitate was collected. It was dissolved in water and this treatment was repeated two times. The precipitate so was obtained dried in vacuo, and the okra mucilage (50 g) was a brown block. The mucilage was a glycoprotein that was a perfect Ca salt and had a molecular weight of about 10,000,000. The reason why the molecular weight of our mucilage is quite different from that of okra mucilage F is due to the difference in their counter cations. Our okra mucilage is a Ca salt, while okra mucilage F must be the Na salt, based on its preparation procedure. The component sugars of our okra mucilage were rhamnose, galacturonic acid, galactose, glucose, and glucuronic acid in the ratio of 1:1.83:2.46:0.33:0.17. Considering these results, our mucilage was not as pure as okra mucilage F. However, our mucilage is of sufficient purity for the lepidimoide synthesis, because it contained rhamnose and galacturonic acid, and hence included a high level of okra mucilage F (Scheme 1).

We began the production of lepidimoide from the okra mucilage according to the process shown in Scheme 1. Since the starting material was an enormous glycoprotein whose purification is difficult, we chose a method fundamentally without purification of these intermediates. The galactose of the side chain was removed from the hexasaccharide repeating unit by heating with 0.5 M trifluoroacetic acid, and the disaccharide repeating unit was produced. It was acetylated with acetic anhydride–sodium acetate and esterified with trimethylsilyldiazomethane, and the resulting poly-disaccharide derivative was treated with sodium methoxide in methanol. During this process, β-eliminative degradation of the poly-disaccharide occurs. Lepidimoide was obtained by hydrolysis of the reaction mixture. The compound was purified by centrifugal partition chromatography, followed by preparative HPLC, and the unsaturated disaccharides (showed one peak and duplicated with authentic lepidimoide upon HPLC analysis under the previous conditions[3]) were obtained in a yield of 2.9% from the okra mucilage.

However, the disaccharides were separated into four peaks under new HPLC conditions (Fig. 1). Peaks 1 and 3 were assigned to lepidimoide by comparison with authentic lepidimoide,[2] but these could not be individually isolated because they were anomers. Therefore, the mixture was acetylated with acetic anhydride–sodium acetate, and then esterified with trimethylsilyldiazomethane, and this reaction mixture showed four peaks upon HPLC analysis (Fig. 2). These derivatives were perfectly separated into four compounds by HPLC. The chemical structures for the individual peaks (Scheme 1) were determined from the ^{1}H NMR data (Karplus–Conroy graph). Peak 1: the coupling constant of $J_{3,2}$ 9.6 Hz suggested the presence of a 6-deoxy-L-glucopyranosyl group, which was epimerized from the L-rhamnopyranosyl group. The coupling constant of $J_{1,2}$ 8.4 Hz showed that H-1 was an axialbond. Therefore, peak 1 was compound **6**. This conclusion was also confirmed by single-crystal X-ray analysis (Fig. 3) of the compound corresponding to peak 1, and the absolute configuration of compound **6** was determined as shown

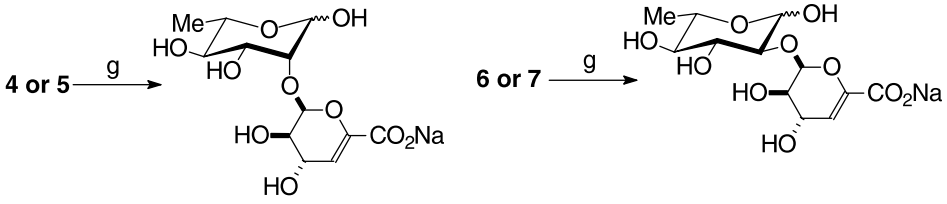


Okra mucilage F **2** Lepidimoic acid **3** epi-Lepidimoic acid



4 $R^1 = H$, $R^2 = OAc$ **6** $R^1 = H$, $R^2 = OAc$

5 $R^1 = OAc$, $R^2 = H$ **7** $R^1 = OAc$, $R^2 = H$



1 Lepidimoide **8** epi-Lepidimoide

Scheme 1. Synthetic route for lepidimoide and its epimer: (a) 0.5 M TFA, reflux; (b) Ac_2O–NaOAc, reflux; (c) $TMSCHN_2$; (d) NaOMe, H_2O. Separation and identification of these derivatives: (e) Ac_2O–NaOAc, reflux; (f) $TMSCHN_2$ saponification of separated derivatives (**4**–**7**): (g) NaOH, Dowex 50W X8 (H^+ form), Amberlite IRC50 (Na^+ form).

in Scheme 1. Peak 2: the coupling constant of $J_{3,2}$ 10.0 Hz suggested the presence of a 6-deoxy-L-glucopyranosyl group. The coupling constant of $J_{1,2}$ 4.0 Hz showed that H-1 was an equatorial bond. It was compound **7**. Peak 3: the coupling constant of $J_{3,2}$ 3.6 Hz showed the presence of an L-rhamnopyranosyl group. The coupling constant of $J_{1,2}$ 2.0 Hz showed that H-1 was an equatorial bond. Therefore, it was compound **5**. Peak 4: the coupling constant of $J_{3,2}$ 3.2 Hz suggested the presence of an L-rhamnopyranosyl group. The coupling constant of $J_{1,2}$ 0 Hz (singlet) showed that H-1 was an axial bond. Therefore, it was compound **4**. When hydrolysis of the compound corresponding to peak 3 (compound **5**) or peak **4** (compound **4**) of Figure 2 was carried out in alkaline medium, the compound corresponding to peak 1 and peak 3 of Figure 1 was obtained, respectively, and these peaks were identical with those of authentic lepidimoide (Scheme 1). Thus these compounds were anomers, and the anomeric ratio was 1:4. We could clearly separate lepidimoide into two peaks on HPLC for the first time (Fig. 4).

The ^1H NMR data (d, 1H, $J_{1,2}$ 1.8 Hz, H-1) showed that the main anomer possessed an axial bond (–OH). In addition, hydrolysis of the compound corresponding to peak 1 (compound **6**) or peak 2 (compound **7**) of Figure 2 produced the compound corresponding to peak 2 and peak 4 of Figure 1, respectively. These peaks were *epi*-lepidimoide **8** (Scheme 1). *epi*-Lepidimoide was identified as the 4-deoxy-β-L-*threo*-hex-4-enopyranuronosyl-(1 → 2)-6-deoxy-L-glucopyranose monosodium

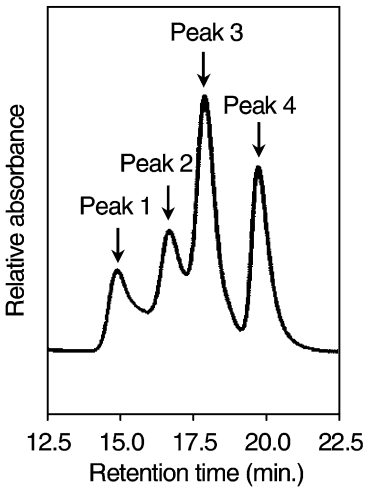


Figure 1. Unsaturated disaccharides separated into four peaks under the new HPLC conditions: Amide-80 (Toso Co., Ltd.), 4.6×250 mm, 9:1 CH_3CN 2 mM aq TFA, 1.0 mL/min, 30 °C.

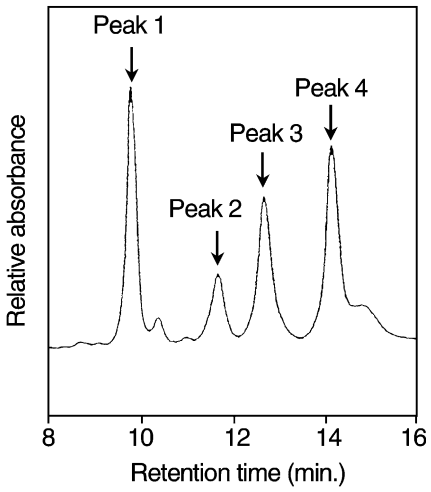


Figure 2. HPLC chart of derivatives. HPLC conditions: YMC-Pack SIL SH-043-5 (YMC Corp.), 20×250 mm, 6:4 AcOEt–*n*-hexane.

salt by its ^{1}H NMR spectrum. *epi*-Lepidimoide, as an anomeric mixture, also showed two peaks (Fig. 4) on HPLC. The anomeric ratio was as the same as that of lepidimoide. The ^{1}H NMR data (d, 1H $J_{1,2}$ 3.6 Hz, H-1) showed that the main anomer is an axial bond (–OH). The ^{1}H NMR data of our obtained lepidimoide,

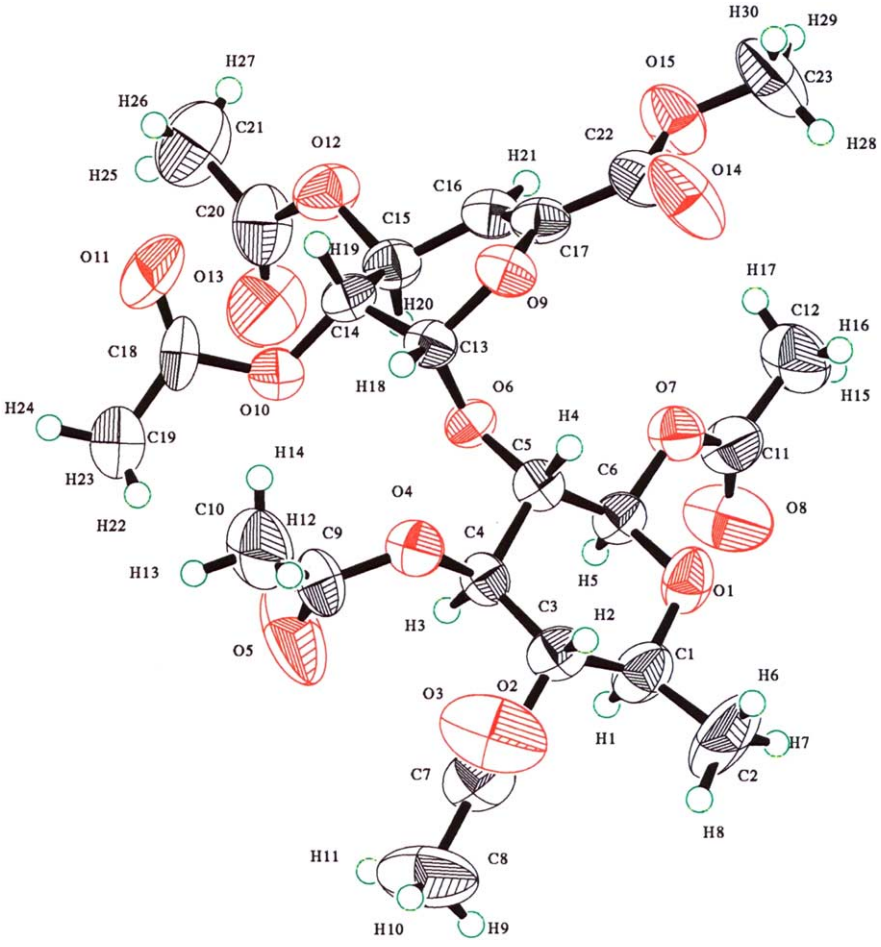


Figure 3. ORTEP of compound **6** ($C_{23}H_{30}O_{15}$).

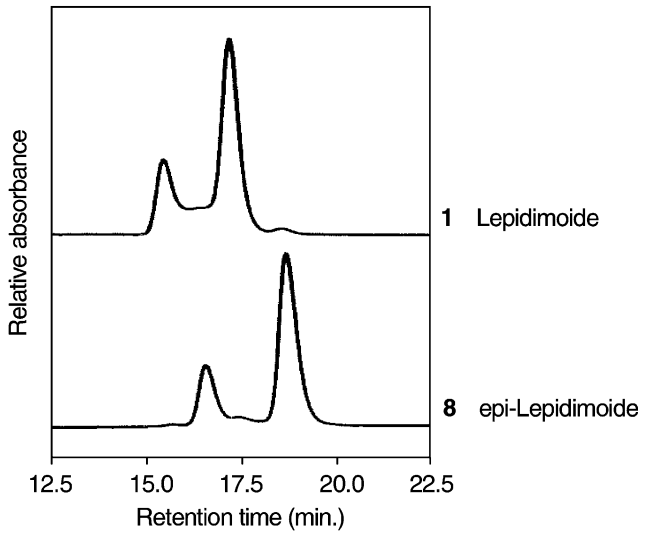


Figure 4. HPLC charts of lepidimoide and *epi*-lepidimoide.

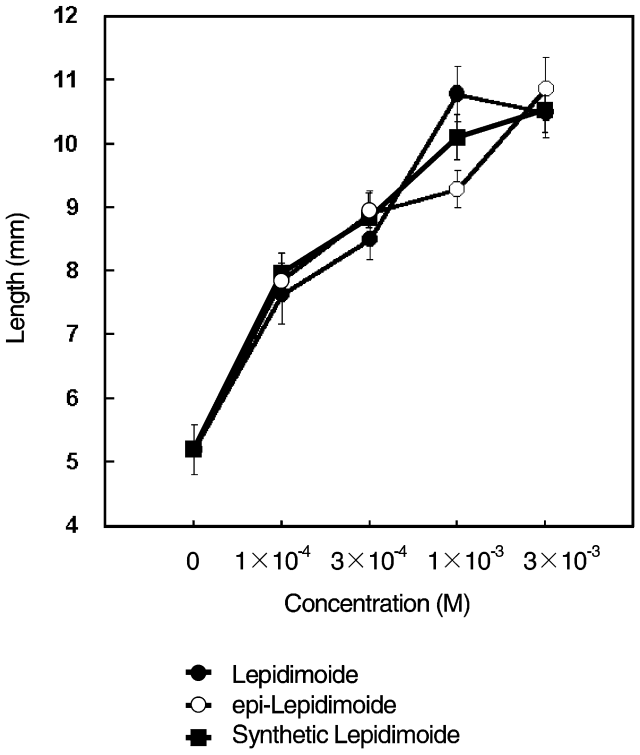


Figure 5. Cockscomb hypocotyls elongation test.

epi-lepidimoide, and the reported synthetic lepidimoide are summarized in Table 1. These data were further supported by the ^{13}C NMR spectrum. Furthermore, the absolute configuration of compound **6** was determined by X-ray analysis, and the result supported the structure of *epi*-lepidimoide (Scheme 1). Part of the L-rhamnosyl group might be epimerized to the 6-deoxy-L-glucosyl group during the process of β-eliminative degradation in alkaline medium. We demonstrated that lepidimoide easily epimerized to *epi*-lepidimoide in alkaline media. Thus, a purified lepidimoide was obtained, and the optical rotation showed the highest value of $[\alpha]_D^{21}$ +138° (*c* 0.034, D_2O) among the reported ones.[1,2]

The biological activities of the compounds obtained above (Fig. 5) were measured by the *Celosia argentea* elongation test. Lepidimoide obtained from okra mucilage showed a similar high growth-promoting activity to that of synthetic lepidimoide. Surprisingly, *epi*-lepidimoide also showed a similar high activity to that of lepidimoide. As a result, this easily obtainable mixture of lepidimoide and *epi*-lepidimoide will be used in further research on plant growth-promoting activity.

3. Experimental

3.1. General methods

Optical rotations were determined with a Horiba High Sensitive Polarimeter SEPA-200. The IR spectra were recorded on a Jasco FTIR-410 spectrophotometer. The HPLC analysis was carried out using a Shimadzu LC-10 system. The ^1H and ^{13}C NMR spectra were measured with a Varian Mercury 400 vx spectrometer in D_2O at

Table 1. ^1H NMR spectral data[a] of *epi*-lepidimoide, lepidimoide and reported lepidimoide[2]

Position	*epi*-Lepidimoide	Lepidimoide	Lepidimoide (reported[b])
1	5.20 (1H, d, 3.7)	5.17 (1H, d, 1.8)	5.17 (1H, d, 1.6)
2	3.56 (1H, dd, 9.2, 3.6)	4.08 (1H, dd, 3.2, 1.8)	4.08 (1H, dd, 3.4, 1.6)
3	3.64 (1H, dd, 9.5, 9.5)	3.77 (1H, dd, 9.6, 3.2)	3.76(1H, dd, 9.7, 3.4)
4	3.10 (1H, dd, 9.5, 9.5)	3.31 (1H, dd, 9.6, 9.6)	3.31(1H, dd, 9.7, 9.7)
5	3.78 (1H, dd, 9.5, 6.4)	3.79 (1H, dq, 9.6, 6.4)	3.79 (1H, dq, 9.7, 6.8)
6	1.14 (3H, d, 6.4)	1.18 (3H, d, 6.4)	1.80(3H, d, 6.8)
7	5.19 (1H, d, 2.6)	5.07 (1H, d, 2.4)	5.07(1H, d, 2.3)
8	3.70 (1H, dd)	3.72 (1H, dd, 6.8, 2.4)	3.72(1H, dd, 6.9, 2.3)
9	4.34 (1H, dd, 8.4, 2.8)	4.26 (1H, dd, 6.8, 3.2)	4.26 (1H, dd, 6.9, 3.2)
10	5.70 (1H, d, 2.8),	5.72 (1H, d, 3.2)	5.72 (1H, d, 3.2)

[a]Reported as δ (ppm) in D_2O at 400 MHz.
[b]See Ref. 1.

room temperature. The mass spectra (MALDITOF-MS) were recorded on a Shimadzu KRATOS KOMPACT MALDI II using a fine cobalt powder as the matrix.

3.2. Extraction of okra mucilage

The immature okra fruit was obtained in October 1996 from plants cultivated in Miyazaki Prefecture. The immature okra fruit (4.5 kg) was crushed with a mixer for 60 s at 1500 rpm. The juice that resulted was extracted with water (10 L) and ice (5 kg) with gentle stirring for 12 h. After filtration under pressure (2–3 kg/cm^2), MeOH (25 L) was added to the filtrate, and the precipitate was collected. This treatment was repeated three times, and the resulting product was then dried in vacuo for 2 days at 55 °C. The okra mucilage thus obtained was a brown block (50 g).

3.3. Quantitative analysis of component sugars

A sample (5 mg) was hydrolyzed with 1.5 M HCl (0.5 mL) in a sealed tube at 100 °C for 6 h, followed by concentration in vacuo. Distilled water (0.2 mL) was added and evaporated, then similarly repeated three times. Dry benzene (0.4 mL) was added and evaporated in vacuo, then repeated three times. TMS (0.5 mL, Tokyo Chemical Industry Co., Ltd.) was added to the residue under nitrogen, and this was treated with supersonic waves for 30 min, then reacted for 2 h at 50 °C. The solution was directly analyzed by gas chromatography (column, OV225, 3.2 mm 2.1 m; column temp, 110–250 °C 3 °C/min; injection temp, 250 °C; detection temp, 250 °C, 100 kPa in N_2). The same mixture by weight of rhamnose, galactose, glucose, glucuronic acid, and galacturonic acid was reacted and treated similar to the test sample. The response factors of the component sugars were measured, and the sugars were determined in the okra mucilage.

3.4. Other analyses of okra mucilage

The N (N: 9%) content of each sample was measured with EPMA by Sumika Chemical Analysis Service, Ltd. The quantity of Ca (2.2%) was measured with ICP by Sumika Chemical Analysis Service, Ltd. The molecular weight was measured with GPC by Toray Research Center, Inc.

3.5. Synthesis of an epimeric mixture of lepidimoic acid

Okra mucilage (20 g) was reacted with 0.5 M aq CF_3CO_2H (1000 mL) under reflux for 1 h. The reaction mixture was concentrated in vacuo) and purified with 600 mL of DEAE-cellulose (eluent: H_2O, 0.15 M HCOOH). After removing the neutral materials, the acidic elements were collected and concentrated in vacuo. The residue (5.8 g) thus obtained was added to a mixture of Ac_2O (150 mL) and NaOAc (2 g), and they were refluxed for 1 h. Distilled water (1000 mL) was added to the reaction mixture. The mixture was extracted with EtOAc (300 mL × 4). The EtOAc layer was washed with brine (300 mL × 2), and after being dried over Na_2SO_4, concentrated in vacuo. The product was dissolved in 9:1 MeOH–benzene (120 mL), and then 2.0 M $TMSCHN_2$ in *n*-hexane (3.3 mL) was added. After stirring for 5 min, the mixture was concentrated in vacuo to produce a brown block (2.7 g). It was added to 1.0 M NaOMe in MeOH (120 mL), and the mixture was stirred at room temperature for 10 h. Distilled water (30 mL) was added to the mixture, and Dowex 50W X8 (H^+ form) was added with stirring. After filtration, the reaction mixture was concentrated in vacuo. The product thus obtained was subjected to centrifugal partition chromatography (eluent: 4:1:5 *n*-BuOH–MeOH–H_2O; flow rate 15 mL/min; rotation, 700 rpm; 25 °C, followed by preparative HPLC (column: Tosoh, Amide-80, 4.6 × 250 mm, solvent: 77:23 CH_3CN 2 mM aq CF_3CO_2H; acid, flow rate 1.0 mL/min; column temp, 80 °C; detector: 214 nm). A colorless powder (580 mg, 2.9% from okra mucilage) was obtained. Under these HPLC conditions the colorless powder showed only one peak that was shown to coelute with synthetic lepidimoide. However, it separated into four peaks (Fig. 3) under the new HPLC conditions (column, Tosoh, Amide-80; 4.6 × 250 mm; solvent, 9:1 CH_3CN 2 mM aq CF_3CO_2H, flow rate, 1.0 mL/min; column temp, 25 °C; detector: 214 nm).

3.6. Compounds 4–7

The colorless powder (1.3 g) that was isolated above was added to a mixture of Ac_2O (43 mL) and NaOAc (2.4 g) and refluxed for 0.5 h. After cooling, distilled water (400 mL) was added, and the mixture was extracted with EtOAc (100 mL × 6). The organic layer was washed with brine (100 mL × 2), dried over Na_2SO_4, and concentrated in vacuo. The resulting brown powder (790 mg) was dissolved with 9:1 MeOH–benzene (27 mL) and 2.0 M $TMSCHN_2$ in *n*-hexane (1.2 mL) was added. The mixture was stirred at room temperature for 0.5 h and concentrated in vacuo to give a brown powder (740 mg). The product was subjected to silica gel chromatography (eluent, 2:8 EtOAc–*n*-hexane), and a mixture of four compounds was produced as a colorless oil (720 mg). The mixture (Fig. 4) was separated into **4** (183 mg) **5** (160 mg), **6** (282 mg) and **7** (57 mg) by preparative HPLC (YMC-Pack SIL SH-043-5, 20 × 250 mm; eluent, 6:4 EtOAc–*n*-hexane; flow rate, 18 mL/min; detector: 254 nm).

3.6.1. Compound 4. $[\alpha]_D^{21}$ +172° (*c* 0.10, $CHCl_3$); IR: 1748 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.16 (d, 1H, $J_{10,9}$ 3.2 Hz, H-10), 5.72 (s, 1H, H-1), 5.71 (d, 1H, $J_{7,8}$ 2.8 Hz, H-7), 5.64 (dd, 1H, $J_{9,8}$ 7.6 Hz, H-9), 5.10 (dd, 1H, H-8), 5.02 (dd, 1H, $J_{4,3}$ 10.0, $J_{4,5}$ 9.8 Hz, H-4), 4.91 (dd, 1H, $J_{3,2}$ 3.2 Hz, H-3), 4.43 (d, 1H, H-2), 3.80 (s, 3H, H-13), 3.59 (dq, 1H, $J_{5,6}$ 6.0 Hz, H-5), 2.14 (s, 3H, H-Ac), 2.13 (s, 3H, H-Ac), 2.11 (s, 3H, H-Ac), 2.04 (s, 3H, H-Ac), 1.95 (s, 3H, H-Ac), 1.25 (d, 3H, H-6); MALDITOF-MS: calcd for $C_{23}H_{30}O_{15}$: *m*/*z* 546. Found: *m*/*z* 546 $[M]^+$.

3.6.2. Compound 5. $[\alpha]_D^{21}$ +135° (*c* 0.10, $CHCl_3$); IR: 1750 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.14 (d, 1H, $J_{10,9}$ 3.6 Hz, H-10), 6.04 (d, 1H, J_{12} 2.0 Hz, H-1), 5.59 (dd, 1H, $J_{9,8}$ 7.6 Hz, H-9), 5.31 (d, 1H, $J_{7,8}$ 2.4 Hz, H-7), 5.18 (dd, 1H, H-8), 5.16 (dd, 1H, $J_{3,4}$ 9.6, $J_{3,2}$ 3.6 Hz, H-3), 5.06 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-4), 4.27 (dd, 1H, H-2), 3.89 (dq, 1H, $J_{5,6}$ 6.0 Hz, H-5), 3.80 (s, 3H, H-13), 2.15 (s, 6H, H-Ac), 2.10 (s, 3H, H-Ac), 2.05 (s, 3H, H-Ac), 2.00 (s, 3H, H-Ac), 1.22 (d, 3H, H-6); MALDITOF-MS: calcd for $C_{23}H_{30}O_{15}$: *m*/*z* 546. Found: *m*/*z* 546 $[M]^+$.

3.6.3. Compound 6. $[\alpha]_D^{21}$ +124° (*c* 0.10, $CHCl_3$); IR: 1754 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.03 (d, 1H, $J_{10,9}$ 2.4 Hz, H-10), 5.59 (d, 1H, J_{12} 8.4 Hz, H-1), 5.58 (dd, 1H, $J_{9,8}$ 8.8 Hz, H-9), 5.52 (d, 1 H, $J_{7,8}$ 2.8 Hz, H-7), 5.29 (dd, 1H, $J_{3,4}$ 9.6, $J_{3,2}$ 9.6 Hz, H-3), 4.95 (dd, 1H, H-8), 4.74 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-4), 4.00 (dd, 1H, H-2), 3.85 (s, 3H, H-13), 3.69 (dq, 1H, $J_{5,6}$ 6.0 Hz, H-5), 2.09 (s, 3H, H-Ac), 2.08 (s, 3H, H-Ac), 2.05 (s, 3H, H-Ac), 2.03 (s, 6H, H-Ac), 1.21 (d, 3H, H-6); MALDITOF-MS: calcd for $C_{23}H_{30}O_{15}$ *m*/*z* 546. Found: *m*/*z* 546 $[M]^+$.

3.6.4. Compound 7. $[\alpha]_D^{21}$ +63.5° (*c* 0.10, $CHCl_3$); IR: 1748 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.13 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 6.02 (d, 1H, $J_{10,9}$ 2.7 Hz, H-10), 5.69 (dd, 1H, $J_{9,8}$ 8.8 Hz, H-9), 5.41 (d, 1H, $J_{7,8}$ 2.9 Hz, H-7), 5.40 (dd, 1H, $J_{3,4}$ 10.0, $J_{3,2}$10.0 Hz, H-3), 5.02 (dd, 1H, H-8), 4.76 (dd, 1H, $J_{4,5}$ 10.0 Hz, H-4), 4.04 (dd, 1H, H-2), 3.92 (dq, 1H, $J_{5,6}$ 6.0 Hz, H-5), 3.84 (s, 3H, H-13), 2.14 (s, 3H, H-Ac), 2.08 (s, 3H, H-Ac), 2.07 (s, 3H, H-Ac), 2.05 (s, 3H, H-Ac), 2.04 (s, 3H, H-Ac), 1.17 (d, 3H, H-6); MALDI-TOF-MS: calcd for $C_{23}H_{30}O_{15}$: *m*/*z* 546. Found: *m*/*z* 546 $[M]^+$.

3.7. Compound 1 (lepidimoide)

To a mixture of MeOH (8.1 mL) and 1.5 M NaOH (1.1 mL), compound **4** (38 mg) and **5** (50 mg) were added, and stirred at room temperature for 5 min. The mixture was decationized using a Dowex 50W X8 (H^+ form) column (eluent: H_2O), and an Amberlite IRC50 (Na^+ form) column (eluent: H_2O) to give upon evaporation of the solvent compound **1** as a colorless hygroscopic oil (52 mg): $[\alpha]_D^{21}$ +138° (*c* 0.034, D_2O); IR: 1592 cm^{-1}; ^{13}C NMR (D_2O): δ 169.19 (C-12), 145.01 (C-11), 107.71 (C-10), 97.99 (C-7), 91.85 (C-1), 77.60 (C-2), 72.46 (C-4), 69.48 (C-8), 68.65 (C-3), 66.18 (C-5), 66.12 (C-9), 17.07 (C-6); MALDITOF-MS: calcd for $C_{12}H_{17}O_{10}Na$: *m*/*z* 344. Found: *m*/*z* 345 $[M+H]^+$, 367 $[M+Na]^+$; Anal. calcd for $C_{12}H_{17}NaO_{10}\cdot 4H_2O$: C, 34.62; H, 6.05. Found: C, 35.10; H, 5.70.

3.8. Compound 8 (*epi*-lepidimoide)

To a mixture of MeOH (21 mL) and 1.5 M NaOH (2.7 mL), compound **6** (207 mg) and **7** (10 mg) were added, and stirred at room temperature for 5 min. The mixture was treated with a Dowex 50W X8 (H^+ form) column (eluent, H_2O), and an Amberlite IRC50 (Na^+ form) column (eluent, H_2O) to give upon evaporation of the solvent compound **8** as a colorless hygroscopic oil (131 mg): $[\alpha]_D^{21}$ +83° (*c* 0.024, D20); IR: 1594 cm^{-1}; ^{13}C NMR (D_2O): δ 174.82 (C-12), 150.08 (C-11), 111.60 (C-10), 98.44 (C-7), 91.85 (C-1), 77.72 (C-2), 72.39 (C-4), 70.14 (C-8), 69.31 (C-3), 68.69 (C-5), 65.93 (C-9), 17.04 (C-6); MALDITOF-MS: calcd for $C_{12}H_{17}O_{10}Na$: *m*/*z* 344. Found: *m*/*z* 345 $[M+H]^+$, 367 $[M+Na]^+$; Anal. calcd for $C_{12}H_{17}NaO_{10}\cdot 3H_2O$: C, 36.19; H, 5.82. Found: C, 37.10; H, 5.90.

3.9. Epimerization of lepidimoide

Lepidimoide (5 mg) was epimerized in 5 M NaOH (5 mL) at 50 °C. After 2 h, the ratio of lepidimoide/*epi*-lepidimoide from the HPLC analysis was 1:4.

3.10. Bioassay *C. argentea* elongation test

Eight cockscomb seeds (*C. argentea* var. *cnstata* L. *Kuntze*) were placed on filter paper moistened with 0.4 mL of test solution in a petri dish (27 mm). The petri dishes were kept in the dark at 25 °C for 4 days, and then the length of the hypocotyls were measured. This experiment was repeated three times. The results are shown in Figure 5.

3.11. X-ray crystallography

The crystal of compound **6** was obtained by crystallization with $CHCl_3$–*n*-hexane. Single-crystal X-ray analysis was conducted on a Rigaku A7C5R diffractometer. Details of the experimental are provided in Table 2, and atomic coordinates and B_{eq} values are provide in Table 3.

Supplementary material

The crystal structure data of compound **6** ($C_{23}H_{30}O_{15}$) have been deposited with the Cambridge Crystallographic Data Centre. These data may be

Table 2. X-ray crystallography

A. Crystal data	
Empirical formula	$C_{23}H_{30}O_{15}$
Formula weight	546.48
Crystal color, habit	Colorless, plate
Crystal dimensions	0.20×0.20×0.20 mm
Crystal system	monoclinic
Lattice type	Primitive
No. of reflections used for unit cell determination (2θ range)	25 (29.3–30.00°)
Omega scan peak width at half-height	0.33°
Lattice parameters	$a = 8.723(2)$ Å $b = 16.433(2)$ Å $c = 9.846(1)$ Å $\beta = 92.99(1)°$ $V = 1409.5(4)$ Å^3
Space group	$P2_1$(#4)
Z value	2
D_{calc}	1.288 g/cm^3
F_{000}	576.00
μ(MoKα)	1.09 cm^{-1}
B. Intensity measurements	
Diffractometer	Rigaku AFC5R
Radiation	MoKα ($\lambda = 0.71069$ Å) Graphite monochromated
Attenuator	Zr foil (factors = 1.00, 3.63, 12.48, 45.10)
Take-off angle	6.0°
Detector aperture	6.0 mm horizontal 6.0 mm vertical
Crystal to detector distance	258 mm
Voltage, current	50 kV, 180 mA
Temperature	23.0 °C
Scan type	ω–2θ
Scan rate	16.0°/min (in ω) (up to 5 scans)
Scan width	(1.05+0.30 tan θ)°
$2\theta_{max}$	55.0°
No. of reflections measured	Total: 3560 Unique: 3353 ($R_{int} = 0.020$) Lorentz-polarization
Corrections	Absorption (trans. factors: 0.9665–0.9998) Secondary extinction (coefficient: 6.50350e−07)
C. Structure solution and refinement	
Structure solution	Direct methods (SIR92)
Refinement	Fulknatrix least-squares
Function minimized	$\sum \omega(\|F_o\| - l F_c\|)^2$
Least squares weights	$\omega = 1/\sigma^2(F_o) = [\sigma_c^2(F_o) + p^2F_o^2/4]^{-1}$
p-Factor	0.0040
Anomalous dispersion	All nonhydrogen atoms
No. observations ($I > 3.00\sigma(I)$)	1692
No. variables	369
Reflection/parameter ratio	4.59
Residuals: R; Rw	0.044; 0.042
Residuals: R1;	0.044
No. of reflections to calc R1	1692
Goodness of fit indicator	1.34
Max shift/error in final cycle	2.71
Maximum peak in final duff. map	0.12 e$^-$/Å^3
Minimum peak in final duff. map	−0.10 e$^-$/Å^3

obtained upon request from The Directory, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 IEZ, UK, (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk; WEB: http://www.csdc.cam.ac.uk. Deposition number: CCDC 200467.

Table 3. Atomic coordinates and B_{isc}/B_{eq}[a]

Atom	*x*	*y*	*z*	B_{eq}
O(1)	0.6331(4)	0.293(1)	0.8375(3)	5.18(9)
O(2)	0.4236(4)	0.104(1)	0.8627(4)	5.51(10)
O(3)	0.2471(7)	0.105(1)	1.0168(6)	10.1(2)
O(4)	0.1946(4)	0.219(1)	0.7406(4)	4.48(8)
O(5)	0.1773(7)	0.115(1)	0.5974(7)	11.5(2)
O(6)	0.3446(4)	0.334(1)	0.5701(3)	3.60(7)
O(7)	0.6171(4)	0.400(1)	0.6943(4)	4.67(8)
O(8)	0.8453(5)	0.358(1)	0.6295(6)	8.7(2)
O(9)	0.2314(4)	0.458(1)	0.6192(3)	4.24(8)
O(10)	0.1312(5)	0.304(1)	0.3666(4)	5.48(10)
O(11)	−0.0912(8)	0.335(1)	0.2640(9)	15.6(3)
O(12)	0.2104(5)	0.459(1)	0.2083(4)	5.7(1)
O(13)	0.3086(7)	0.357(1)	0.0898(5)	9.4(2)
O(14)	0.3741(7)	0.575(1)	0.7548(4)	8.4(1)
O(15)	0.4543(5)	0.631(1)	0.5669(4)	6.4(1)
C(1)	0.6093(7)	0.208(1)	0.8628(7)	5.5(1)
C(2)	0.6936(10)	0.189(1)	0.9957(8)	9.7(3)
C(3)	0.4383(7)	0.191(1)	0.8616(6)	4.6(1)
C(4)	0.3589(6)	0.222(1)	0.7313(5)	3.68(9)
C(5)	0.4017(6)	0.311(1)	0.7043(5)	3.58(8)
C(6)	0.5741(6)	0.317(1)	0.7087(5)	3.85(9)
C(7)	0.3268(8)	0.068(1)	0.9457(8)	6.7(2)
C(8)	0.329(1)	−0.022(1)	0.933(1)	9.9(3)
C(9)	0.1189(8)	0.160(1)	0.6670(8)	6.5(2)
C(10)	−0.0481(8)	0.162(1)	0.6961(10)	8.9(2)
C(11)	0.7626(6)	0.412(1)	0.6579(6)	5.4(1)
C(12)	0.8014(8)	0.500(1)	0.6605(8)	7.3(2)
C(13)	0.2078(6)	0.378(1)	0.5636(6)	3.80(10)
C(14)	0.1556(6)	0.385(1)	0.4136(6)	4.2(1)
C(15)	0.2791(6)	0.427(1)	0.3348(5)	4.3(1)
C(16)	0.3440(6)	0.499(1)	0.4142(6)	4.4(1)
C(17)	0.3192(6)	0.509(1)	0.5459(5)	4.0(1)
C(18)	−0.0001(9)	0.285(1)	0.2982(9)	8.6(2)
C(19)	−0.001(1)	0.197(1)	0.2606(10)	9.3(2)
C(20)	0.2281(8)	0.414(1)	0.0939(6)	6.7(2)
C(21)	0.1366(10)	0.451(1)	−0.0230(7)	9.1(3)
C(22)	0.3841(7)	0.575(1)	0.6345(6)	4.8(1)
C(23)	0.523(1)	0.696(1)	0.6503(8)	8.2(2)
H(1)	0.642(7)	0.173(4)	0.786(6)	8.1470
H(2)	0.397(7)	0.209(4)	0.948(6)	8.1470
H(3)	0.387(7)	0.187(4)	0.654(7)	8.1470
H(4)	0.363(7)	0.348(4)	0.770(6)	8.1470
H(5)	0.627(7)	0.285(4)	0.634(6)	8.1470
H(6)	0.6504	0.2213	1.0660	11.6674
H(7)	0.7984	0.2025	0.9909	11.6674
H(8)	0.6820	0.1335	1.0165	11.6674
H(9)	0.4300	−0.0414	0.9561	11.9116
H(10)	0.2597	−0.0453	0.9941	11.9116
H(11)	0.2996	−0.0372	0.8430	11.9116
H(12)	−0.0590	0.1514	0.7906	10.5416
H(13)	−0.1008	0.1209	0.6442	10.5416
H(14)	−0.0900	0.2135	0.6738	10.5416
H(15)	0.9034	0.5066	0.6350	8.6911
H(16)	0.7918	0.5200	0.7506	8.6911
H(17)	0.7329	0.5283	0.5998	8.6911
H(18)	0.121(7)	0.351(4)	0.620(7)	8.1470
H(19)	0.058(7)	0.412(5)	0.406(6)	8.1470
H(20)	0.3604	0.3882	0.3165	5.1851
H(21)	0.420(8)	0.535(4)	0.372(7)	8.1470
H(22)	0.0084	0.1642	0.3397	11.1342
H(23)	0.0812	0.1859	0.2038	11.1342
H(24)	−0.0960	0.1842	0.2116	11.1342
H(25)	0.1498	0.4182	−0.1028	10.9105

(*continued on next page*)

Table 3 (*continued*)

Atom	*x*	*y*	*z*	B_{eq}
H(26)	0.0309	0.4504	−0.0041	10.9105
H(27)	0.1699	0.5040	−0.0387	10.9105
H(28)	0.5993	0.6732	0.7153	9.8186
H(29)	0.5750	0.7338	0.5952	9.8186
H(30)	0.4487	0.7233	0.6991	9.8186
O(1*)	0.3669(4)	0.793(1)	0.1625(3)	5.18(9)
O(2*)	0.5764(4)	0.604(1)	0.1373(4)	5.51(10)
O(3*)	0.7529(7)	0.605(1)	−0.0168(6)	10.1(2)
O(4*)	0.8054(4)	0.719(1)	0.2594(4)	4.48(8)
O(5*)	0.8227(7)	0.615(1)	0.4026(7)	11.5(2)
O(6*)	0.6554(4)	0.834(1)	0.4299(3)	3.60(7)
O(7*)	0.3829(4)	0.900(1)	0.3057(4)	4.67(8)
O(8*)	0.1547(5)	0.858(1)	0.3705(6)	8.7(2)
O(9*)	0.7686(4)	0.958(1)	0.3808(3)	4.24(8)
O(10*)	0.8688(5)	0.804(1)	0.6334(4)	5.48(10)
O(11*)	1.0912(8)	0.835(1)	0.7360(9)	15.6(3)
O(12*)	0.7896(5)	0.959(1)	0.7917(4)	5.7(1)
O(13*)	0.6914(7)	0.857(1)	0.9102(5)	9.4(2)
O(14*)	0.6259(7)	1.075(1)	0.2452(4)	8.4(1)
O(15*)	0.5457(5)	1.131(1)	0.4331(4)	6.4(1)
C(1*)	0.3907(7)	0.708(1)	0.1372(7)	5.5(1)
C(2*)	0.3064(10)	0.689(1)	0.0043(8)	9.7(3)
C(3*)	0.5617(7)	0.691(1)	0.1384(6)	4.6(1)
O(4*)	0.6411(6)	0.722(1)	0.2687(5)	3.68(9)
C(5*)	0.5983(6)	0.811(1)	0.2957(5)	3.58(8)
C(6*)	0.4259(6)	0.817(1)	0.2913(5)	3.86(9)
C(7*)	0.6732(8)	0.568(1)	0.0543(8)	6.7(2)
C(8*)	0.671(1)	0.478(1)	0.067(1)	9.9(3)
C(9*)	0.8811(8)	0.660(1)	0.3330(8)	6.5(2)
C(10*)	1.0481(8)	0.662(1)	0.3039(10)	8.9(2)
C(11*)	0.2374(6)	0.912(1)	0.3421(6)	5.4(1)
C(12*)	0.1986(8)	1.000(1)	0.3395(8)	7.3(2)
C(13*)	0.7922(6)	0.878(1)	0.4364(6)	3.80(10)
C(14*)	0.8444(6)	0.885(1)	0.5864(6)	4.2(1)
C(15*)	0.7209(6)	0.927(1)	0.6652(5)	4.3(1)
C(16*)	0.6560(6)	0.999(1)	0.5858(6)	4.4(1)
C(17*)	0.6808(6)	1.009(1)	0.4541(5)	4.0(1)
C(18*)	1.0001(9)	0.785(1)	0.7018(9)	8.6(2)
C(19*)	1.001(1)	0.697(1)	0.7394(10)	9.3(2)
C(20*)	0.7719(8)	0.914(1)	0.9061(6)	6.7(2)
C(21*)	0.8634(10)	0.951(1)	1.0230(7)	9.1(3)
C(22*)	0.6159(7)	1.075(1)	0.3655(6)	4.8(1)
C(23*)	0.477(1)	1.196(1)	0.3497(8)	8.2(2)
H(1*)	0.358(7)	0.673(4)	0.214(6)	8.1470
H(2*)	0.603(7)	0.709(4)	0.052(6)	8.1470
H(3*)	0.613(7)	0.687(4)	0.346(7)	8.1470
H(4*)	0.637(7)	0.848(4)	0.230(6)	8.1470
H(5*)	0.373(7)	0.785(4)	0.366(6)	8.1470
H(6*)	0.3496	0.7213	−0.0660	11.6674
H(7*)	0.2016	0.7025	0.0091	11.6674
H(8*)	0.3180	0.6335	−0.0165	11.6674
H(9*)	0.5700	0.4586	0.0439	11.9116
H(10*)	0.7403	0.4547	0.0059	11.9116
H(11*)	0.7004	0.4628	0.1570	11.9116
H(12*)	1.0590	0.6514	0.2094	10.5416
H(13*)	1.1008	0.6209	0.3558	10.5416
H(14*)	1.0900	0.7135	0.3262	10.5416
H(15*)	0.0966	1.0066	0.3650	8.6911
H(16*)	0.2082	1.0200	0.2494	8.6911
H(17*)	0.2671	1.0283	0.4002	8.6911
H(18*)	0.879(7)	0.851(4)	0.380(7)	8.1470
H(19*)	0.942(7)	0.912(5)	0.594(6)	8.1470
H(20*)	0.6396	0.8882	0.6835	5.1851
H(21*)	0.580(8)	1.035(4)	0.628(7)	8.1470

Table 3 (*continued*)

Atom	*x*	*y*	*z*	B_{eq}
H(22*)	0.9916	0.6642	0.6603	11.1342
H(23*)	0.9188	0.6859	0.7962	11.1342
H(24*)	1.0960	0.6842	0.7884	11.1342
H(25*)	0.8502	0.9182	1.1028	10.9105
H(26*)	0.9691	0.9504	1.0041	10.9105
H(27*)	0.8301	1.0040	1.0387	10.9105
H(28*)	0.4007	1.1732	0.2847	9.8186
H(29*)	0.4250	1.2338	0.4048	9.8186
H(30*)	0.5513	1.2233	0.3009	9.8186
O	0.0000	0.0000	0.0000	0.0000
A	1.0000	0.0000	0.0000	0.0000
B	0.0000	1.0000	0.0000	0.0000
C	0.0000	0.0000	1.0000	0.0000
(F)	1.0000	1.0000	0.0000	0.0000
(F)	1.0000	0.0000	1.0000	0.0000
(F)	0.0000	1.0000	1.0000	0.0000
(F)	1.0000	1.0000	1.0000	0.0000

[a] $B_{eq} = 8/3\pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^*\cos\gamma + 2U_{13}aa^*cc^*\cos\beta + 2U_{23}bb^*cc^*\cos\alpha)$.

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