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THREE NAPHTHALENES FROM ROOT BARK OF *HIBISCUS SYRIACUS*

ICK-DONG YOO*, BONG-SIK YUN, IN-KYOUNG LEE, IN-JA RYOO, DONG-HO CHOUNG and KYOU-HOON HAN

Korea Research Institute of Bioscience and Biotechnology, KIST, P.O. Box 115, Yusong, Taejon 305-600, Korea

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Key Word Index—*Hibiscus syriacus*; Malvaceae; root bark; syriacusins; naphthalene; naphthalenecarbaldehyde; naphthalenecarbolactone; lipid peroxidation inhibitory activity; cytotoxicity.

Abstract—Three new naphthalenes, designated as syriacusins A–C, were isolated from the root bark of *Hibiscus syriacus*. These compounds were identified as 2,7-dihydroxy-6-methyl-8-methoxy-1-naphthalenecarbaldehyde, 2-hydroxy-6-hydroxymethyl-7,8-dimethoxy-1-naphthalenecarbaldehyde, 1-carboxy-2,8-dihydroxy-6-methyl-7-methoxynaphthalenecarbolactone ($1 \rightarrow 8$), respectively, on the basis of various spectral studies. The compounds inhibited lipid peroxidation with IC_{50}s of 0.54, 5.90 and 1.02 $\mu g\ ml^{-1}$, respectively. The first compound also showed cytotoxicity against some human cancer cell lines with an ED_{50} of 1.5–2.4 $\mu g\ ml^{-1}$.

INTRODUCTION

The genus *Hibiscus* is widely distributed over Korea, China, India and Siberia. The dried flowers and root bark of *H. syriacus* are used as a folk medicine in the Orient [1, 2]. From the stem and root bark of *H. syriacus*, saponarin [1], polyphenol compounds [3], betulin [4], canthin-6-one [5], carotenoids [6] and anthocyanins [7, 8] have been isolated previously. During screening for lipid peroxidation inhibitors from traditional Chinese medicines, we have isolated three naphthalene compounds, syriacusins A–C, from the $CHCl_3$ extract of the root bark of *H. syriacus*. This paper describes the isolation and structural elucidation and biological activities of these compounds.

RESULTS AND DISCUSSION

The methanolic extract of *H. syriacus* was purified by solvent partition, silica gel and Sephadex LH-20 column chromatography, preparative TLC and HPLC to give syriacusins A–C.

The high resolution FAB-mass spectrum of syriacusin A (**1**) indicated its molecular formula to be $C_{13}H_{12}O_4$. The IR spectrum suggested the presence of hydroxyl (3245 cm^{-1}), internally hydrogen-bonded carbonyl (1620 cm^{-1}) and an oxygenated aromatic ring (1295 cm^{-1}). The ^1H NMR spectrum of **1**, in $CDCl_3+CD_3OD$ (1:1), showed signals for an aromatic methyl group at δ 2.37, a methoxyl group at δ 3.75, three aromatic methines at δ 6.87 (*d*, $J = 9.0$ Hz), 7.37 (*s*) and 7.84 (*d*, $J = 9.0$ Hz), two of which were *ortho*-coupled and an aldehyde at δ 11.13. The spectrum in DMSO-d_6 also had two additional broad peaks at δ 9.66 (phenolic OH) and 13.82 (hydrogen-bonded OH), which was collapsed on shaking with D_2O. The ^{13}C NMR and DEPT spectra suggested the presence of an aldehyde carbonyl, seven sp^2 quaternary carbons, three sp^2 methines, one methoxyl and one methyl (Table 1). These NMR data and the UV maxima at 228, 274 and 363 nm demonstrated that **1** possessed a highly conjugated naphthalene system. Its structure was further assigned by HMBC (Fig. 1), which established the respective placement of the substituents of aldehyde, hydroxyl, methoxyl and methyl groups on the naphthalene ring system. A hydroxyl group was placed *ortho* to the aldehyde function to account for the intramolecular hydrogen-bond. The long-range correlations from the aldehyde proton to sp^2 carbons at δ 166.3, 116.4 and 112.3, and from two *ortho*-coupled aromatic methines at δ 7.84 and 6.87 to an oxygenated quaternary carbon at δ 166.3, assigned the substituents attached to C-1, 2, 3 and 4 as CHO, OH, H and H, respectively. Also, the aromatic methyl protons were long-range coupled with three carbons at δ 151.2, 127.7 and 126.3, and an aromatic methine at δ 7.37 was correlated with the sp^2 carbons at δ 151.2, 140.0 and 125.9, revealing that C-6 and 7 were

* Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR spectral data for compounds **1**–**3**

No.	1	2	3
1	112.3	112.6	119.2
1-CO	199.2	198.6	167.8
2	166.3	166.5	158.5
3	116.4	118.9	119.0
4	140.0	138.9	134.8
4a	124.3	126.0	100.3
5	127.7	124.7	122.4
6	126.3	132.2	129.9
6-CH_3	16.3		17.9
6-CH_2		61.4	
7	151.2	152.0	140.2
7-OCH_3		61.2	59.9
8	141.7	148.0	131.6
8-OCH_3	59.5	59.7	
8a	125.9	127.3	131.7

1 and **3** were measured in $CDCl_3$+CD_3OD (1:1), **2** in $CDCl_3$.

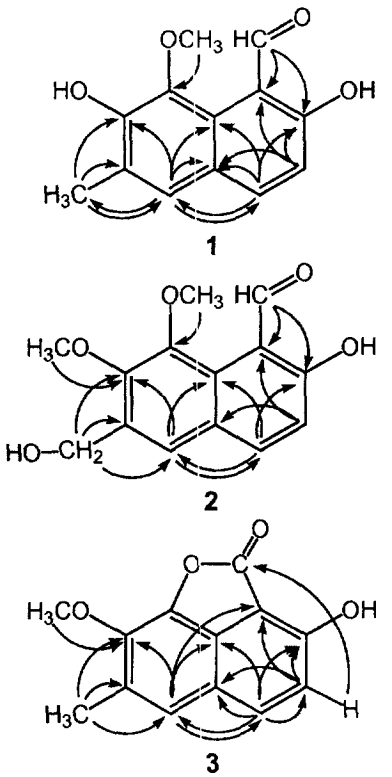

Fig. 1. Structures of syriacusins A (**1**), B (**2**) and C (**3**). Arrows indicate correlations between ^1H and ^{13}C.

connected with methyl and hydroxyl, respectively. By a process of elimination, the remaining quaternary carbon at δ 141.7, that was long-range coupled with methoxyl protons at δ 3.75, should be C-8. Therefore, the structure of **1** was assigned as 2,7-dihydroxy-6-methyl-8-methoxy-1-naphthalenecarbaldehyde.

Syriacusin B (**2**) was closely related to **1** in its physicochemical properties and NMR spectra, suggesting that it was also a highly substituted naphthalene. The molecular formula was determined to be $C_{14}H_{14}O_5$ by high resolution FAB-mass spectrometry. The ^1H NMR spectrum revealed the presence of two methoxyls, an hydroxymethyl, three aromatic methines (two of which were *ortho*-coupled), an aldehyde and an hydrogen-bonded hydroxyl. Direct comparison of NMR spectra between **1** and **2** indicated that a hydroxymethyl (δ 4.84) and a methoxyl (δ 4.09) in **2** replaced the methyl (δ 2.29) and the hydroxyl (δ 9.66) groups in **1**. HMBC data (Fig. 1) were analysed in order to clarify the location of the substituents and to assign all NMR signals. The hydroxymethyl protons at δ 4.84 showed long-range correlations with three sp^2 carbons at δ 152.0, 132.2 and 124.7, and the methoxyl protons at δ 4.09 were long-range correlated to a quaternary carbon at δ 152.0, revealing that the hydroxymethyl and methoxyl groups conjugated with C-6 and C-7, respectively. In addition, long-range correlations from the aromatic proton at δ 7.58 to carbons at δ 152.0, 138.9 and 127.3, and from the aldehyde proton at δ 11.22 to carbons at δ 112.6 and 166.5, were observed. The structure of **2** was thus concluded to be 2-hydroxy-6-hydroxymethyl-7,8-dimethoxy-1-naphthalenecarbaldehyde.

The molecular formula of syriacusin C (**3**) was determined to be $C_{13}H_{10}O_4$ from the high resolution FAB-mass spectrum, in combination with ^1H and ^{13}C NMR spectral data. The IR spectrum showed absorptions due to a hydroxyl group at 3315 cm^{-1} and a carbonyl group at 1735 cm^{-1}. The ^1H NMR spectrum of **3** in DMSO-d_6 was similar to that of **1**, except for the absence of the hydrogen-bonded hydroxyl and aldehyde proton signals. The spectral spectra suggested that **3** was a syriacusin analogue. The substituents at C-2 to C-7 composed of a hydroxyl, three hydrogens, an aromatic methyl and a methoxyl were assigned unambiguously by HMBC data (Fig. 1). Also a carboxyl carbon at δ 167.8 was attached to C-1, as evidenced by a four-bonded long-range correlation from H-3 at δ 7.05. By a process of elimination, the remaining quaternary carbon at δ 131.6 should be C-8, which is attached to the lactone oxygen. Therefore, the structure of **3** was determined as a highly substituted naphthalenecarbolactone.

Syriacusins A–C inhibited lipid peroxidation with IC_{50} values of 0.54, 5.90 and 1.02 $\mu g\ ml^{-1}$, respectively. Syriacusin A was ca three times as active as vitamin E. Also, the cytotoxicity of the major component **1** against several human tumor cell lines was examined (Table 2); **1** was the most effective against UACC62, ACHN, SW620 and SF539, but none of them were as effective as adriamycin.

EXPERIMENTAL

General

Mps: uncorr. UV: MeOH. IR: KBr discs. NMR: 300 and 500 MHz for ^1H, and 75 and 125 MHz for

Table 2. ED_{50} values ($\mu g\ ml^{-1}$) of compound 1 against human cancer cell lines

Cell lines	1	Adriamycin
UACC62 (melanoma)	1.73	0.18
MCF7 (breast)	2.35	0.13
NCI-H23 (lung)	2.06	0.14
ACHN (renal)	1.46	0.33
PC-3 (prostate)	2.29	0.41
SW620 (colon)	1.56	0.14
SF539 (central nervous system)	1.53	0.19

^{13}C in $CDCl_3+CD_3OD$ (1:1), $CDCl_3$ and DMSO-d_6 with TMS as int. standard. The 2D-NMR expts were carried out at 500.05/1125 MHz with $^nJ_{CH} = 8.3$ Hz for HMBC. Analytical TLC: silica gel (Merck, Kiesel gel $60F_{254}$) Prep. TLC: silica gel (Merck, Kiesel gel $60F_{254}$, 0.5 mm). HPLC was performed on a Senshu pak ODS column (20 × 250 mm) using a H_2O–MeOH system and by monitoring with a photodiode-array detector (190–650 nm).

Plant material

Root bark of *H. syriacus* was collected at Yusong, Chungnam Province, Korea, in October 1995, and identified by staff at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea. Fr. root bark was dried in a dark, well-ventilated place. A voucher specimen is deposited in the herbarium of KRIBB.

Extraction and isolation

Dried root bark (1.6 kg) was ground into powder and extracted with MeOH at room temp. for two days. The extract was filtered and concd under red. pres. The residue obtained was extracted with *n*-hexane and then $CHCl_3$. The $CHCl_3$ layer was concd *in vacuo* and the residue chromatographed on a silica gel column eluted with *n*-hexane and *n*-hexane–EtOAc (10:1 to 1:1). Frs were collected and combined by monitoring with lipid peroxidation inhibitory activity in combination with analytical TLC to yield frs 1 and 2. Fr. 1 was rechromatographed on a silica gel column eluting with the same solvents as described above and on Sephadex LH-20 with $CHCl_3$–MeOH (1:1). Syriacusin A (35.5 mg) was finally purified from fr. 1 by HPLC on a Senshu pak ODS column (20 × 250 mm) with 70% aq. MeOH at 8 ml min^{-1}. Fr. 2 was also rechromatographed on a silica gel column eluting with $CHCl_3$–MeOH (100:1 to 1:1) followed by Sephadex LH-20 CC with MeOH, to give syriacusin C (3 mg) and another active fr. 2a. Syriacusin B (3.5 mg) was purified from fr. 2a by silica gel prep. TLC developed with $CHCl_3$–MeOH (10:1).

Syriacusin A (**1**)

Yellow needles, mp 173–177°. UV λ_{max}^{MeOH} nm (log ε): 228 (4.84), 274 (3.82), 363 (4.11). IR ν_{max} cm^{-1}: 3245, 1620, 1480, 1295, 1235, 1160, 815. ^1H NMR (DMSO-d_6): δ 13.82 (1H, *s*, 2-OH), 11.02 (1H, *s*, 1-CHO), 9.66 (1H, *s*, 7-OH), 7.99 (1H, *d*, *J* = 9.0 Hz, 4-H), 7.51 (1H, *s*, 5-H), 6.93 (1H, *d*, *J* = 9.0, 3-H), 3.67 (3H, *s*, 8-OCH_3), 2.29 (3H, *s*, 6-CH_3), ^{13}C NMR: Table 1. HRFAB-MS *m/z*: $[M+H]^+$ 233.0808 ($C_{13}H_{12}O_4$ requires 233.0814).

Syriacusin B (**2**).

Yellow solid. UV λ_{max}^{MeOH} nm (log ε): 226 (4.46), 270 (3.76 sh), 340 (3.70). IR ν_{max} cm^{-1}: 3375, 1625, 1465, 1350, 1275, 1165, 830. ^1H NMR (DMSO-d_6): δ 14.05 (1H, *s*, 2-OH), 11.22 (1H, *s*, 1-CHO), 7.91 (1H, *d*, *J* = 9.0 Hz, 4-H), 7.58 (1H, *brs*, 5-H), 7.08 (1H, *d*, *J* = 9.0, 3-H), 4.09 (3H, *s*, 7-OCH_3), 3.92 (3H, *s*, 8-OCH_3), 4.84 (2H, *s*, 6-CH_2), ^{13}C NMR: Table 1. HRFAB-MS *m/z*: $[M+H]^-$ 263.0915 ($C_{14}H_{14}O_5$ requires 263.0920).

Syriacusin C (**3**).

Yellow solid, mp 230–235°. UV λ_{max}^{MeOH} nm (log ε): 220 (4.64), 256 (4.49), 360 (4.11). IR ν_{max} cm^{-1}: 3315, 1735, 1640, 1505, 1340, 1290, 1260, 960. ^1H NMR ($CDCl_3+CD_3OD$ (1:1)): δ 7.86 (1H, *d*, *J* = 8.7 Hz, 4-H), 7.36 (1H, *s*, 5-H), 7.05 (1H, *d*, *J* = 8.7, 3-H), 4.31 (3H, *s*, 7-OCH_3), 2.37 (3H, *s*, 6-CH_3), ^{13}C NMR: Table 1. HRFAB-MS *m/z*: $[M+H]^+$ 231.0638 ($C_{13}H_{10}O_4$ requires 231.0658).

Antioxidative activity

These were evaluated by the inhibitory activities of compounds against lipid peroxidation in rat liver microsomes according to the method of ref. [9], with minor modification. Reaction was initiated by the addition of 100 μM $FeSO_4 \cdot 7H_2O$ into a mixt. of ascorbic acid (0.2 mM) and microsomal suspension (0.5 μg protein ml^{-1}). Lipid peroxidation was assessed by measuring the thiobarbituric acid reaction products at 532 nm.

Cytotoxicity

Cytotoxic activity against some human tumor cell lines was estimated according to NCI protocols [10].

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