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ALIPHATIC COMPOUNDS FROM *HYBISCUS ROSA-SINENSIS*

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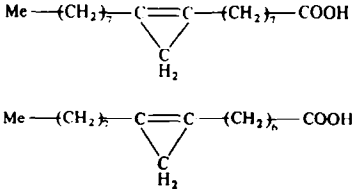
Key Word Index—*Hibiscus rosa-sinensis*; Malvaceae; stem bark; conjugated ynone; conjugated terminal enone; germination inhibition.

Abstract—Four novel aliphatic esters have been isolated in trace amounts from the stem bark of *Hibiscus rosa-sinensis* and are characterized as methyl 10-oxo-11-octadecynoate, methyl 8-oxo-9-octadecynoate, methyl 9-methylene-8-oxoheptadecanoate and methyl 10-methylene-9-oxooctadecanoate. The first two compounds inhibited the germination of lettuce seeds.

INTRODUCTION

The leaves and roots of hibiscus are widely used for medicinal 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 8-nonynoic and 9-decynoic acids (**1** and **2**), and their methyl esters (**3** and **4**), which inhibited the germination of lettuce seeds [1]. Studies of the fatty acid constituents of hibiscus have been confined mainly to the seed oils in which palmitic, stearic, oleic and linoleic acids were the main compounds [2, 3]. Bu'Lock *et al.* first isolated two acetylenic compounds, stearolic (9-octadecynoic) and 8-heptadecynoic acids, from the seeds of *H. syriacus* [4]. They also isolated two novel cyclopropene acids of sterculic and malvalic acids (**5** and **6**) from the same seeds and studied the biogenesis of these compounds to propose mechanisms that the CH_2 group of the cyclopropene ring in **5** was introduced by an alkylation reaction similar to that leading to cyclopropane acids and in **6** by a chain-shortening following α-oxidation [5]. On the other hand, Gopalakrishnan *et al.* studied fatty acid changes in *H. esculentus* tissues during growth and found that the cyclopropene fatty acids were present at some stages in roots and seeds [6].

In our studies of the stem bark of *H. rosa-sinensis*, we could not obtain the acids **5** and **6**, but we isolated two unique isomeric conjugated ynone esters (**7** and **8**) possessing moderate inhibitory activity on lettuce seed germination and two novel esters (**9** and **10**) having a conjugated terminal enone group, easily correlated to the cyclopropene acids. β-Sitosterol was also isolated and identified. The isolation and structural elucidation of the novel fatty acid methyl esters is reported in the present paper.



5

6

RESULTS AND DISCUSSION

After fractionation of the methanolic extract of the stem bark with hexane, ether and ethyl acetate, in succession, the hexane extract (4.3 g) was fractionated by silica gel CC to give three fractions which showed strong germination inhibition of lettuce seeds. Fractions I and III contained the acetylenic methyl esters **3** and **4**, and the corresponding acids **1** and **2**, identified in previous studies [1]. Rechromatography and subsequent HPLC in hexane–dichloromethane of fraction II (95 mg) afforded four oily compounds: **7** (5 mg), **8** (5 mg), **9** (3 mg) and **10** (10 mg). From the next fraction of II, β-sitosterol (53 mg) was obtained.

Compound **7** showed IR absorption bands for acetylene (2200 cm^{-1}), ester carbonyl (1730 cm^{-1}) and conjugated ketone (1660 cm^{-1}) groups. The UV spectrum also showed the presence of a conjugated ynone system at 222 nm (ε5000). The mass spectrum of **7** displayed an $[M]^+$ at *m/z* 308 corresponding with $C_{19}H_{32}O_3$. The ^1H NMR spectrum showed the presence of a carbomethoxy group at δ3.67, which was confirmed by a peak at *m/z* 276 $[M - MeOH]^+$, and methylene groups adjacent to carbonyl and acetylene groups at δ2.52 (2H, *t*, *J* = 7.5 Hz), 2.30 (2H, *t*, *J* = 7.5 Hz) and 2.36 (2H, *t*, *J* = 7.5 Hz). Homonuclear decoupling of the 360 MHz ^1H NMR spectrum allowed the assignment of all peaks in the spectrum to derive structure **7** (Table 1). Unequivocal support for the structure was also obtained from the mass spectrum (Scheme 1) in which the peaks corresponding to fission α to the CO group at C-10 are observed at *m/z* 137, 171 and 109. The intense peaks at *m/z* 95 (67%), 81 (base peak) and 67 (34%) produced from further successive losses of one, two and three CH_2 units from the *m/z* 109 peak revealed the location of the acetylenic linkage at C-11 and C-12 [7]. We therefore assign structure **7** as methyl 10-oxo-11-octadecynoate.

Compound **8** had an $[M]^+$ at *m/z* 308 ($C_{19}H_{32}O_3$) the same as that of **7** and the IR and UV spectra of this compound also showed signals for ester carbonyl (1730 cm^{-1}) and conjugated ynone groups (2200, 1660 cm^{-1} and 222 nm: ε5000) very similar to those of **7**, suggesting that **8** only differs from **7** in the location of the ynone group. The ^1H NMR spectrum showed a terminal

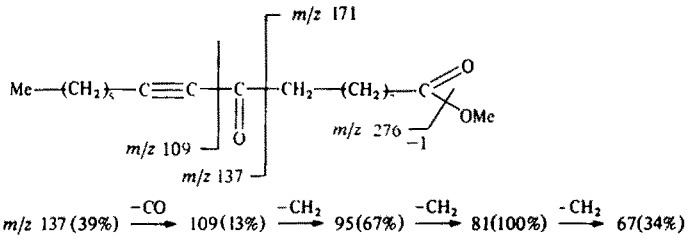
Table 1. ^{1}H NMR data for **7** (360 MHz, $CDCl_3$, TMS int. standard)

18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

$MeCH_2CH_2CH_2CH_2CH_2C{\equiv}C{-}\underset{\underset{O}{\|}}{C}{-}CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CO_2Me$

H-2	2.30 *t* (7.5)	H-7	1.34 *m*	H-15	1.39 *m*
H-3	1.64 *m*	H-8	1.65 *m*	H-16	1.28 *m*
H-4	1.34 *m*	H-9	2.52 *t* (7.5)	H-17	1.28 *m*
H-5	1.28 *m*	H-13	2.36 *t* (7.5)	H-18	0.88 *t* (7.2)
H-6	1.28 *m*	H-14	1.57 *quint* (7.5)	CO_2Me	3.67 *s*

Coupling constants (Hz) in parentheses.



Scheme 1. Mass spectral fragmentation of **7**.

methyl and a carbomethoxy group at δ0.88 and 3.67, and three methylene groups adjacent to carbonyl and acetylene groups at δ2.51, 2.31 and 2.36. The location of the ynone system was determined by the mass spectral peaks at *m/z* 137, 171, 165 (base peak) and 143 corresponding to fission α to the CO group at C-8, and the peaks at *m/z* 137, 123, 109, 95, 81 and 67 suggesting the position of an acetylene group at C-9 and C-10. The structure of **8** was finally proved by homonuclear decoupling of the 360 MHz ^{1}H NMR spectrum as methyl 8-oxo-9-octadecynoate (Fig. 1). The compounds **7** and **8** showed moderate inhibition of the germination of lettuce seeds; concentration for 50% germination inhibition (mM in H_2O): **7**; 2.4, **8**; 2.4.

The third novel compound **9**, $C_{19}H_{34}O_3$ ($[M]^+$ *m/z* 310), had an IR spectrum which showed the presence of an ester carbonyl (1730 cm^{-1}) and a conjugated enone (1670 and 1620 cm^{-1}). The UV spectrum showed absorption for a conjugated enone at 228 nm (ε4800). The ^{1}H NMR spectrum exhibited a terminal methyl and carbomethoxy groups at δ0.88 and 3.67, and a terminal methylene group at δ5.69 and 5.95 coupling with 0.6 Hz to each other [8].

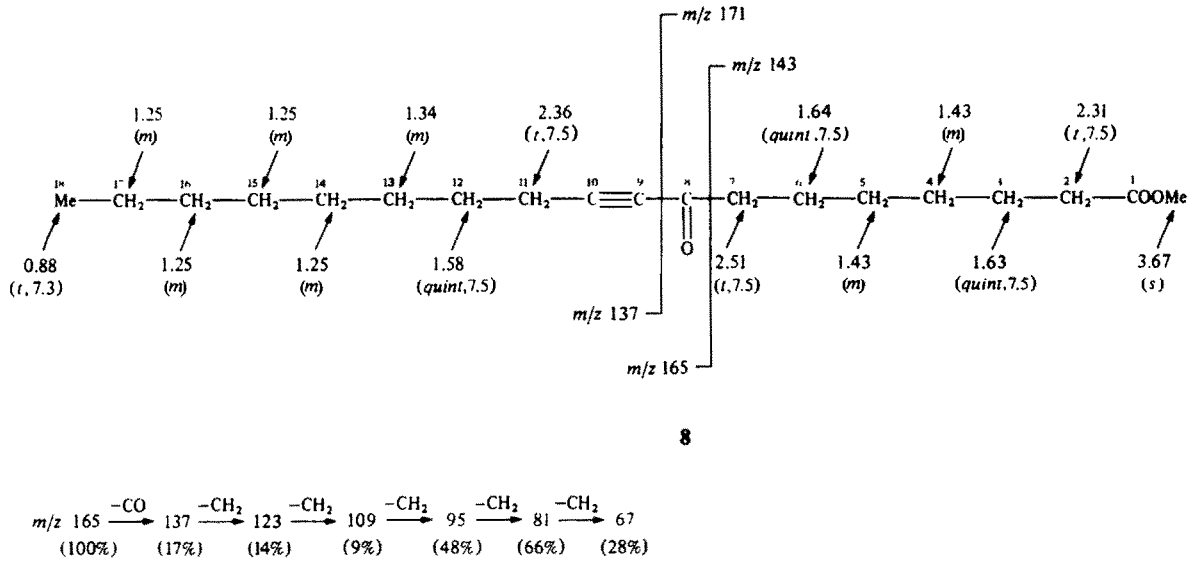
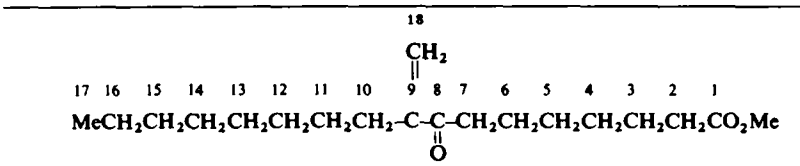


Fig. 1. ^{1}H NMR data [$CDCl_3$, 360 MHz, δ-values (ppm)] with multiplicities and *J* values (in Hz) given in parentheses, and mass spectral fragmentation of **8**.

Table 2. ^{1}H NMR data for **9** (360 MHz, $CDCl_3$, TMS int. standard)

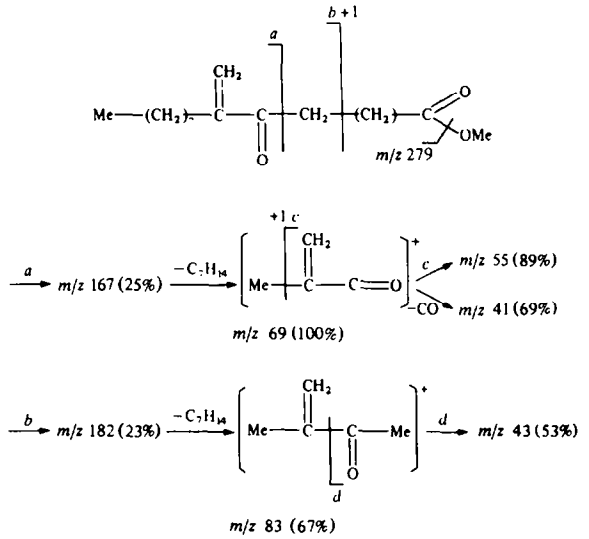


H-2	2.30 *t* (7.5)	H-10	2.25 *dt* (1.0, 7.5)	H-16	1.27 *m*
H-3	1.62 *quint* (7.4)	H-11	1.37 *m*	H-17	0.88 *t* (7.0)
H-4	1.33 *m*	H-12	1.27 *m*	Ha-18	5.69 *dt* (0.6, 1.0)
H-5	1.33 *m*	H-13	1.27 *m*	Hb-18	5.95 *br s*
H-6	1.62 *quint* (7.4)	H-14	1.27 *m*	CO_2Me	3.67 *s*
H-7	2.66 *t* (7.5)	H-15	1.27 *m*		

Coupling constants (Hz) in parentheses.

The 5.69 signal showed an allylic coupling (J = 1.0 Hz) with H-10 at δ2.25 coupled to H-11 at δ1.37 with 7.5 Hz. On the other hand, two triplets at δ2.66 (H-7, J = 7.5 Hz) and 2.30 (H-2, J = 7.5 Hz) are coupled with H-3 and H-6 at δ1.62 coupled to the signals (H-4 and H-5) at δ1.33 (Table 2). Unequivocal support for the structure was obtained from its mass spectrum. The CO group has been assigned to C-8 as the significant α- and β-fission ions are obtained at m/z 167 and 182, which further afforded the base peak at m/z 69 $[CH_2{=}CMeC{=}O]^+$ and the peak at m/z 83 $[CH_2{=}CMeCOMe]^+$, respectively [9]. The mass fragmentation is represented in Scheme 2. These data strongly corroborated the structure **9**.

The fourth compound **10**, $C_{20}H_{36}O_3$ ($[M]^+$ m/z 324), exhibited similar IR and UV spectra to those of compound **9**; ν_{max} 1730 (ester carbonyl), 1670 and 1620 cm^{-1} (conjugated enone); λ_{max} 228 nm (ε4500). The ^{1}H NMR spectrum also showed characteristic signals at δ0.85 (3H, *t*, J = 7.3 Hz), 2.16 (2H, *br t*, J = 7.5 Hz), 2.26 (2H, *t*, J = 7.5 Hz), 2.60 (2H, *t*, J = 7.5 Hz) and 3.61 (3H, *s*) together with terminal methylene signals at δ5.65 and 5.92. The mass spectrum showed the same fragment ions as **9** suggesting that the position of the conjugated enone system at m/z 182, 167, 83, 69, 55 (base peak), 43 and 41.



Scheme 2. Mass spectral fragmentation of **9**.

These data led to the assignment of the structure as **10** (Fig. 2).

Compounds **9** and **10** would be easily converted biogenetically from the cyclopropene acids **6** and **5** by oxidation and successive dehydration. Compounds **6** and **5** are considered to be precursors of **9** and **10**, but we have not yet succeeded in isolating them from the extract.

EXPERIMENTAL

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

Plant material. Stem bark of *H. rosa-sinensis* was collected in August 1982 at Tokuno-shima and Amami-Oshima, Kagoshima and identified by Mr. M. Fukuyama (Kagoshima Agricultural Experiment Station).

Germination inhibition. Lettuce seeds (*cv* Great Lakes) (20) were placed on filter paper in a 9 cm Petri dish with 1 ml test soln per dish; control seeds were treated with H_2O alone. Each of dishes was placed in a 15 cm Petri dish satd with H_2O vapour and placed in a room at 23° for 72 hr. The expt was repeated × 3 and results represent the average concns at which % germination of seeds was 50% of control value.

Extraction and isolation. Fresh stem bark (1.3 kg) was extracted with MeOH (3 × 3 l.). After conc to 200 ml, H_2O (300 ml) was added. Extraction with hexane yielded 4.3 g extract. Subsequent extraction with Et_2O and EtOAc gave 2.5 and 1.0 g of extracts, respectively. The hexane and Et_2O extracts inhibited the germination of lettuce seeds. The hexane extract was chromatographed on silica gel with hexane–CH_2Cl_2 to give three active fractions, I (65 mg), II (95 mg) and III (130 mg). Fractions I and III were found to contain two terminal acetylenic esters, **3** and **4**, and the corresponding acids, **1** and **2**, respectively, by IR, ^{1}H NMR spectra and co-HPLC with authentic samples. Fraction II was rechromatographed on silica gel with 30% C_6H_6 in CH_2Cl_2 to give 47 mg of an active fraction, which was separated into three parts by HPLC on a Whatman Partisil M9 semiprep column, using 25% hexane in CH_2Cl_2. Repeated passage of each part through an HPLC column using 5–10% Et_2O in hexane finally gave **7** (5 mg), **8** (5 mg) and **10** (10 mg). Similar treatment of a non-active fraction (25 mg) gave **9** (3 mg). From the next fraction of II, β-sitosterol (53 mg) was obtained by recrystallization from MeOH.

Compound **7**. Oil, $C_{19}H_{32}O_3$; IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2200, 1730 and 1660. UV λ_{max} nm (ε): 222 (5000). EIMS m/z (rel. int.): 308 $[M]^+$

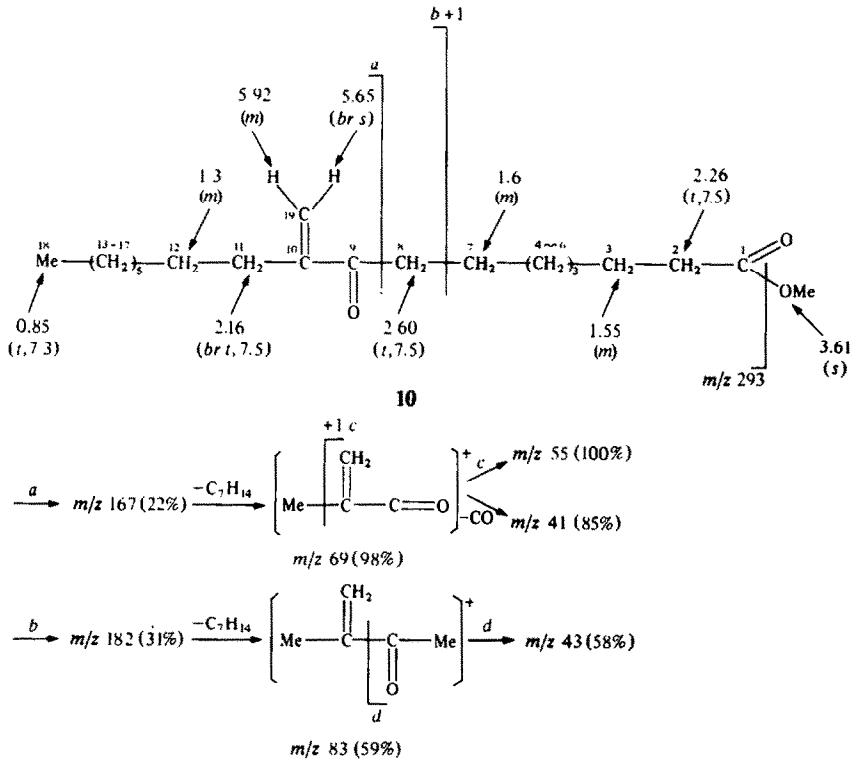


Fig. 2. ^{1}H NMR data [$CDCl_3$, 100 MHz, δ-values (ppm)] with multiplicities and J values (in Hz) given in parentheses, and mass spectral fragmentation of **10**.

(1), 276 (3), 249 (4), 209 (16), 171 (8), 150 (35), 137 (39), 136 (45), 135 (52), 110 (24), 109 (13), 107 (48), 93 (87), 81 (100), 67 (34).

Compound **8**. Oil, $C_{19}H_{32}O_3$; IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2200, 1730 and 1660. UV λ_{max} nm(ε): 222 (5000). EIMS *m/z* (rel. int.): 308 $[M]^+$ (1), 276 (4), 249 (4), 210 (24), 171 (14), 165 (100), 143 (3), 138 (33), 137 (17), 123 (14), 110 (17), 109 (9), 95 (48), 84 (32), 81 (66), 67 (28).

Compound **9**. Oil, $C_{19}H_{34}O_3$; IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1730, 1670 and 1620. UV λ_{max} nm (ε): 228 (4800). EIMS *m/z* (rel. int.): 310 $[M]^+$ (17), 279 (10), 182 (23), 167 (25), 111 (45), 97 (32), 83 (67), 74 (10), 69 (100), 55 (89), 43 (53), 41 (69).

Compound **10**. Oil, $C_{20}H_{36}O_3$; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1670 and 1620. UV λ_{max} nm (ε): 228 (4600). EIMS *m/z* (rel. int.): 324 $[M]^+$ (14), 293 (8), 182 (25), 167 (16), 111 (30), 97 (27), 83 (46), 74 (6), 69 (76), 55 (100), 43 (41), 41 (65).

β-Sitosterol. Crystallized from MeOH, $C_{29}H_{50}O$, mp 135°. EIMS *m/z* (rel. int.): 414 $[M]^+$ (100), 396 $[M-H_2O]^+$ (60), 255 (59), 159 (50), 145 (61), 133 (49), 107 (70), 105 (52), 95 (73), 93 (51), 83 (70), 81 (92), 69 (73). Acetate, mp 129–129.5°. EIMS *m/z* (rel. int.): 456 $[M]^+$ (8), 396 $[M-AcOH]^+$ (100). ^{1}H NMR (60 MHz): δ 2.03 (3H, *s*, OAc). The IR, ^{1}H NMR and MS of these compounds were identical with those of authentic samples.

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