HETEROCYCLES, Vol. 74, 2007, pp. 977 - 981. © The Japan Institute of Heterocyclic Chemistry
Received, 23rd August, 2007, Accepted, 26th September, 2007, Published online, 28th September, 2007. COM-07-S(W)42

NEW SESQUITERPENOIDS FROM *CURCUMA* AFF. *AERUGINOSA* ROXB.

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Abstract–Two new natural sesquiterpenoids, designated aeruginolactone (**3**) and aeruginone (**10**), and eight known sesquiterpenoids were isolated from the rhizomes of *Curcuma* aff. *aeruginosa* Roxb. (Zingiberaceae). Their structures were determined using spectroscopic analyses.

Curcuma aff. *aeruginosa* Roxb. (Zingiberaceae) is a herb, 1-2 m high, and known under local name Nghe xanh. The plant is cultivated in several areas in northern Vietnam in Hanoi, Hai Phong, and Province Vinh Phuc to produce rhizomes for domestic medicinal uses. According to local healers the rhizomes are used in the treatment of stomach ache and in the enhancement of digestion.[1] In our previous communication the detection of eighteen volatile sesquiterpenoids using GC and GC-MS techniques and the isolation and structure determination of seven sesquiterpenoids, furanodiene, germacrone, furanodienone, curdione, curzerenone, curcumol, and curcumenol were reported.[2] The present paper reports the isolation of two new natural sesquiterpenoids, designated aeruginolactone (**3**) and aeruginone (**10**), and eight known sesquiterpenoids, 13-hydroxygermacrone (**1**),[3] curdione (**2**),[4] guiaianediol (**4**),[5] isoprocurcumenol (**5**),[4] procurcumenol (**6**),[4] aerugidiol (**7**),[6] curcolonol (**8**)[7] and zedoarofuran (**9**),[8] from the rhizomes of *C*. aff. *aeruginosa*, which were collected at the same place as in the previous work.[2] The structures of the known compounds **1**, **2**, **4**–**8**, and **9**, including their stereochemistries, were determined by comparing their physical ($[\alpha]_D$) and spectroscopic data (^{1}H-, ^{13}C-NMR, and CD) with literature values.[3–8] This paper deals with the isolation and structure detremination of the two new natural sesquiterpenoids **3** and **10**.

This paper is dedicated to Professor Dr. Ekkehard Winterfeldt on the occasion of his 75th birthday.

RESULTS AND DISCUSSION

The dried rhizomes of *C.* aff. *aeruginosa* were extracted with MeOH and sequentially fractionated using solvents of increasing polarity to give *n*-hexane-, ethyl acetate-, and 1-BuOH-soluble fractions. The *n*-hexane-soluble fraction was separated by using silica (Si) gel open-column chromatography (CC), octadecyl Si (ODS) gel CC, and preparative ODS gel HPLC resulting in the isolation of compounds **1**–**10**.

Aeruginolactone (**3**) was isolated as an amorphous powder, $[\alpha]_D^{25}$ –8.1 °, and had a molecular formula $C_{15}H_{20}O_3$, which was determined by negative-ion high-resolution (HR)-FABMS (*m/z* 247.1324, $[M–H]^-$). The IR spectrum indicated the presence of hydroxyl (3381 cm^{-1}) and γ-lactone (1760 cm^{-1}) functional groups. The ^{1}H- and ^{13}C-NMR (Table 1) spectroscopic data and heteronuclear single quantum correlation (HSQC) spectrum of **3** showed the presence of 15 carbons assignable to three olefinic methyl groups [δ_H 1.60 (6H, s) and 1.80 (3H, s); δ_C 16.8 (q), 17.3 (q), and 8.6 (q), respectively], a ketone group [δ_C 171.9 (s)], a ketal carbon [δ_C 109.8 (s)], two trisubstituted [δ_C 123.4 (d), 131.7 (d), 133.0 (s), and 134.2 (s)] and a tetrasubstituted olefins [δ_C 162.4 (s) and 127.4 (s)], and four methylene groups [δ_C 25.3 (t), 25.6 (t), 38.4 (t), and 51.3 (t)]. On the basis of the NMR analysis the structure of **3** was deduced to be of the germacrane lactone type. The presence of the γ-lactone ring fused with the ten-membered ring at C-7 and C-8 was clarified by using HMBC correlations between H-13 (δ_H 1.80) and C-7 [δ_C 162.4 (s)], C-11 [δ_C 127.4 (s)], and C-12 [δ_C 171.9 (s)], and between H_2-9 (δ_H 2.31 and 3.05) and C-8 [δ_C 109.8 (s)]. The trisubstituted double bonds were located in the 1(10) and 4 positions and the ketal carbon was found at C-8 as evidenced by the analysis of the HMBC correlations (Figure 2) and comparison of the ^{13}C-NMR data with those of germacrone.[9] Thus the planar structure of **3** was found to be similar to a germacranolide, which was transformed from isofuranodiene by an oxidation reaction.[10] The lack of NMR data on this compound as well as information on its absolute configuration led us to verify the stereochemistry of **3** at C-8 using circular dichroism (CD) spectroscopy. As a result an 8*R*-configuration was determined from the negative

Table 1. ^{13}C- and ^{1}H-NMR Spectroscopic Data for **3** (δ in ppm, *J* in Hz, $CDCl_3$)

C/H	**3**	
	C	H
1	131.7	4.77 br s
2a	25.6	2.26 m
b		1.46 m
3a	38.4	2.24 m
b		1.89 m
4	133.0	
5	123.4	4.24 br d (9.5)
6a	25.3	3.08 m
b		2.07 m
7	162.4	
8	109.8	
9a	51.3	3.05 m
b		2.31 m
10	134.2	
11	127.4	
12	171.9	
13	8.6	1.80 s
14	16.8	1.60 s
15	17.3	1.60 s

at 221 nm (Δε –0.79) and positive Cotton effects at 256 nm (Δε +0.17) in α,β-unsaturated γ-lactones.[7] Therefore the absolute structure of **3** was determined as depicted in Figure 1.

Compound **10** was isolated as an amorphous powder and had the ^{1}H- and ^{13}C-NMR data superimposable with those of 2*β*-hydroxycurdione, which was biotransformed from curdione by suspension cultured cells of *Lonicera japonica*.[10] The optical rotation of **10** $[\alpha]_D^{25}$ +173 ^{o} confirmed the same stereochemistry of **10** with that of 2β-hydroxycurdione ($[\alpha]_D$ +185.6 ^{o}),[10] however **10** was isolated for the first time from Nature.

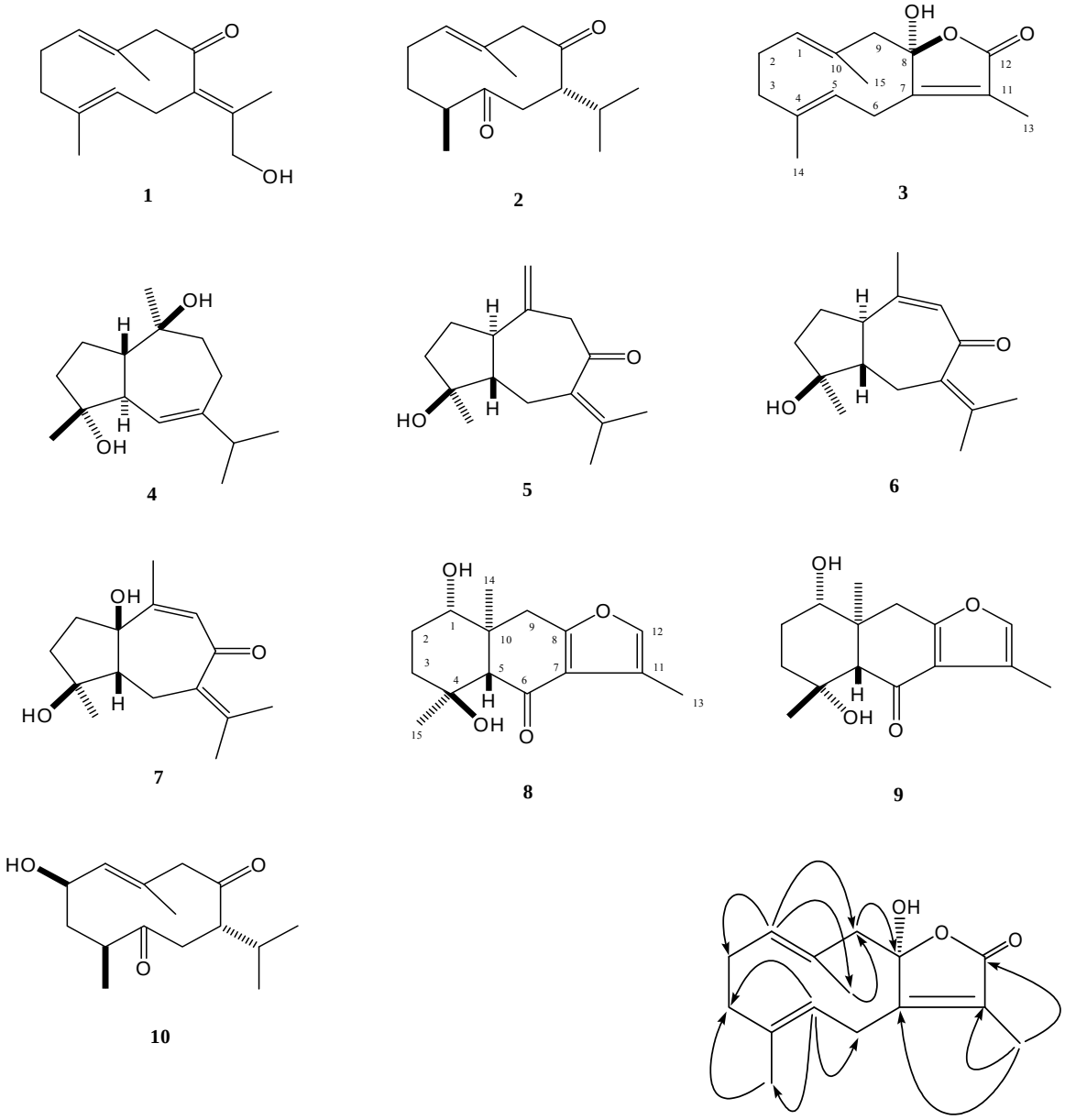

Figure1. Structures of Isolates **1**–**10** from *C*. aff. *Aeruginosa*

Figure 2. Selective HMBC Correlations of **3**

EXPERIMENTAL

General Procedure Optical rotations were measured on a JASCO P-1030 digital polarimeter. FT-IR spectra were recorded on a Horiba FT-710 spectrophotometer. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using a JEOL JNM-α 400 NMR spectrometer. Negative-ion HR-FABMS were measured on a JEOL SX-102 mass spectrometer with PEG-400 as a calibration matrix. HPLC was carried out with a JASCO PU-1580 pump and a UV-2075 Plus detector (set at 210 nm) on YMC ODS columns (150 × 4.6 mm i.d. in analytical and 150 × 20 mm i.d. in preparative scales) at the corresponding flow rates of 0.5 and 5 ml/min. Silica gel 60 (0.063–0.200 mm, Merck, Germany) and reversed-phase octadecyl Si (ODS) gel (YMC, Japan) were used for CC. TLC was carried out on Merck TLC glass plates (Si gel 60 F_{254}), and detected by spraying with 10 % H_2SO_4 in 50 % EtOH, followed by heating on a hot plate at 200 ^{o}C.

Plant Material The rhizomes of *C.* aff. *aeruginosa* were collected from Phu Thuy village, Gia Lam district, Hanoi, Vietnam, and identified by Dr. Tran Ngoc Ninh, a botanist of the Institue of Ecology and Natural Resources, Vietnam Academy of Natural Science and Technology (Hanoi, Vietnam), in February 2004. A voucher specimen (no. HCTN 2004-2) is deposited in the Laboratory of Chemistry of Natural Products, Faculty of Chemistry, Vietnam National University (Hanoi, Vietnam).

Extraction and Isolation of 1–10 The fresh rhizomes of *C.* aff. *aeruginosa* were sliced and air-dried, then oven-dried at 40–50 ^{o}C. The dried rhizomes (2.0 kg) were powdered and extracted with MeOH by percolation at rt (3 times, for 3 days each). After evaporation the MeOH extract was suspended in H_2O and subjected to sequential fractionation with *n*-hexane, EtOAc, and 1-BuOH. The corresponding *n*-hexane- (157 g), EtOAc- (17.8 g), and 1-BuOH-soluble (6.8 g) fractions. Part of the *n*-hexane-soluble fraction (83 g) was chromatographed on a Si gel open column using stepwise gradients of *n*-hexane with increasing amounts of EtOAc (19:1, 9:1, 4:1, 2:1, and 1:1) to afford eight pooled fractions. Separation of fraction 5 (3.1 g) by ODS gel CC (MeOH–H_2O, 3:2 and 7:3) followed by ODS gel preparative HPLC (MeOH–H_2O, 7:3) afforded **1** (53.9 mg), **2** (5 mg), and **3** (21.2 mg). Fraction 6 (4 g) was separated by ODS gel CC (MeOH–H_2O, 3:2 and 7:3) and ODS gel preparative HPLC (MeOH–H_2O, 3:2) to afford **4** (18.2 mg), **5** (42.3 mg), and **6** (95.7 mg). Fractions 7 (1.7 g) and 8 (1.7 g) were fractionated separately by ODS gel CC (MeOH–H_2O, 3:2 and 7:3) and ODS gel preparative HPLC (MeOH–H_2O, 1:1) to afford **7**

(96.3 mg), **8** (2.1 mg), **9** (21.0 mg), and **10** (19.1 mg).

Aeruginolactone (**3**): Amorphous powder, $[\alpha]_D^{25}$ –8.1 ^{o} (*c* 1.0, $CHCl_3$). UV (MeOH) λ_{max} (log ε): 217 (4.05). IR ν_{max} (film) cm^{-1}: 3381, 1760. ^{1}H- and ^{13}C- NMR: see Tables 1 and 2. CD (MeOH): $\Delta\varepsilon$ (nm): –0.79 (221), +0.17 (256) (c=6.4×10^{-5} M). Negative-ion HR-FABMS: *m/z* 247.1324 $[M–H]^-$ (Calcd for $C_{15}H_{19}O_3$: 247.1334).

Aeruginone (**10**): Amorphous powder, $[\alpha]_D^{25}$ +173 ^{o} (*c* 0.76, $CHCl_3$) [Lit.,[11] $[\alpha]_D$ +185.6 ^{o} (*c* 0.6, MeOH)]. ^{1}H- and ^{13}C-NMR were superimposable with those reported in the literatue.[11]

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS) and Research Grant to P. M. G. from the International Foundation for Science (Stockholm, Sweden) and Vietnam National University (Hanoi, Vietnam). We thank the Research Center of the Graduate School of Biomedical Sciences, Hiroshima University, Japan, for the use of its 400 MHz NMR instrument. This work was also supported in part by grant-in-aids from the Ministry of Education, Science, Sports, Culture and Technology of Japan. Thanks are also due to Astellas Foundation for Research on Medicinal Resources and Takeda Science Foundation for financial supports.

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