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Absolute Stereochemistry and Novel Ring-Opening Oxidation of Methyl (*R*)-2-Hydroxysterculate

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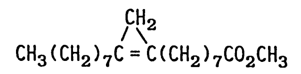
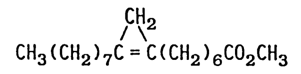
A cyclopropene-ring-containing fatty acid ester, methyl (*R*)-2-hydroxysterculate, was isolated from *Hibiscus rosa-sinensis* along with methyl sterculate and malvalate. The stereochemistry of the asymmetric carbon at C-2 was determined as *R* by applying a dibenzoate chirality method, and a mild air oxidation afforded two conjugated enone isomers accompanying a ring opening.

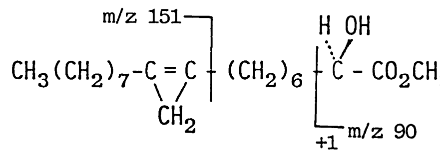
In continuous study on the seed germination inhibitors from *Hibiscus rosa-sinensis* (Malvaceae),[1,2] we isolated a third cyclopropene-ring-containing fatty acid ester, methyl (*R*)-2-hydroxysterculate (**1**), from the methanol extract of the root bark along with two known cyclopropenoids, methyl sterculate (**2**)[3] and malvalate (**3**).[4] Although these compounds **1**—**3** may be present as free acids or glycerides in the plant, they also showed inhibitory activites to lettuce seed germination.


$CH_3(CH_2)_7\overset{CH_2}{C=C}(CH_2)_7CO_2CH_3$

2


$CH_3(CH_2)_7\overset{CH_2}{C=C}(CH_2)_6CO_2CH_3$

3



$CH_3(CH_2)_7\underset{CH_2}{C=C}(CH_2)_6\overset{H\ OH}{C}-CO_2CH_3$

1

Compounds containing cyclopropane or cyclopropene ring in a fatty acid chain have been known to affect strongly insect metabolism.[5—9] The cyclopropenoids of sterculic acid, malvalic acid and their methyl esters (**2** and **3**), when fed to some noxious insects, sterilized their females[5,6] and reduced pupation and pupal weight of their larvae.[7,8] Therefore, 2-hydroxysterculate (**1**) is also expected to have similar activities against insects.

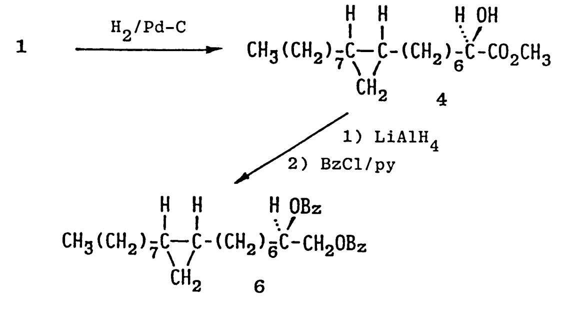
2-Hydroxysterculic acid was first isolated from alkaline hydrolysis products of glycerides from *Pachra* and *Bombacopsis* oil, and the stereochemistry at C-2 of the methyl ester **1** was inferred as *R* from the specific rotation ($[\alpha]_D$ $-5.1°$) by analogy with some α-hydroxy acid methyl esters.[10] We have now confirmed it unambiguously from the CD spectrum of a 1,2-*O*-dibenzoyl derivative **6** by applying the dibenzoate chirality method of Harada and Nakanishi.[11]

Some cyclopropenoids were known to be sensitive to air and to give polymerized mixtures.[12] A mild oxidation of **1** with air afforded a pair of conjugated enone isomers, methyl 2-hydroxy-10-methylene-9-oxooctadecanoate (**7**) and methyl 2-hydroxy-9-methylene-10-oxooctadecanoate (**8**). We also describe this novel ring opening oxidation.

Results and Discussion

Compound **1** ($C_{20}H_{36}O_3$; $[\alpha]_D$ $-10°$), 2.8 mg, was isolated from the root bark (1.5 kg) and identified as methyl 2-hydroxysterculate by spectrometry. It showed the presence of hydroxyl (3500 cm^{-1}) and methoxycarbonyl (1740 cm^{-1}) groups and a 1,2-disubstituted cyclopropene ring [^{1}H NMR: δ=0.77 (2H, s) and ^{13}C NMR: δ=7.4 (t), 109.2 (s), and 109.5 (s)]. The presence of the hydroxyl group on 2-position was deduced from the ^{1}H NMR and MS spectra (see Experimental). The position of the cyclopropene ring in the chain was determined by a prominent peak at m/z 151, corresponding to fission α to the cyclopropene ring, which was also observed (see Experimental) in the MS of methyl sterculate (**2**) and malvalate (**3**).

Stereochemistry at C-2. Several approaches to determine the configuration of chiral alcohols have been reported,[13—15] in which CD method provides better means to determine the absolute configuration of a small amount of chiral compound having suitable UV chromophores which give Cotton effects reflecting the absolute stereochemistry. We prepared a 1,2-dibenzoate **6** from **1** according to Scheme 1 and applied the dibenzoate chirality method of Harada and Nakanishi[11] for the determination of the stereochemistry of the asymmetric carbon at C-2.



Scheme 1.

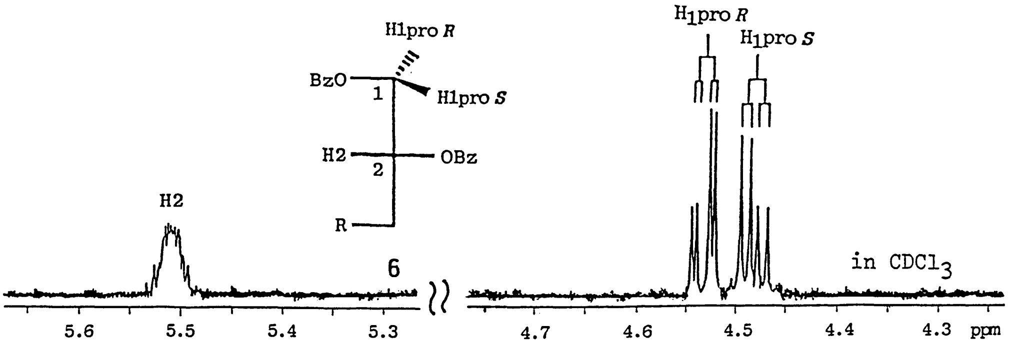


Fig. 1. The 400 MHz ^{1}H NMR spectrum of compound **6**.

The cyclopropenoid **1** was hydrogenated over palladium catalyst to give a saturated ester **4**, which should be a diastereoisomeric mixture but it showed one spot on TLC. Signals of a 1,2-disubstituted cyclopropane ring were observed at $\delta=-0.33$ and 0.56 (1H each, $\overset{\mathrm{C\underline{H}_2}}{\overset{/\ \backslash}{\text{-CH-CH-}}}$) and 0.65 (2H, $\overset{\mathrm{CH_2}}{\overset{/\ \backslash}{\text{-C}\underline{\text{H}}\text{-C}\underline{\text{H}}\text{-}}}$) in the ^{1}H NMR and at $\delta=11.2$, 16.3, and 16.3 in the ^{13}C NMR. The ester **4** was in turn reduced with lithium aluminium hydride to give a 1,2-diol **5** [^{1}H NMR: $\delta=3.46$ and 3.65 (1H each, $-\mathrm{CH(OH)C\underline{H}_2OH}$) and 3.72 (1H, $-\mathrm{C\underline{H}(OH)}$-$\mathrm{CH_2OH}$)]. The 1,2-dibenzoate **6** was prepared by acylation of **5** with benzoyl chloride in pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine.

The ^{1}H NMR spectrum of the dibenzoate **6** showed two geminal protons due to the benzoyloxymethylene group at $\delta=4.48$ (1H, dd, $J=11.7$ and 6.8 Hz) and 4.53 (1H, dd, $J=11.7$ and 3.4 Hz) (Fig. 1). The proton at higher field and with larger vicinal coupling constant was assigned to the H_1-pro-*S* in the case having 2*R* configuration and to the H_1-pro-*R* in 2*S*.[16] The assignment of the two prochiral protons at C_1 and an application of the Karplus type equation proposed by Haasnoot et al.[17,18] revealed that **6** existed predominantly in a *gt* conformer in the solution (see Fig. 2) and its population was 58% in $CDCl_3$ and 62% in CD_3OD (Table 1).

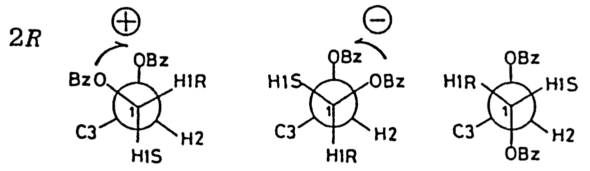


Fig. 2. Three possible conformers, *gg, gt,* and *tg,* in 2*R* about the C_1–C_2 bond.

The CD spectrum (Fig. 3) of **6** gave a negative exciton coupled CD having the first band at 237 nm with a negative sign and the second band at 224 nm with a positive one. The CD peaks can be attributed to the interaction between the di-*O*-benzoyl chromophores at C_1 and C_2, and the negative sign of the Cotton effect indicates that C_1–O and C_2–O bonds adopt a negative helicity, namely, *gt* conformation in 2*R* and *gg* in 2*S* (Fig. 2): there exist three kinds of staggered conformations in each case of 2*R* and 2*S*, namely, *gg*, *gt*, and *tg* about C_1–C_2 axis. In 2*R*, conformer *gt* of the C_1–C_2 bond should have a negative contribution, while conformer *gg* should have a positive contribution to the exciton coupling. On the other hand, the reverse contributions should be predictable in 2*S*. Here, the contribution of conformers *tg* with the antiperiplanar disposition of the two benzoyloxy groups will be negligible.

The results of the conformational analysis by NMR spectroscopy and the CD study revealed the absolute

Table 1. ^{1}H NMR Data of 1,2-Dibenzoate **6** (400 MHz)

Solvent	Chem. shift δ	Assign	$^1J_1$/Hz	$^1J_2$/Hz	Population (%)[a]		
					gg	*gt*	*tg*
$CDCl_3$	4.48	H_1-pro-*S*	11.7	6.8	37	58	5
	4.53	H_1-pro-*R*		3.4			
CD_3OD	4.46	H_1-pro-*S*	12.1	6.8	35	62	3
	4.62	H_1-pro-*R*		3.2			

a) Calculation from $J(H_1\text{-pro-}R,\ H_2)$ and $J(H_1\text{-pro-}S,\ H_2)$ values according to the following equations of Haasnoot et al. based on the Karplus equation (Refs. 17, 18).

$$2.8gg+3.1gt+10.7tg=J_{H_1\text{-pro-}R,\ H_2} \quad (1)$$

$$0.9gg+10.7gt+5.7tg=J_{H_1\text{-pro-}S,\ H_2} \quad (2)$$

$$gg+gt+tg=1 \quad (3)$$

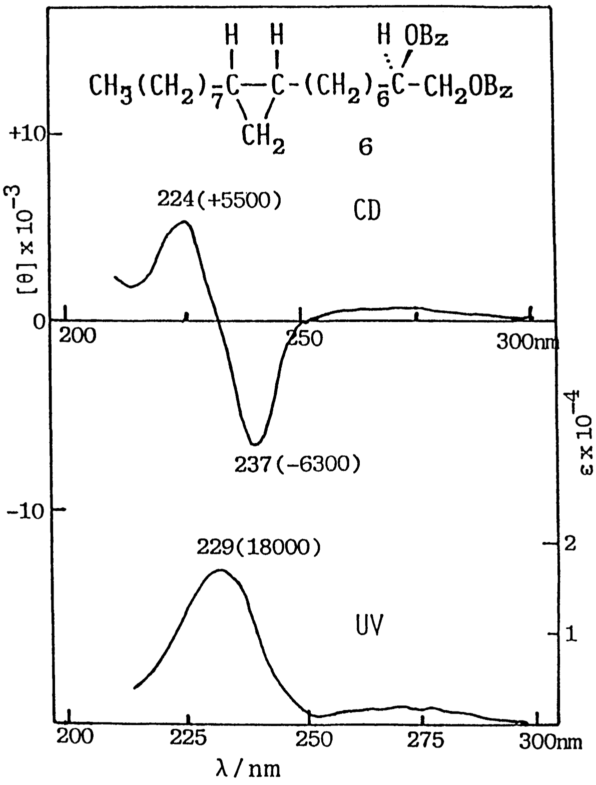


Fig. 3. CD and UV spectra of **6** in MeOH.

configuration of C-2 to be *R*.

Ring-Opening Air Oxidation. Without the bubbling air, compound **1** was slowly oxidized by air at room temperature and, after 2 weeks, completely changed to give a 1 : 1 mixture of isomers **7** and **8** (Scheme 2).

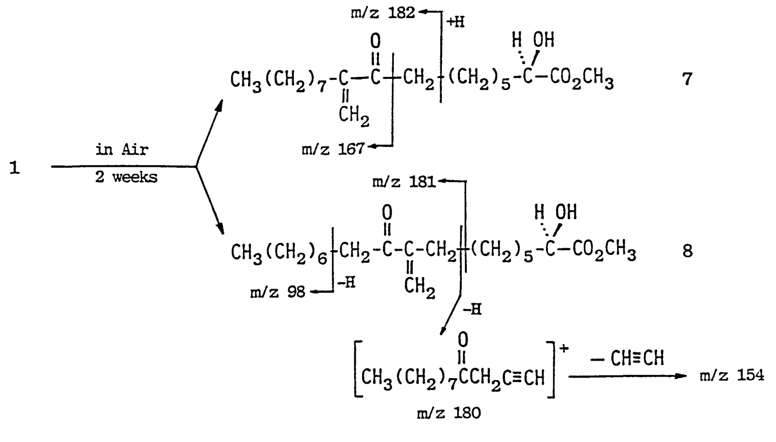
The MS spectra of **7** and **8** both indicated the same molecular formula of $C_{20}H_{36}O_4$ and their UV spectra also resembled each other to show the presence of a conjugated enone system at λ_{max} 228 nm. Signals in the ^1H and ^{13}C NMR spectra of **7** suggested the presence of a 1,1-disubstituted ethylene double bond [δ=5.70 (1H, s) and 5.97 (1H, s), and δ=124.1 and 149.2] and two carbonyl groups [δ=175.9 and 202.6] as well as a methine bearing an OH group [δ=4.21 (1H, m) and 70.4]. These data suggested that **7** had a conjugated enone system containing a side-chain methylene group instead of the cyclopropene ring in **1**. A comparison of the ^1H and ^{13}C NMR of **8** with those of **7** indicated that the two compounds had nearly identical structures and differed from one another only in the location of the enone system, and they were similar type isomers with the air-oxidation products from methyl sterculate (**2**) and malvalate (**3**) reported recently by us.[19]

Unequivocal support for their structures were obtained from their MS fragmentations. The position of the CO group in **7** has been assigned to C-9 since the significant α- and β-fission ions were obtained at *m*/*z* 167 and 182, respectively. On the other hand, **8** showed a different fragmentation to give a characteristic rearranged peak at *m*/*z* 180 and a peak at *m*/*z* 154 due to a successive loss of HC≡CH, along with a β-cleaved ion peak to the CO group at *m*/*z* 98.

These enone isomers would have been produced by a singlet oxygen attack to either of the two olefinic carbons of cyclopropene ring in **1**. Compounds **7** and **8** were also sensitive to air to give gradually a complex mixture at room temperature.

Cyclopropenoid fatty acids, sterculic acid and malvalic acid, are constituents of the seed oils of many species of the natural order Malvales and have been recognized to occur generally together, one or other being predominant according to species. The structures of these cyclopropenoid acids suggest that malvalic acid may be derived from sterculic acid by a biological α-oxidation mechanism. Evidence for this suggestion has been obtained from an incubation experiment using radioactivity labelled acetate[20] so that, if malvalic acid was derived from sterculic acid in this way, 2-hydroxysterculate (**1**) would be an intermediate to malvalate (**3**).

The occurrence of the oxidation products, **7** and **8**, in *Hibiscus* has not been observed yet, but the possibility is present at least on compound **7** because two analogs, methyl 10-methylene-9-oxooctadecanoate and 9-meth-



Scheme 2.

ylene-8-oxoheptadecanoate, respectively derived by air oxidation from sterculate (**2**) and malvalate (**3**), have been isolated from it.[2] The isolation study of **7** is now in progress.

Experimental

IR and UV spectra were recorded on Shimadzu IR-408 and UV-210A spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 400 and 100 MHz using a JEOL FX-400 spectrometer. Mass spectra were recorded on a JEOL D-300 spectrometer. Optical rotation and circular dichroism (CD) were measured using a JASCO J-20A spectropolarimeter.

Plant Material. The root bark of *H. rosa-sinensis* was collected at Amami-Oshima (Amami Islands) in August 1988.

Isolation. The fresh root bark (1.5 kg) was extracted with MeOH. The acetone-soluble part afforded an oil containing a viscous material (Fraction A) to be separated, each of which was fractionated twice by flash chromatography on SiO_2 with CH_2Cl_2–hexane. Final purification by repeated passage through an HPLC, Waters μ-bondasphere semiprep column, using a solvent system of $MeCN$–H_2O gave 2.8 mg of **1** and two known cyclopropene esters **2**[3] (54.2 mg) and **3**[4] (81.6 mg). The solvent evaporation was performed at 40 °C under reduced pressure under nitrogen and the resulting products were also stored under nitrogen atmosphere.

Methyl 2-Hydroxysterculate (1). A colorless sirup; $[\alpha]_D^{24}$ $-10°$ (c 0.083, $CHCl_3$); IR (liq. film) 3500, 1740, 1200 cm^{-1}; UV (MeCN) 212 nm (ε 2700); ^{1}H NMR δ=0.77 (2H, s, $C\underline{H}_2$ of cyclopropene ring), 0.88 (3H, t, J=6.6 Hz, 18-H), 1.25—1.37 (16H, m), 1.54 (4H, m, 7- and 12-H), 1.65 (1H, m, 3-H), 1.80 (1H, m, 3-H), 2.37 (4H, t, J=7.0 Hz, 8- and 11-H), 2.67 (1H, d, J=5.9 Hz, $O\underline{H}$, exchangeable with D_2O), 3.79 (3H, s, $OC\underline{H}_3$), 4.20 (1H, dt, J=4.4 and 7.0 Hz, 2-H); ^{13}C NMR δ=7.4 ($C\underline{H}_2$ of cyclopropene ring), 14.1 (C-18), 22.7 (C-17), 24.7 (C-4), 2×26.0 (C-8 and 11), 27.3 (C-7), 27.4 (C-12), 29.1, 29.3, 2×29.4, 31.9 (C-16), 34.4 (C-3), 52.5 ($O\underline{C}H_3$), 70.5 (C-2), 109.2 (C-9), 109.5 (C-10), 175.8 (C-1); EIMS m/z 324 (4, M$^+$), 265 (5), 165 (11), 151 (14), 149 (12), 109 (34), 95 (81), 90 (54), 81 (base peak), 68 (73), 67 (59), 55 (52), 42 (75). Found: m/z 324.2612. Calcd for $C_{20}H_{36}O_3$: M, 324.2613.

Methyl Sterculate (2). ^{13}C NMR δ=7.4, 14.1, 22.7, 25.0, 26.0, 26.1, 27.3, 27.4, 29.0, 29.1, 29.2, 29.3, 2×29.4, 31.9, 34.1, 51.5, 109.2, 109.5, 174.3; EIMS m/z 308 (M)$^+$, 277, 151, 109, 95, 81, 68, 55, 42.

Methyl Malvalate (3). ^{13}C NMR δ=7.4, 14.1, 22.7, 24.9, 26.0, 27.3, 27.4, 29.0, 29.1, 29.2, 29.3, 2×29.4, 31.9, 34.1, 51.4, 109.2, 109.5, 174.3; EIMS m/z 294 (M)$^+$, 263, 196, 151, 122, 95, 81, 68, 67, 55, 42.

Cyclopropaneoctanoic Ester 4. Cyclopropenoid **1** (1.2 mg) was hydrogenated over Pd–C in EtOH for 12 h. After removal of catalyst, the filtrate was purified by prep. TLC using ether–hexane (1:1) to give **4**. ^{1}H NMR δ=−0.33 (1H, dt, J=3.6 and 4.5 Hz, $>C<^{\underline{H}}_{H}$ of cyclopropane ring), 0.56 (1H, ddd, J=3.6, 8.1 and 8.2 Hz, $>C<^{H}_{\underline{H}}$ of cyclopropane ring), 0.65 (2H, m, 9- and 10-H), 0.89 (3H, t, J=6.7 Hz, 18-H), 1.13 (4H, m, 8- and 11-H), 1.23—1.55 (20H, m), 1.65 (1H, m), 1.79 (1H, m), 2.67 (1H, d, J=6.1 Hz, $O\underline{H}$), 3.79 (3H, s, $OC\underline{H}_3$, 4.20 (1H, m, 2-H); ^{13}C NMR δ=11.2 ($\underline{C}H_2$ of cyclopropane ring), 14.5 (C-18), 2×16.3 (C-9 and 10), 23.1 (C-17), 25.2 (C-4), 2×29.1, 2×29.7, 29.8, 2×30.0, 2×30.6, 32.2 (C-16), 34.9 (C-3), 53.0 ($O\underline{C}H_3$), 71.0 (C-2), 175.0 (C-1). Found: m/z 326.2815. Calcd for $C_{20}H_{38}O_3$: M, 326.2821.

8-Cyclopropyl-1,2-octanediol (5). The cyclopropane ester **4** was reduced with $LiAlH_4$ in ether to give the diol **5**. ^{1}H NMR δ=−0.32 (1H, dt, J=4.3 and 5.4 Hz), 0.58 (1H, dt, J=4.3 and 8.1 Hz), 0.66 (2H, m), 0.89 (3H, t, J=6.8 Hz), 1.1—1.8 (26H, m), 3.46 (1H, m, 1-H), 3.65 (1H, m, 1-H), 3.72 (1H, m, 2-H); EIMS m/z 298 (M)$^+$.

8-Cyclopropyl-1,2-octanediol Dibenzoate (6). A mixture of the diol **5** and 4-dimethylaminopyridine (0.3 mg) in pyridine (0.2 ml) was stirred for 0.5 h at room temperature. To the solution was added benzoyl chloride (0.2 ml), and the mixture was stirred for 12 h. TLC separation of the product using hexane–AcOEt (3:1) gave the dibenzoate **6**. ^{1}H NMR δ=−0.34 (1H, dt, J=4.2 and 5.5 Hz), 0.57 (1H, dt, J=4.1 and 8.3 Hz), 0.64 (2H, m), 0.89 (3H, t, J=6.7 Hz), 1.12 (4H, m), 4.48 (1H, dd, J=11.7 and 6.8 Hz, 1-H-pro-*S*), 4.53 (1H, dd, J=11.7 and 3.4 Hz, 1-H-pro-*R*), 5.51 (1H, m, 2-H), 7.34 (2H, t, J=8.2 Hz), 7.37 (2H, t, J=8.2 Hz), 7.47 (2H, dd, J=8.2 and 7.5 Hz), 7.93 (2H, dd, J=7.5 and 0.8 Hz), 7.98 (2H, dd, J=7.5 and 0.8 Hz); ^{1}H NMR (CD_3OD) δ=−0.35 (1H, dt, J=4.1 and 5.5 Hz), 0.58 (1H, ddd, J=4.2, 8.1, and 8.2 Hz), 0.66 (2H, m), 0.90 (3H, t, J=6.7 Hz), 1.15 (4H, m), 4.46 (1H, dd, J=12.1 and 6.8 Hz, 1-H-pro-*S*), 4.62 (1H, dd, J=12.1 and 3.2 Hz, 1-H-pro-*R*), 5.50 (1H, m, 2-H), 7.43 (2H, t, J=7.7 Hz), 7.46 (2H, t, J=7.7 Hz), 7.58 (1H, t, J=7.7 Hz), 7.60 (1H, t, J=7.7 Hz), 7.95 (2H, d, J=7.7 Hz), 8.01 (2H, d, J=7.7 Hz). Found: m/z 506.3412. Calcd for $C_{33}H_{46}O_4$: M, 506.3397.

Air Oxidation of 1. i) When air was bubbled into a CH_2Cl_2 solution of **1** (0.2 mg) for 30 min, the product was a complex mixture on HPLC. ii) Compound **1** (0.9 mg) changed to a mixture of two compounds when exposed to air at room temperature for two weeks. The products were separated by HPLC using MeCN containing 10% H_2O to give **7** (0.4 mg) and **8** (0.4 mg).

7; ^{1}H NMR δ=0.91 (3H, t, J=6.7 Hz, 18-H), 1.67 (1H, m, 3-H), 1.80 (1H, m, 3-H), 2.23 (2H, t, J=7.7 Hz, 11-H), 2.66 (2H, t, J=7.4 Hz, 8-H), 3.79 (3H, s, $OC\underline{H}_3$), 4.21 (1H, m, 2-H), 5.70 (1H, s, $=C<^{\underline{H}}_{H}$), 5.97 (1H, s, $=C<^{H}_{\underline{H}}$); ^{13}C NMR δ=14.1 (C-18), 22.7 (C-17), 24.6 (C-7), 28.5 (C-11), 29.0, 2×29.1, 29.3, 2×29.4, 30.9, 31.9, 34.3 (C-3), 37.8 (C-8), 52.3 ($O\underline{C}H_3$), 70.4 (C-2), 124.1 ($=\underline{C}H_2$), 149.2 (C-10), 175.9 (C-1), 202.6 (C-9); EIMS m/z 340 (5, M$^+$), 324 (6), 195 (8), 182 (36), 167 (28), 139 (7), 125 (12), 111 (47), 97 (32), 90 (2), 83 (52), 69 (75), 55 (base peak).

8; ^{1}H NMR δ=0.90 (3H, t, J=6.6 Hz, 18-H), 1.66 (1H, m, 3-H), 1.80 (1H, m, 3-H), 2.24 (2H, t, J=7.6 Hz, 8-H), 2.66 (2H, t, J=7.5 Hz, 11-H), 3.71 (3H, s, $OC\underline{H}_3$), 4.21 (1H, m, 2-H), 5.70 (1H, s, $=C<^{\underline{H}}_{H}$), 5.96 (1H, s, $=C<^{H}_{\underline{H}}$); ^{13}C NMR δ=14.1 (C-18), 22.7 (C-17), 24.7 (C-12), 28.4 (C-8), 2×29.1, 29.2, 29.3, 2×29.4, 30.9, 31.8, 34.1 (C-3), 37.9 (C-11), 51.6 ($O\underline{C}H_3$), 70.5 (C-2), 123.4 ($=\underline{C}H_2$), 149.1 (C-9), 176.6 (C-1), 202.5 (C-10); EIMS m/z 340 (3, M$^+$), 181 (30), 180 (33), 154 (38), 151 (44), 137 (10), 123 (30), 109 (28), 98 (26), 95 (31), 90 (3), 81 (48), 67

(59), 42 (base peak).

Seed Germination Test. Twenty seeds of lettuce (*Lactuca sativa* L.) were placed on a filter paper in a 9-cm Petri dish containing 2 ml of test solution. The dish was incubated at 23 °C in the dark room. After 3 d, the percentage germination was determined; **1**: 55% (*c* 1.5 mM), **2**: 20% (*c* 2.3 mM), and **3**: 15% (*c* 2.3 mM).

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