Phytochemistry, Vol 24, No 1, pp 39–42, 1985
Printed in Great Britain

0031–9422/85 \$3.00 + 0.00

STRUCTURES AND SYNTHESIS OF SEED-GERMINATION INHIBITORS FROM *HIBISCUS ROSA-SINENSIS*

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(*Received* 8 *May* 1984)

Key Word Index—*Hibiscus rosa-sinensis*; Malvaceae; seed germination; inhibition; acetylenic acids; *o*-nitrophenyl selenide.

Abstract—8-Nonynoic, 9-decynoic acids and their methyl esters were isolated for the first time as natural products from *Hibiscus rosa-sinensis*. They were inhibitors of the germination of lettuce seeds and were synthesized via *o*-nitrophenyl selenides.

INTRODUCTION

The leaves and roots of hibiscus are widely used for medical purposes. In the course of an investigation of bioactive compounds of the plant, the stem bark of Japanese *Hibiscus rosa-sinensis* has been found to yield two acetylenic straight-chain acids, 8-nonynoic acid (**3**) and 9-decynoic acid (**4**), and their methyl esters, **1** and **2**, which were inhibitors of the germination of lettuce seeds. They are one of the few classes of aliphatic compounds possessing a terminal acetylenic linkage from higher plants. The present paper describes the isolation, characterization and synthesis of these compounds.

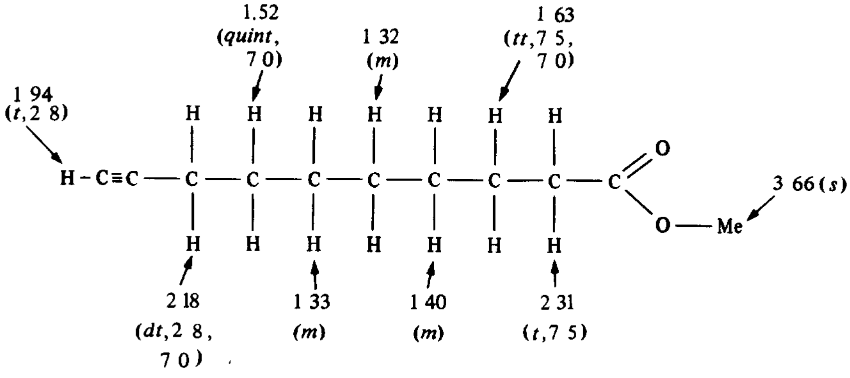
RESULTS AND DISCUSSION

The isolation of **1**–**4**, guided by bio-assay against lettuce seed, required careful use of HPLC. Compounds **1** and **2** were finally obtained by using 50% dichloromethane in hexane as the solvent: **1**, oil, $C_{10}H_{16}O_2$ (FDMS *m/z*: 168, $[M]^+$); **2**, oil, $C_{11}H_{18}O_2$ (FDMS *m/z*: 182, $[M]^+$). On the other hand, the use of 0.4–0.6% methanol in dichloromethane as the solvent gave a mixture of **3** and **4**, which, on methylation with diazomethane, afforded **1** and **2** in a ratio of 4:1. Isolation of **3** and **4** was achieved using HPLC with a water–methanol solvent system: **3**, $C_9H_{14}O_2$ mp 22–23°; **4**, $C_{10}H_{16}O_2$, mp 24.5–25.5°.

The IR absorptions of 3300 and 2100 cm^{-1} in the spectra of **1** and **2** indicated the presence of a terminal acetylenic linkage, which was also supported by the EIMS fragments of *m/z* 54 $[CH_2{=}CH{-}CH{=}CH_2]^+$ and 68 $[Me{-}(CH{=}CH)_2{-}H]^+$ [1]. Their ^1H NMR spectra showed acetylenic protons at δ1.94 (1H, *t*, $J = 2.5$ Hz and 1H, *t*, $J = 2.8$ Hz), coupling with H-7 and H-8, respectively, and carbomethoxyl groups at δ3.66. Homonuclear decoupling of the 360 MHz ^1H NMR spectra allowed us to assign all of the peaks in the spectra and derive structures **1** and **2** (Fig. 1). On hydrogenation with palladium–carbon, **1** and **2** gave methyl nonanoate and methyl decanoate, respectively.

Compounds **1**–**4** showed 50% inhibition to lettuce seed germination at 5, 10, 20 and 40 μg/ml of water, respectively. These effects are comparable to those of a well-known acetylenic growth inhibitor, *cis*-dehydromatricaric acid [2]. Aliphatic compounds have been reported to have inhibitory activity on lettuce seed germination [3]. Reynolds showed that inhibitory activity increased with carbon chain length and the main structure–activity correlation was with lipophilic properties [3, 4]. Increased lipophilicity usually leads to increased inhibitory activity; thus the methyl esters **1** and **2** showed increased activities. Recently, a variety of organic acids have also been reported to inhibit pollen germination in *Camellia sinensis* [5], and inhibition was greatest with the C_{10}- and C_{11}-acids. It is of interest that the C_{10}-compounds **2** and **4** have lower activities than the C_9-compounds **1** and **3**.

There are some reports on the synthesis of 9-decynoic acid (**4**) [6, 7], but we now deal with another approach to the synthesis of compounds **1**–**4** from 1,9- and 1,10-diols via *o*-nitrophenyl selenides **9** and **10** (Scheme 1). Recently, gentle formation of terminal olefins via the decomposition of primary alkyl phenyl selenides has been demonstrated by two groups [8, 9]. From 1,9-nonanediol and 1,10-decanediol, 8- and 9-bromo-esters **5** and **6** were prepared in three steps in *ca* 35% yields by mono-bromination with hydrobromic acid followed by oxidation and methylation. Treatment of **5** and **6** with *o*-nitrophenyl selenocyanate in the presence of sodium borohydride under nitrogen in ethanol at room temperature [8] gave *o*-nitrophenyl selenides **9**, mp 61.5–62.5°, and **10**, mp 44–45.5°, in 79 and 76% yields, respectively. On the other hand, alcohols **7** and **8** obtained by hydrolysis of **5** and **6** were also converted into the *o*-nitrophenyl selenides in 73 and 78% yields, respectively, using *o*-nitrophenyl selenocyanate with tri-*n*-butylphosphine in tetrahydrofuran at room temperature [9]. Decomposition of these selenides by 30% hydrogen peroxide afforded olefins **11** and **12** in 85 and 86% yields, respectively. The synthesis was completed by converting **11** and **12** into 8-nonynoic acid (**3**) and 9-decynoic acid (**4**), by bromination with bromine and subsequent dehydrobromination with alcoholic potassium hydroxide under reflux, in 75 and 78% yields, respectively. Both methyl esters **11** and **12** inhibited the germination of lettuce seeds with only 50% germination at 1 μg/ml of water.



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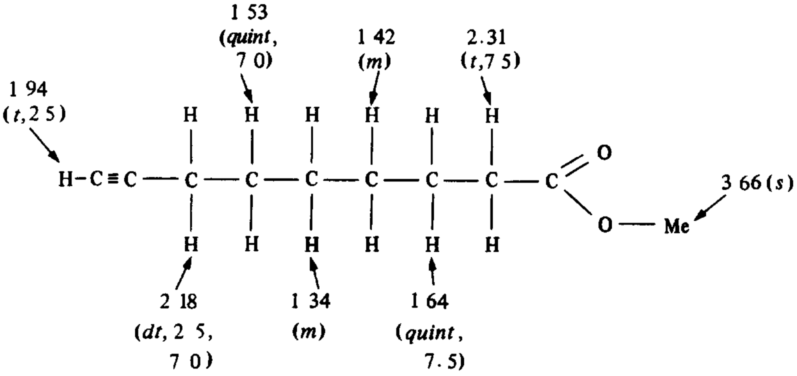

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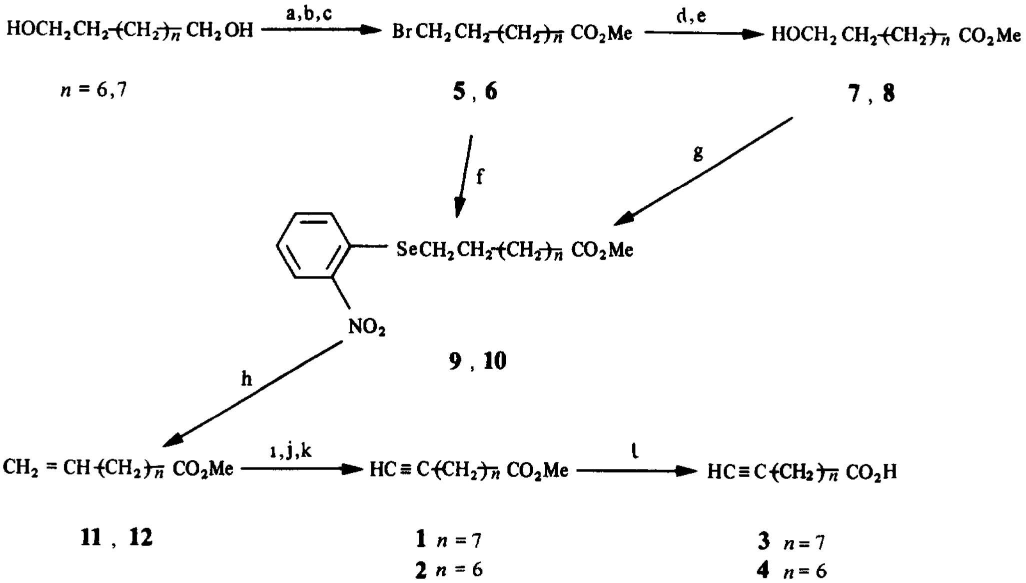
Fig. 1. ^{1}H NMR data [CDCl$_3$, 360 MHz, δ-values (ppm)] for **1** and **2**, with multiplicities and *J* values (in Hz) given in parentheses.



Scheme 1. Synthesis of acetylinic acids. a, Aq. HBr; b, KMnO$_4$, H$_2$SO$_4$; c, CH$_2$N$_2$; d, alcoholic KOH; e, CH$_2$N$_2$; f, *o*-nitrophenyl selenocyanate, NaBH$_4$, EtOH; g, *o*-nitrophenyl selenocyanate, (*n*-Bu)$_3$P, THF; h, H$_2$O$_2$, THF; i, Br$_2$, CH$_2$Cl$_2$; j, alcoholic KOH; k, CH$_2$N$_2$; l, alcoholic KOH.

EXPERIMENTAL

Mps are uncorr. UV spectra were recorded in MeOH solns. ^{1}H NMR spectra of $CDCl_3$ solns with TMS as internal standard ($\delta = 0$ ppm) were measured at 60 and 360 MHz.

Plant material. Stem bark of *H. rosa-sinensis* L. was collected in August 1980 at Amami-Oshima, Kagoshima and identified by Mr. M. Fukuyama (Kagoshima Agricultural Experiment Station).

Extraction and isolation. Fresh stem bark (2.6 kg) was extracted with MeOH (3 × 10 l.) and, after concn to 1.5 l., the MeOH extract was extracted with hexane to yield 24 g of an extract. This extract was chromatographed on silica gel with MeOH–CH_2Cl_2 and the active fractions were rechromatographed on silica gel with Et_2O–hexane to give two active fractions. Repeated passage of a non-polar fraction through an HPLC, Whatman Partisil M9 semiprep. column, using 50–70% CH_2Cl_2 in hexane as solvent finally gave the following compounds: Me 8-nonynoate (**1**), 46 mg: oil, $C_{10}H_{16}O_2$; FDMS m/z: 168 $[M]^+$; EIMS m/z (rel. int.): 137 (5), 94 (53), 81 (23), 74 (100), 68 (13), 54 (22); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2100, 1725; UV λ_{max} nm (ε): 206 (140). Me 9-decynoate (**2**), 12 mg: oil, $C_{11}H_{18}O_2$; FDMS m/z: 182 $[M]^+$; EIMS m/z (rel. int.): 151 (8), 108 (66), 87 (64), 81 (94), 74 (100), 68 (56), 54 (19); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2120, 1725; UV λ_{max} nm (ε): 206 (140). By use of 0.4–0.6% MeOH in CH_2Cl_2 as solvent, the polar fraction gave a mixture (15 mg) of **3** and **4**, which was isolated on a Waters μ-Bondapack C_{18} column with 53% H_2O in MeOH: 8-nonynoic acid (**3**): mp 22–23°; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3300, 3200–2500, 2100, 1703. 9-Decynoic acid (**4**): mp 24.5–25.5°; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3300, 3200–2500, 2100, 1700.

Hydrogenation of **1** *and* **2**. Compounds **1** (5 mg) and **2** (5 mg) were treated with Pd–C under H_2 gas in hexane to give Me nonanoate and Me decanoate, respectively. These compounds were identified by direct comparison with authentic samples (IR and ^{1}H NMR).

Methylation of a mixture of **3** *and* **4**. A mixture (10 mg) of **3** and **4** obtained from CC was methylated with CH_2N_2–Et_2O to give **1** (8 mg) and **2** (2 mg).

Hydrolysis of **1** *and* **2**. In EtOH, **1** (5 mg) and **2** (5 mg) were gently refluxed with a small amount of 30% KOH soln for 30 min to give quantitatively the corresponding acids **3** and **4**, respectively.

Synthesis of 9- and 10-bromo-esters **5** *and* **6**. 1,9-Nonanediol (5 g) was treated in a soln of 48% HBr (6.5 g) and conc. H_2SO_4 (1.1 ml) for 3 hr under reflux. Et_2O extraction gave a crude product, which was oxidized with $KMnO_4$ (2.5 g) in 20% H_2SO_4 (28 ml) at 15–20°. After methylation of the crude product with CH_2N_2, the product was chromatographed over silica gel to give **5** (2.9 g): oil; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 1740. ^{1}H NMR (60 MHz): δ2.27 (2H, *t*, J = 7 Hz, $-CH_2-CO-$), 3.38 (2H, *t*, J = 6.5 Hz, $-CH_2Br$), 3.65 (3H, *s*, $-CO_2Me$). In a similar manner as above, 1,10-decanediol (5 g) afforded **6** (2.8 g): oil; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 1740. ^{1}H NMR (60 MHz): δ2.28 (2H, *t*, J = 7 Hz, $-CH_2CO-$), 3.38 (2H, *t*, J = 6.7 Hz, $-CH_2Br$), 3.66 (3H, *s*, $-CO_2Me$).

Methyl 9-hydroxynonanoate (**7**) and *methyl 10-hydroxydecanoate* (**8**). The bromo-esters **5** and **6** were hydrolysed with aq. 20% NaOH at 50° and the products were methylated with CH_2N_2 to give **7** and **8** in *ca* 90% yields, respectively. Compound **7**: mp 56.5–57.5°; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3400, 1740; ^{1}H NMR (60 MHz): δ2.27 (2H, *t*, J = 7 Hz, $-CH_2CO-$), 3.33 (2H, *t*, J = 6 Hz, $-CH_2OH$), 3.60 (3H, *s*, $-CO_2Me$). (Found: C, 63.56; H, 11.06. Calc. for $C_{10}H_{20}O_3$: C, 63.83; H, 10.72%.) Compound **8**: mp 59.5–60.5°; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3350, 1740; ^{1}H NMR (60 MHz): δ2.31 (2H, *t*, J = 7 Hz, $-CH_2CO-$), 3.32 (2H, *t*, J = 6 Hz, $-C\underline{H}_2OH$), 3.67 (3H, *s*, $-CO_2Me$); EIMS (rel. int.): 203 $[M+1]^+$ (19), 185 (12), 153 (25), 97 (44), 83 (100), 74 (13), 69 (92). (Found: C, 65.28; H, 10.88. Calc. for $C_{11}H_{22}O_3$: C, 65.35; H, 10.98%.)

Preparation of selenides **9** *and* **10**. (i) a. To a cooled (0°) suspension of *o*-nitrophenyl selenocyanate (1.03 g, 4.5 mmol) in EtOH (20 ml) under N_2, $NaBH_4$ (0.19 g, 5 mmol) was added with stirring. Me 9-bromononanoate (**5**) (1.1 g, 4.4 mmol) was added and the soln stirred at room temp. for 13 hr. After removal of EtOH, the crude product was chromatographed over silica gel to give 1.3 g (79%) of selenide **9** as a yellow crystalline compound: mp 61.5–62.5°; IR ν_{max}^{Nujol} cm^{-1}: 1727, 1587, 1560. ^{1}H NMR (60 MHz): δ2.28 (2H, *t*, J = 7 Hz, $-CH_2CO-$), 2.90 (2H, *t*, J = 7 Hz, $-C\underline{H}_2Ph$), 3.65 (3H, *s*, $-CO_2Me$), 7.27–7.69 (3H, *m*, Ph-$\underline{H}$), 8.38 (1H, *d*, J = 8 Hz, Ph-$\underline{H}$). (Found: C, 51.65, H, 6.23; N, 3.75. Calc. for $C_{16}H_{23}NO_4Se$: C, 51.61; H, 6.23; N, 3.76%.) b. In a similar manner as above, Me 10-bromodecanoate (**6**) (1.45 g, 7.3 mmol) afforded 1.55 g (76%) of **10** as a yellow crystalline compound: mp 44–45.5°; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 1733, 1590, 1560. ^{1}H NMR (60 MHz): δ2.32 (2H, *t*, J = 7 Hz, $-CH_2CO-$), 2.96 (2H, *t*, J = 7.5 Hz, $-C\underline{H}_2Ph$), 3.69 (3H, *s*, $-CO_2Me$), 7.36–7.72 (3H, *m*, Ph-$\underline{H}$), 8.35 (1H, *d*, J = 8 Hz, Ph-$\underline{H}$). (Found: C, 52.77; H, 6.61; N, 3.65 Calc. for $C_{17}H_{25}NO_4Se$: C, 52.89; H, 6.53; N, 3.63%.)

(ii) a. A soln of Me 9-hydroxynonanoate (**7**) (450 mg, 2.4 mmol) in 8 ml of THF containing *o*-nitrophenyl selenocyanate (672 mg, 2.9 mmol) under N_2 was treated dropwise with tri-*n*-butylphosphine (600 mg, 2.9 mmol) at room temp. After the reaction was stirred for 30 min, the solvent was removed *in vacuo*. Chromatography of the residue on silica gel gave 753 mg (73%) of **9**. b. In a similar manner as above, **8** (725 mg, 3.65 mmol) afforded 1.1 g (78%) of **10**.

Preparation of methyl 8-nonenoate (**11**) *and methyl 9-decenoate* (**12**). (i) Into a cooled (0°) soln of **9** (582 mg, 1.56 mmol) in THF (15 ml), 30% H_2O_2 (4 ml, 60 mmol) was added dropwise The ice bath was removed and the soln stirred for an additional 15 hr. The mixture was diluted with H_2O and extracted with hexane. Chromatography of the hexane extract gave 225 mg (85%) of **11** as a very smelling oil: IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3070, 1740, 1640. ^{1}H NMR (60 MHz): δ2.27 (2H, *t*, J = 6.5 Hz, $-CH_2CO-$), 3.66 (3H, *s*, $-CO_2Me$), 4.78 (1H, *br d*, J = 8 Hz, ${}^{H}{>}C{=}C{<}^{\underline{H}}$), 4.83 (1H, *br d*, J = 16 Hz, ${}^{H}{>}C{=}C{<}^{H}_{\underline{H}}$), 5.40–6 10 (1H, ${}^{\underline{H}}{>}C{=}C{<}^{H}_{H}$). FDMS m/z: 170 $[M]^+$. (ii) In a similar manner as above, **10** (1.1 g) afforded **12** (440 mg, 84%) as a smelling oil; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3060, 1740, 1638. ^{1}H NMR (60 MHz): δ2.27 (2H, *t*, J = 6.5 Hz, $-CH_2CO-$), 3.63 (3H, *s*, $-CO_2Me$), 4.78 (1H, *br d*, J = 9 Hz, ${}^{H}{>}C{=}C{<}^{\underline{H}}_{H}$), 4.83 (1H, *br d*, J = 16 Hz, ${}^{H}{>}C{=}C{<}^{H}_{\underline{H}}$), 5 40–6.11 (1H, ${}^{\underline{H}}{>}C{=}C{<}^{H}_{H}$). FDMS m/z: 184 $[M]^+$.

Preparation of **1** *and* **2** *from* **11** *and* **12**. To a soln of **11** (480 mg) in $CHCl_3$ (10 ml), excess Br_2 was added and the soln stirred at room temp. for 1 hr. After usual work-up, the product was treated with KOH (670 mg) in EtOH under reflux for 1.5 hr. After acidification, the soln was extracted with hexane and the extract methylated with CH_2N_2 to give **1** (356 mg, 75%), which showed the same spectral properties (IR and ^{1}H NMR) and biological activity as the natural product. In a similar manner as above, **12** afforded **2** (78%).

Acknowledgements—We are grateful to Dr. H. Akita (The Institute of Physical and Chemical Research) for helpful discussions and suggestions, and to Dr. T. Iwashita and Dr. H. Naoki (Suntory Institute for Bioorganic Research) for ^{1}H NMR (360 MHz) and FD-mass spectra. Our thanks are also due to Professor T. Tokoroyama (Osaka City University) for elemental analysis and to Mr. M. Fukuyama (Kagoshima Agricultural Experiment Station) for the identification of plant material.

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