HETEROCYCLES, Vol. 75, No. 5, 2008, pp. 1241 - 1246. © The Japan Institute of Heterocyclic Chemistry
Received, 26th December, 2007, Accepted, 5th February, 2008, Published online, 8th February, 2008 . COM-07-11316

ENT-SAUCHINONE FROM *SAURURUS CHINENSIS*

Lishu Wang,[a,b,c] Daqing Zhao,[a] Dongyan Cheng,[b] and Yonghong Liu*[,c]

[a]College of Pharmacy, Changchun University of Chinese Medicine, Changchun 130117, China

[b]Jilin Province Academy of Chinese Medicine Sciences, Changchun 130021, China

[c]Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510-301, China

*To whom correspondence should be addressed. Tel: 0086-20-89023244, Fax: 0086-20-84451672. E-mail address: yonghongliu@scsio.ac.cn

Abstract—A new lignan *ent*-sauchinone (**1**) was isolated from *Saururus chinensis*. The structure has been established on the basis of spectral methods and confirmed by X-ray crystallographic analysis.

INTRODUCTION

Saururus chinensis (Lour.) Baill (Saururaceae) has been used in Chinese folk medicine for treatment of various diseases, such as edema, jaundice, gonorrhea, antipyretic, diuretic, and anti-inflammatory agents.[1] Previous biological studies of this species extract have shown antiasthmatic,[2] antioxidative, antiinflammatory,[3] and hypertensive effects.[4] Lignans,[5–17] alkaloids,[18] diterpenes,[19] flavonoids,[20] and lipids[21] were isolated from *S. chinensis*, which exhibited hepatoprotective,[5] antioxidant,[6] antiinflammatory,[9] immunosuppressive activities,[11] inhibition of TNF-α-induced cell adhesion molecule expression of human umbilical vein endothelial cells,[12] Human ACAT-1 and -2 inhibitory activities,[13] inhibition of the LPS-induced production of nitric oxide and prostaglandin E_2 in macrophage RAW264.7 cells,[14] inducement of the differentiation of human acute promyelocytic leukemia HL-60 cells,[15] Inhibitory effects on PMA-induced ICAM-1 expression,[16] cytotoxicity against human cancer cell lines,[17] attenuation of glutamate-induced neurotoxicity in rat cortical cultures probably by inhibiting nitric oxide production,[18] peroxisome proliferator-activated receptor γ (PPARγ) agonist,[19] free radical-scavenging activity,[20] Lp-PLA_2 inhibitory activities.[21]

RESULTS AND DISCUSSION

Sauchinone, a lignan from *S. chinensis*, significantly reduced the level of glutamic pyruvic transaminase released by the damaged,[5] attenuates CCl_4-induced toxicity in primary cultures of rat hepatocytes,[22] inhibits staurosporine-induced apoptosis in C6 rat glioma cells,[23] inhibit production of NO in LPS-stimulated RAW264.7 cells through the suppression of NF-KB by inhibiting transactivation activity of ReIA subunit.[24]

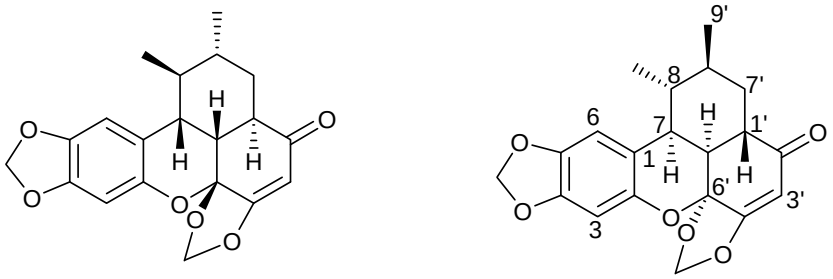

Figure 1. Structures of sauchinone and *ent* –sauchinone.

Compound **1** was identified as *ent*-sauchinone by comparison to previously reported spectroscopic data of sauchinone. Sauchinone is a unique lignan with a structure closely related to that of carpanone. Wang et al.[4] isolated sauchinone from *S. chinensis* and evaluated its stereostructure using NMR spectroscopy and X-ray crystallography. Compound **1** was obtained as colorless needles. The molecular formula $C_{20}H_{20}O_6$ was established by HRESIMS. The spectral data closely resembled those of sauchinone (Table 1). The NMR spectra showed the presence of two aromatic protons (δ 6.45 and 6.85) and two methylenedioxy groups, one (δ_H5.93, 5.90; δ_C101.2) attached to an aromatic ring and the other (δ_H 5.68, 5.63; δ_C 98.5) attached to aliphatic carbons. ^{13}C NMR signals at δ_C 143.1, 144.9, and 146.6 indicated that, in addition to aromatic carbon-oxygen bonds for a methylenedioxy group, there is additional oxygen attached to the aromatic ring. The ^{13}C NMR signal at δ_C 199.5 was attributed to the carbonyl group of an enone, while the ^{13}C NMR signal at δ_C 168.5 indicated the presence of a methylenedioxy group attached to the γ-carbon of an enone. ^{1}H NMR signals at δ_H 1.22 (*d*, 7.2 Hz) and 0.74 (*d*, 7.2 Hz) showed two methyl groups, each coupled to a vicinal proton. Comparison of ^{1}H and ^{13}C NMR spectral data between **1** and sauchinone suggested that two compounds are stereoisomers. The NMR and MS data of **1** were almost identical to those reported for sauchinone isolated from herb-medicine *S. chinensis* in Taiwan,[4] except the carbonyl group (**1**: δ_C 199.5; sauchinone: δ_C 193.3) of ^{13}C NMR However, the optical rotation of **1** was opposite in sign of that reported (**1**: $[\alpha]^{25}_D$ -112.8°, 0.022, $CHCl_3$; sauchinone: $[\alpha]_D$ +97.8°, 0.022, $CHCl_3$). This suggested that **1** is enantiomeric to sauchinone. The relative stereochemistry of **1** was established unequivocally by single-crystal X-ray analysis. Colorless crystals of **1** in the form of needles were obtained by slow evaporation from MeOH at room temperature. The X-ray structure of **1** was presented in

Figure 1. Thus, **1** is a sauchinone diastereomer, which designated *ent*–sauchinone.

Cytotoxicity MTT assays showed that compound **1** was not the active (LC_{50}>100 μg/mL) against HeLa (human ovarian cancer), SGC-7901 (human stomach cancer), HepG2 (human liver cancer), and normal cell lines L02 (human fetal hepatocytes).

EXPERIMENTAL

General Melting point was determined using an X4 micromelting point apparatus and is uncorrected. Optical rotation was recorded using a JASCO DIP-370 digital polarimeter. NMR spectra were recorded on a Bruker AC500 spectrometer in $CDCl_3$. Chemical shifts were referenced to the residual solvent peaks $CDCl_3$ (δ_H 7.26 and δ_C77.0). ESIMS data were obtained using a Finnigan DecaXP. MALDI-TOFMS data were obtained on an Applied Biosystems Voyager-DESTR. X-Ray crystallographic analysis performed using a Bruker smart APEX CCD area-detector.

Plant Material The herbs were purchased at the local herb-drug store and identified as *S. chinensis* with the assistance of Professor M. L. Deng of Department of Pharmacy, Changchun University of Chinese Medicine.

Extraction and Isolation The dried herbs (5 Kg) were extracted twice with aq. 70% EtOH under reflux. Concentration of the solvent until weight ratio (EtOH/dried herb) was 1:1, standing at rt overnight, brown yellow precipitates were obtained, after filtration of the solution and drying the precipitates at 55 ºC, to get residue 105 g . The residue was subjected to a silica gel column, eluted by Petroleum ether/ EtOAc, 20:1→3:1, to get 20 fractions, combined fractions 6:1 and 5:1, to give crude crystals, and recrystallized by MeOH, yielded colorless needles compound **1** (300 mg).

Compound **1**: Colorless needle crystals; mp 205.6-206.8 °C; $[\alpha]^{25}_D$ -112.8°, 0.022, $CHCl_3$; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1. ESI-MS *m/z* 379 $[M + Na]^+$; HRESIMS $[M]^+$*m/z* 356.1254 (calcd for $C_{20}H_{20}O_6$, 356.1260). $[M + H]^+$*m/z* 357.1338 (calcd for $C_{20}H_{21}O_6$, 357.1333). $[M + Na]^+$ 379.1150 (calcd for $C_{20}H_{21}O_6Na$, 379.1152).

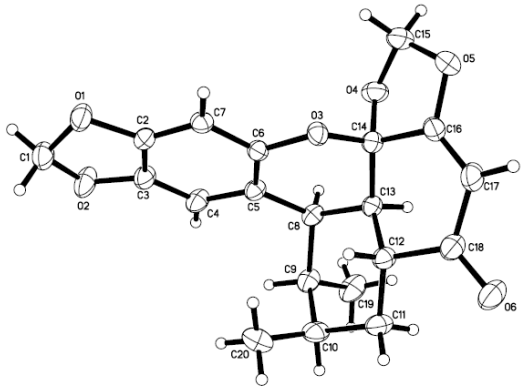

Figure 2. X-Ray diffraction structure of *ent*–sauchinone, with the numbering of the atom.

Table 1. The ^{1}H and ^{13}C NMR of sauchinone[4] and *ent*–sauchinone (in $CDCl_3$)

	sauchinone		*ent* -sauchinone	
	^{13}C (100 MHz)	^{1}H (400 MHz)	^{13}C (100 MHz)	^{1}H (400 MHz)
1	115.5		115.6	
2	144.8		144.9	
3	99.0	6.38 (1H, S)	99.1	6.45 (1H, S)
4	143.0		143.1	
5	146.5		146.6	
6	106.3	6.82 (1H, S)	106.4	6.85 (1H, S)
7	34.8	3.03 (1H, d, 3.9)	34.9	3.05 (1H, d, 5.2)
8	34.6	2.47 (1H, m)	34.7	2.47 (1H, m)
9	21.0	1.21 (3H, d, 7.3)	21.2	1.22 (3H, d, 7.2)
1'	37.3	2.47 (1H, m)	37.4	2.53 (1H, m)
2'	193.3		199.5	
3'	100.0	5.50 (1H, S)	100.1	5.52 (1H, S)
4'	168.4		168.5	
5'	100.2		100.3	
6'	37.3	2.47 (1H, m)	37.5	2.50 (1H, d, 5.6)
7'eq	25.0	1.64 (1H, m)	25.1	1.65 (1H, m)
7'ax		1.91 (1H, m)		1.92 (1H, m)
8'	33.2	1.91 (1H, m)	33.3	1.89 (1H, m)
9'	20.7	0.71 (3H, d, 7.4)	20.8	0.74 (3H, d, 72)
Aromatic	101.1	5.91 (1H, d, 1.3)	101.2	5.93 (1H, d, 1.2)
OCH_2O		5.87 (1H, d, 1.3)		5.90 (1H, d, 1.2)
Aliphatic	98.4	5.65 (1H, S)	98.5	5.68 (1H, S)
OCH_2O		5.60 (1H, S)		5.63 (1H, S)

Crystal data: Molecular formula: $C_{20}H_{20}O_6$. The crystal used for the diffraction study shows no decomposition during data collection. A well-shaped 0.44 mm × 0.37 mm × 0.31 mm individual crystal was chosen for X-ray data collection which performed on a SMART APEX CCD diffractometer using graphite-monochromated MoKα radiation (λ = 0.071073 nm) at rt (293K). The title complex crystallizes in orthorhombic, space group $P2_12_12_1$, with a = 0.9838(2) nm, b = 1.1779(3) nm, c = 1.4431(3) nm, V = 167.23(7) nm, Z=4, D =1.415 mg/m^3, F(000)=752, linear absorption coefficient μ = 0.105mm^{-1}. A total of 8964 reflections were collected in the range $2.23° \leq \theta \leq 25.57°$ of which 3129 were independent (R_{int} = 0.0169). The structure was solved by direct methods with SHELXS and SHELXL software package and the all non-hydrogen atoms were refined anistropically by the full-matrix least-squares method. The finally R_1 and ωR_2 was 0.0304, and 0.0851 respectively (Figure 2).

Measurement of Cytotoxicity The cytotoxicity of the compound **1** was tested in the HeLa (human ovarian cancer), SGC-7901 (human stomach cancer), HepG2 (human liver cancer), and normal cell lines L02 (human fetal hepatocytes) using the MTT method. Cell suspensions (180 μL) were seeded in 96-well plates at densities of 1.0×10^5 cells per well with test compound (20 μL) added from DMSO stock solution. After 3 d of culture, attached cells were incubated with MTT (10 μL, 4 h) and subsequently solubilized in 10% SDS–*N*, *N*-dimethylformaldehyde (DMF) solution (100 μL, 10 h). The absorbance was measured at 570 nm using a microplate reader.

ACKNOWLEDGEMENTS

This study was supported by grants from National Natural Science Foundation of China (No. 40706046), Knowledge Innovation Program of Chinese Academy of Sciences (LYQY200703), and Guangdong Key Laboratory of Marine Materia Medica Foundation.

REFERENCES AND NOTES

1. National Pharmacopoeia Committee, "Chinese Pharmacopoeia," Vol. I, Chemical Industrial Press, Beijing, 2005.
2. E. Lee, K. Hae, J. M. Yook, M. H. Jin, C. S. Seo, K. H. Son, H. P. Kim, K. H. Bae, S. S. Kang, J. K. Son, and H.W. Chang, *Biol. Pharm. Bull.*, 2006, **29**, 211.
3. H. Y. Cho, C. W. Cho, and Y. S. Song, *J. Med. Food*, 2005, **8**, 190.
4. E. C. Wang, M. H. Shih, M. C. Liu, M. T. Chen, and G. H. Lee, *Heterocycles*, 1996, **43**, 969.
5. S. H. Sung and Y. C. Kim, *J. Nat. Prod.*, 2000, **63**, 1019.
6. W. S. Lee, Y.- I. Baek, J.-R. Kim, K.-H. Cho, D.-E. Sokb, and T.-S. Jeong, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 5623.
7. S. H. Sung, M. S. Huh, and Y. C. Kim, *Chem. Pharm. Bull.*, 2001, **49**, 1192.
8. B.-T. Ahn, S. Lee, S.-B. Lee, E.-S. Lee, J.-G. Kim, S.-H. Bok, and T.-S. Jeong, *J. Nat. Prod.*, 2001, **64**, 1562.
9. B.Y. Hwang, J.-H. Lee, J. B. Nam, Y.-S. Hong, and J. J. Lee, *Phytochemistry*, 2003, **64**, 765.
10. S. H. Sung, *Fitoterapia*, 2006, **77**, 487.
11. S. Y. Park, S. H. Lee, W. H. Choi, E. M. Koh, J. H. Seo, S. Y. Ryu, Y. S. Kim, D. Y. Kwon, and W. S. Koh, *Planta Med.*, 2007, **73**, 674.
12. O. E. Kwon, H. S. Lee, S. W. Lee, M. Y. Chung, K. H. Bae, M. C. Rho, and Y. K. Kim, *Arch. Pharm. Res.*, 2005, **28**, 55.

13. W. S. Lee, D. W. Lee, Y. I. Baek, S. J. An, K. H. Cho, Y. K. Choi, H. C. Kim, H. Y. Park, K. H. Bae, and T. S. Jeong, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 3109.
14. H.-J. Park, R.-G. Kim, B.-R. Seo, J. Ha, B.-T. Ahn, and K.-T. Lee, *Planta Med.*, 2003, **69**, 947.
15. B. Seo, C. Yoo, H. Park, J. Choi, K. Seo, S. Choi, and K. Lee, *Biol. Pharm. Bull.*, 2004, **27**, 1594.
16. M. C. Rho, O. E. Kwon, K. Kim, S. W. Lee, M. Y. Chung, Y. H. Kim, M. Hayashi, H. S. Lee, and Y. K. Kim, *Planta Med.*, 2003, **69**, 1147.
17. J. Hahm, I. Lee, W. Kang, S. Kim, and Y. Ahn, *Planta Med.*, 2005, **71**, 464.
18. S. R. Kim, S. H. Sung, S. Y. Kang, K. A. Koo, S. H. Kim, C. J. Ma, H. S. Lee, M. J. Park, and Y. C. Kim, *Planta Med.*, 2004, **70**, 391.
19. B. Y. Hwang, J.-H. Lee, J. B. Nam, H. S. Kim, Y. S. Hong, and J. J. Lee, *J. Nat. Prod.*, 2002, **65**, 616.
20. T. H. Kang, H. Cho, H. Oh, D. H. Sohn, and Y. C. Kim, *Fitoterapia*, 2005, **76**, 115.
21. W. S. Lee, M. J. Kim, Y.-I. Beck, Y.-D. Park, and T.-S. Jeong, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3573.
22. S. H. Sung, E. J. Lee, J. H. Cho, H. S. Kim, and Y.-C. Kim, *Biol. Pharm. Bull.*, 2000, **23**, 666.
23. H. Song, Y. C. Kim, and A. Moon, *Biol. Pharm. Bull.*, 2003, **26**, 1428.
24. B. Y. Hwang, J.-H. Lee, H. S. Kim, J. B. Nam, Y. S. Hong, S.-G. Park, and J. J. Lee, *Planta Med.*, 2003, **69**, 1096.