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AGASTAQUINONE, A NEW CYTOTOXIC DITERPENOID QUINONE FROM *AGASTACHE RUGOSA*

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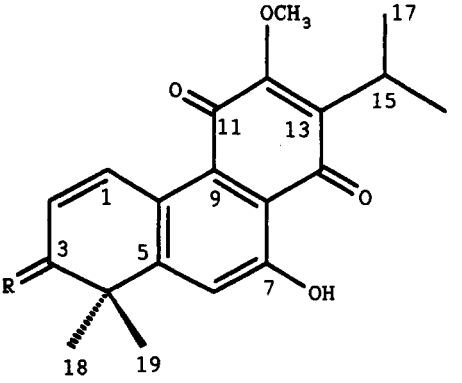
ABSTRACT.—A new diterpenoid quinone, agastaquinone [**1**], was isolated from the roots of *Agastache rugosa*. An oxime derivative [**2**] of agastaquinone was prepared with hydroxylamine hydrochloride. The structure of agastaquinone [**1**] was established as 7-hydroxy-12-methoxy-20-norabieta-1,5(10),6,8,12-pentaene-3,11,14-trione by spectroscopic techniques. Compounds **1** and **2** showed nonspecific cytotoxic activities against several human cancer cell lines in vitro (A549, SK-OV-3, SK-MEL-2, XF498, and HCT15).

Agastache rugosa O. Kuntze is a perennial herb that belongs to the Labiatae, and is distributed throughout Korea, the People's Republic of China, Taiwan, and Japan (1,2). The whole plant has been used as an agent for the treatment of cholera, vomiting, and miasma (noxious air), and is known as Agastachis Herba ("kwag-hyang" in Korean). The leaves are used as a spice for fish-based foods, and the flower is a good source of honey (3–6). Various types of solvent extract of *A. rugosa* have shown antifungal activity, and the H_2O extract has inhibitory activity against a *Leptospira* strain (7). Monoterpenes (α-pinene, β-pinene, and *p*-cymene), sesquiterpenes (α-ylangene, caryophyllene, and calamenene), and flavonoids (agastachoside, agastachin, acacetin, and tilianin) have been isolated from the aerial parts of this plant (8–11). From the roots, triterpenes (erythrodiol-3-*O*-acetate, 3-*O*-acetyl oleanolic aldehyde, 3-*O*-acetyl oleanolic acid, oleanolic acid, and maslinic acid) and diterpenes (dehydroagastol, 18(4→3)-*abeo*-11,14,15-trihydroxy-12-methoxyabieta-4(18),8,11,13-tetraen-7-one and agastanol) have also been reported (12–16).

The *n*-hexane extract of *A. rugosa* roots showed several colored spots on tlc. We report herein the isolation, structure elucidation, and cytotoxicity of one of these compounds.

The roots of *A. rugosa* were extracted successively with MeOH and a mixture of *n*-hexane/EtOAc/Me_2CO. After concentrating the combined extract the resulting solution was partitioned with *n*-hexane. The *n*-hexane extract was chromatographed repeatedly on a Si gel column to yield compound **1**.

Compound **1** was obtained as dark-red needles, mp 121–122°, from *n*-hexane. The molecular formula of $C_{20}H_{20}O_5$



1 R=O
2 R=NOH

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was established by ms and elemental analysis data. The uv (λ max 235, 296, 340, 454 nm) and ir (ν max 1668, 1658, 1629, 1610 cm^{-1}) spectra indicated the presence of an aromatic quinone. In the ^1H-nmr spectrum of compound **1**, one cis-coupled olefinic proton (8.80 and 6.29 ppm, $J=10.8$ Hz), one isolated olefinic proton (7.31 ppm), an isopropyl group (3.40 ppm, sept. and 1.29 ppm, d, $J=7.0$ Hz), and a geminal dimethyl group (1.49 ppm, 6H, s) were observed. The ^{13}C-nmr spectrum exhibited the expected 20 carbon peaks (Table 1), assigned by the DEPT pulse sequence as containing three protonated olefinic (139.66, 126.33, and 121.82 ppm), three carbonyl (201.67, 190.87, and 183.39 ppm), and five methyl (60.89, 27.78 [×2], and 20.25 [×2] ppm) carbons. From the COLOC correlations of H_3-18(19) to C-3, C-4, and C-5, OH-7 to C-6, C-7, and C-8, H_3-16(17) to the aromatic C-13, H-15 to C-12, C-13, and C-14, and OCH_3 to C-12 (Table 1), it was deduced that the geminal dimethyl group (^1H, 1.49 ppm, ^{13}C, 27.78 ppm) was at C-4 (48.31 ppm), the hydroxyl group (13.12 ppm) at C-7 (162.54 ppm), the isopropyl group at C-13 (137.44 ppm), and the methoxyl group at C-12 (159.87 ppm). The paramagnetic shift of the hydroxyl proton (13.12 ppm) at C-7 suggested that the hydroxyl group must be strongly hydrogen-bonded with a peri ketone oxygen of the quinone moiety.

The oxime derivatization of the C-3 carbonyl with $NH_2OH.HCl$ caused a diamagnetic shift of the C-3 chemical shift (201.67→154.97 ppm), also affecting C-2 (126.33→116.68 ppm) and C-4 (48.31→40.89 ppm), and therefore the position of the carbonyl (201.67 ppm) between C-2 and C-4 and the geminal dimethyl group at C-4 was confirmed.

Consequently, the structure of compound **1** was established as 7-hydroxy-12-methoxy-20-norabieta-1,5(10),6,8,12-pentaene-3,11,14-trione, to which we have assigned the trivial name agastaquinone. This is the first report of this compound from a natural source.

TABLE 1. Nmr Data of Agastaquinone [**1**] and Its Oxime Derivative [**2**] (δ values, $CDCl_3$).

Carbon	1			2		
	^{13}C (75 MHz)	^1H (300 MHz)	COLOC[c] (125 MHz)	^{13}C (75 MHz)	^1H (300 MHz)	L-R HETCOR[d] (75 MHz)
1	139.66 (CH)[a]	8.80 (d, 10.8)[b]	H-1	129.25 (CH)	8.18 (d, 10.8)	H-1
2	126.33 (CH)	6.29 (d, 10.8)	—	116.68 (CH)	7.11 (d, 10.8)	—
3	201.67 (C)	—	H_3-18(19)	154.97 (C)	—	H_3-18(19)
4	48.31 (C)	—	H_3-18(19)	40.89 (C)	—	H_3-18(19)
5	157.17 (C)	—	H_3-18(19)	158.29 (C)	—	H_3-18(19)
6	121.82 (CH)	7.31 (s)	OH-7	120.99 (C)	—	OH-7
7	162.54 (C)	—	H-6, OH-7	162.12 (C)	—	H-6, OH-7
8	113.89 (C)	—	H-6, OH-7	113.43 (C)	—	H-6, OH-7
9	122.31 (C)	—	—	124.59 (C)	—	—
10	128.44 (C)	—	H-6	126.99 (C)	—	H-6
11	183.39 (C)	—	—	183.24 (C)	—	—
12	159.87 (C)	—	OCH_3, H-15	159.86 (C)	—	OCH_3, H-15
13	137.44 (C)	—	H_3-16(17), H-15	137.39 (C)	—	H-15, H_3-16(17)
14	190.87 (C)	—	H-15	190.77 (C)	—	H-15
15	24.29 (CH)	3.40 (sept., 7.0)	H-15, H_3-16(17)	24.22 (CH)	3.40 (sept., 7.2)	H_3-16(17)
16	20.25 (CH_3)	1.29 (d, 7.0)	—	20.26 (CH_3)	1.28 (d, 7.2)	—
17	20.25 (CH_3)	1.29 (d, 7.0)	—	20.26 (CH_3)	1.28 (d, 7.2)	—
18	27.78 (CH_3)	1.49 (s)	—	29.28 (CH_3)	1.51 (s)	—
19	27.78 (CH_3)	1.49 (s)	—	29.28 (CH_3)	1.51 (s)	—
OH-7.....	—	13.12 (s)	—	—	13.17 (s)	—
OCH_3	60.89 (CH_3)	4.08 (s)	OCH_3	60.83 (CH_3)	4.06 (s)	—
N-OH	—	—	—	—	8.85 (br s)	—

[a]Each carbon was characterized by DEPT spectra.
[b]J value in Hz.
[c]COLOC spectra were recorded for coupling constants of 4, 8, and 12 Hz.
[d]Long-range HETCOR spectrum was recorded for $J=5$ Hz.

The cytotoxic activities of **1** and its oxime derivative [**2**] have been evaluated in vitro with five human cancer cell lines. As indicated in Table 2, these compounds showed general nonspecific cytotoxicity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp was obtained with an Electrothermal Series IA9100 apparatus and was not corrected. Uv spectra were taken in MeOH on a Milton-Roy Spectronic 3000 spectrophotometer. Ir spectra were recorded on a Precision Analect RFX-65 spectrophotometer. Ms were obtained on a Kratos Concept-1S spectrometer at 70 eV. ^{1}H- and ^{13}C-nmr experiments were run in $CDCl_3$ containing TMS as the internal standard, using Varian Unity-300 and Bruker AM-500 spectrometers. Elemental analysis was carried out with a Carlo Erba EA1108 elemental analyzer. Si gel 60 (Merck, 230–400 mesh) was used for cc and Si gel 60F_{254} (0.25 mm) and RP-18 F_{254} (0.2 mm) (Merck) for tlc.

PLANT MATERIAL.—The roots of *Agastache rugosa* were collected at Yangsan (Kyongnam Province, Korea) in October 1992. A voucher specimen is maintained in the Korea Research Institute of Bioscience and Biotechnology (KIST), Korea.

EXTRACTION AND ISOLATION.—The air-dried roots (4.5 kg) of *A. rugosa* were ground and extracted with MeOH (10 liters, 3×) followed by a mixture of *n*-hexane-EtOAc-Me_2CO (4:4:2) (8 liters, 3×) at room temperature. The combined extracts were concentrated to about 1.5 liters and the resulting solution was extracted with *n*-hexane (500 ml, 4×). The *n*-hexane extract (50 g) was chromatographed on a Si gel column (600 g) using an EtOAc gradient system (0–100%) in *n*-hexane and fractions were monitored by tlc. Fraction 5 was further chromatographed on a Si gel column eluted with a mixture of *n*-hexane-$CHCl_3$-MeOH (40:10:1), and followed by crystallization of the fourth subfraction with *n*-hexane to give agastaquinone [**1**] (150 mg).

Agastaquinone [**1**].—Dark red needles; mp 121–122°; uv λ max (MeOH) (log ε) 235 sh (3.91), 296 (3.93), 340 (3.51), 454 (3.46) nm, λ max (MeOH+KOH) (log ε) 211 (4.51), 289 (3.78), 395 (3.66), 537 (3.53) nm; ir (KBr) ν max 2973, 1668, 1658, 1630, 1610, 1292, 1275, 1259 cm^{-1}; eims *m/z* $[M]^+$ 340 (100), 325 (27), 312 (35), 297 (58), 279 (13), 239 (8), 187 (10), 165 (15); *anal.*, found C 70.78%, H 6.05% (calcd C 70.59%, H 5.88% for $C_{20}H_{20}O_5$); ^{1}H- and ^{13}C-nmr data, see Table 1.

Oxime derivative [**2**].—Hydroxylamine HCl (20 mg) and pyridine (0.5 ml) were added to a MeOH solution (2 ml) of **1** (50 mg). The mixture was refluxed for 2 h and concentrated *in vacuo*. The residue was partitioned with CH_2Cl_2/H_2O and the CH_2Cl_2 layer was concentrated and chromatographed repeatedly over Si gel columns with *n*-hexane-EtOAc (4:1) to yield the oxime derivative as needles (35 mg); ^{1}H- and ^{13}C-nmr data, see Table 1.

CYTOTOXICITY ASSAYS.—The SRB colorimetric determination procedure was employed according to NCI protocols (17,18) against five human cancer cell lines, A549 (non-small cell lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-2 (melanoma), XF498 (CNS cancer), and HCT15 (colon cancer), with cisplatin as a control substance.

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TABLE 2. Cytotoxic Activity (ED_{50} Values) of Agastaquinone [**1**] and Its Oxime Derivative [**2**].[a]

Compound	Cell Line (μg/ml)				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
Agastaquinone [**1**]	6.8	12.8	4.1	1.8	5.2
Oxime derivative [**2**]	9.1	18.7	11.8	8.5	7.0
Cisplatin	1.0	1.0	0.8	0.5	1.4

[a]Means of triplicate tests.

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