

STUDIES ON CONSTITUENTS OF SAURURUS CHINENSIS

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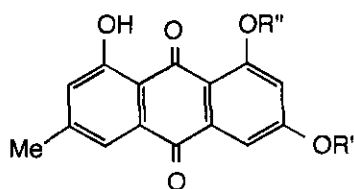
Abstract --- Extracts of *Saururus chinensis* (Lour.) Baill. (Saururaceae) plants were partitioned. The chloroform fraction showed the most potent anti-hypertension effect. Constituents of this fraction include a new lignan, sauchinone, three anthraquinone derivatives and an alkaloid.

It was known that both *Limonophilia rugosa* and *Saururus chinensis* were traded in local herb-drug stores as substitutes for "Shui-ding-xiang", a popular Formosan herb-medicine for hypertension treatment.¹ Previous investigations of *L. rugosa* in our laboratory² revealed that the chloroform fraction from partitions of crude extracts has the most potent anti-hypertension effect on spontaneous hypertensive rats. We also found that crude extracts of the whole plant of *S. chinensis* demonstrated the same effect.¹

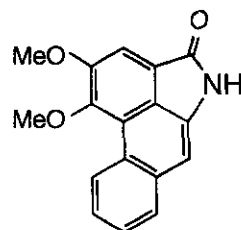
S. chinensis is a fetid, perennial herb with an erect stem. It has petiole, ovate or ovate-oblong shaped leaves and small dense flowers.³ The herb in whole or in parts is commonly used to treat edema, gonorrhoea and also as an uretics in traditional oriental medicine.⁴ Prior literature⁵⁻⁹ showed that the herb contains flavonoids, amino acids, fatty acids and some volatile ingredients. In this study, the crude extracts of *S. chinensis* were partitioned to yield CHCl₃, EtOAc, n-BuOH and water layers. The characterization of chloroform layer, where the most anti-hypertension effect was detected, led to three anthraquinone derivatives, an alkaloid and a new lignan.

RESULTS AND DISCUSSION

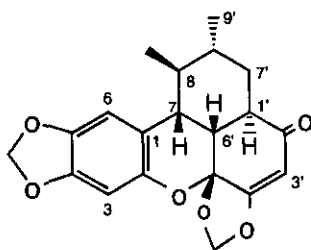
The dried herbs of *S. chinensis* were refluxed repeatedly with 50% aqueous methanol. The combined extracts were condensed and then partitioned sequentially with chloroform, ethyl acetate and n-butyl alcohol to yield CHCl₃, EtOAc, n-BuOH and water layers. Examination of rats' blood pressures under influence of pentobarbital anesthetization revealed that the CHCl₃ fraction has the most potent anti-hypertension effect. Repetitive column chromatographic separation of this fraction resulted in isolation of five crystalline compounds (I, II, III, IV and V).



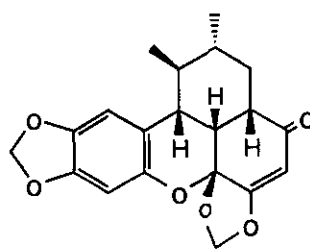
	R'	R''
I	Me	H
II	Me	H
III	Me	glucosyl



IV



V



VI

Compounds (I) (m/z 284) and (II) (m/z 270) shared many common features with quinones in their ir, uv and ms spectra. Their $^1\text{H-nmr}$ spectra showed that each compound has two sets of meta-position aromatic hydrogens (two doublets at δ 6.68 and 7.36, $J=2.4$ Hz and two doublets at δ 7.08 and 7.62, $J=1.0$ Hz for I; two doublets at δ 6.64 and 7.21, $J=2.4$ Hz and two doublets at δ 7.10 and 7.52, $J=1.3$ Hz for II) and one C-3 methyl group at δ 2.45. Furthermore, the $^1\text{H-nmr}$ signals of compound (I) at δ 12.30, 12.11 and 3.94 indicated two hydroxyl groups at C-1 and C-8 and one methoxy group at C-6. With comparisons of the $^1\text{H-}$ and $^{13}\text{C-nmr}$ spectra to an authentic sample, compound (I) was identified as 1,8-dihydroxy-6-methoxy-3-methylantraquinone (physcion). Regarding compound (II), the presence of three hydroxyl signals (δ 12.15, 12.03 and 11-9.5) suggested that the structure is 1,6,8-trihydroxy-3-methylantraquinone (emodin). This was later confirmed by examining the spectra of an authentic sample.

Compound (III) is similar to compound (I) in the sense of methoxy and methyl chemical shifts (δ 3.96 and 2.41, respectively) in their $^1\text{H-nmr}$ spectra. With $^1\text{H-nmr}$ hydroxyl signals at δ 5.11 and δ 4.68, the compound appeared to be a physcion glucoside. The anomeric proton signal at δ 5.17 (d, $J=8.0$ Hz) suggested that a β -glucosyl group is attached to either C-1 or C-8. Comparisons of $^1\text{H-nmr}$ signals at 7-H (δ 7.18, d, $J=2.3$ Hz) and 2-H (δ 7.19, s) to data in literature¹⁰ concluded the structure of III being physcion 8-O- β -D-glucopyranoside.

In compound (IV), an uv absorption pattern of phenanthrenic chromophores was observed. This together with ^1H -nmr spectra (δ 9.75, 1H, br., N-H; δ 4.14, 6H, s, OMe; δ 7.24-9.26, 6H, m, aromatic protons) implied that the compound is a 3,4-dimethoxyaristololactam. The compound was identified as cepharanone B with verification of ms and nmr spectra in literature.¹¹⁻¹³

Compound (V), with formula $\text{C}_{20}\text{H}_{20}\text{O}_6$ ($[\text{M}]^+$ m/z 356.1334), has ir absorption bands of a phenyl (3072 and 3052 cm^{-1}), a conjugated carbonyl (1676 and 1664 cm^{-1}) and two methylenedioxy groups (2784 and 2880 cm^{-1}). The nmr spectra confirmed that it has two aromatic protons (δ 6.82 and δ 6.38) and two methylenedioxy groups, one (^1H -nmr: δ 5.91 and δ 5.87; ^{13}C -nmr: δ 101.08, $-\text{OCH}_2\text{H}_b\text{O}-$) attaches to an aromatic ring and another (^1H -nmr: δ 5.65 and δ 5.60; ^{13}C -nmr: δ 98.38, $-\text{OCH}_2\text{H}_b\text{O}-$) attaches to aliphatic carbons. ^{13}C -Nmr signals at δ 146.49 (C), 144.76 (C) and 143.02 (C) indicated that in addition to two aromatic carbon-oxygen bonds for the methylenedioxy group there is one more oxygen attached to the aromatic ring. The ^{13}C -nmr signal at δ 193.31 (C=O) was attributed to the carbonyl group of an enone, while the ^{13}C -nmr signal at δ 168.41(C) implied that there is a methylenedioxy group attached to γ -carbon of enone. ^1H -Nmr signals at δ 1.21(d, $J=7.3$ Hz) and δ 0.71 (d, $J=7.4$ Hz) showed two methyl groups, each coupled by a vicinal proton. Correlations of ^1H -nmr spectra between compound (V) and a known lignan, dihydrocarpanone (VI),¹⁴ suggested that the two compounds are isomers. Their ^1H -nmr spectra are in reasonable agreement except for proton shifts at 7'a-H, 7'e-H, 6'-H and 8'-H (Table 1). ^1H -Nmr spectral assignments of compound (V)

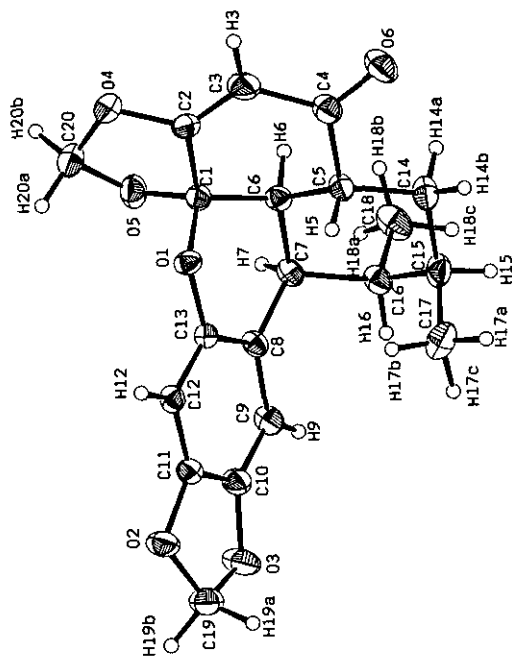
Table 1 ^1H -Nmr Data (δ , in ppm) of Sauchinone and Dihydrocarpanone¹⁴

H	Sauchinone	Dihydrocarpanone
3	6.38	6.35
6	6.82	6.78
7	3.03	3.20
8	2.41	2.26
1'	2.49	2.66
3'	5.50	5.62
6'	2.47	2.89
7'a	1.93	1.16
7'e	1.64	2.05
8'	1.88	1.58
Aromatic $-\text{OCH}_2\text{H}_b\text{O}-$	5.91, 5.87	5.90
Aliphatic $-\text{OCH}_2\text{H}_b\text{O}-$	5.65, 5.60	5.61, 5.58
C-9 Me	1.21	1.23
C-9' Me	0.71	0.70

Table 2 Crystal Data of Sauchinone

Molecular Formula	C ₂₀ H ₂₀ O ₆
Crystal System	orthorhombic
Cell Constants	
a (Å)	9.834 (4)
b	11.7528 (10)
c	14.4275 (16)
V (Å ³)	1667.5 (7)
Space Group	P2 ₁ 2 ₁ 2 ₁
z Value	4
Dx g/cm ³	1.420
X-Ray λ (Å)	0.7107
F(0 0 0)	752

Figure 1 ORTEP drawing of Sauchinone



were based on its COSY and NOESY data in accordance with the stereo structure. It is noteworthy that the upper field of 7'a-H relative to 7'e-H in dihydrocarpanone was most likely caused by proximity of 7'a-H to the oxygen atom in tetrahydropyran ring, whereas shielding effect of C-4 carbonyl group on 7'e-H led to a upper field shift for 7'e-H compared to 7'a-H in sauchinone. X-Ray crystallographic analysis, performed using an Enraf-Nonius CAD4 diffractometer with graphite-monocromated MoK α radiation ($\lambda = 0.7107$ Å), confirmed that these two compounds are configuration isomers. The structure of compound (V) (Figure 1) was solved by MULTAN and computations of X-ray data were aided by Program NRCCVAX in a DEC 3000 computer and refined by full-matrix least-square method to yield crystal constants (Table 2). The notable difference between V and VI is the configurations of their 7-H, 6'-H and 1'-H. The three protons in compound (V) are in a cis-trans form by comparison with the cis-cis form in compound (VI).

EXPERIMENTAL

Melting points were measured with a Yanaco micro-melting point apparatus without calibrations. A JOEL JMS-D 100 or JOEL JMS-HX 110 mass spectrometer was used for obtaining mass spectra. Infrared and ultraviolet spectra were obtained with a Perkin-Elmer 2000 FTIR or JASCO A-100 Infrared spectrophotometer using potassium bromide disc sample and with a Hitachi 270-30 UV spectrophotometer using MeOH as solvent, respectively. ¹H- and ¹³C-nmr spectra were recorded with a Bruker WP-100sy, a AM-400 or a DPX-200 model using

tetramethylsilane as an internal standard. A JASCO DIP-370 digital polarimeter was utilized for measuring optical activities of crystal. Microanalyses were performed at Elemental Analysis Laboratory of National Cheng Kung University, Taiwan. The blood pressures of rats were recorded by a Nihon Kohden RM 25 I polygraph.

The herbs were purchased at the local herb-drug store and identified as *Saururus chinensis* with the assistance of Professor C. S. Kuoh of Department of Biology, National Cheng Kung University. Three kilograms of dried herbs were refluxed for six hours with 50% aqueous MeOH (15 l) each time for six times. The crude extracts were then partitioned in sequence between three solvents (CHCl₃, EtOAc, n-BuOH) and water (3000 g) to yield four fractions: CHCl₃ layer (87 g), EtOAc layer (11 g), n-BuOH layer (26 g) and water layer (452 g). Each fraction was dissolved in Tween 80 (20 mg/kg) for intravenous injections of spontaneous hypertensive rats. The rats were under sodium pentobarbital anesthetization while the solutions were injected into their femoral veins. Their carotid arteries were cannulated for blood pressure records. The results showed that the chloroform fraction had the most potent anti-hypertension effect. Repetitive chromatographic separations of the this fraction over Si-gel column resulted in isolation of five crystalline compounds:

Compound (I) (Physcion, 1,8-dihydroxy-6-methoxy-3-methylantraquinone)

Orange red color crystals (26 mg) from CHCl₃-C₆H₁₄, mp 205-206°C (lit.,¹⁵ mp 207°C). Ir ν_{\max} (KBr) cm⁻¹: 1670, 1620, 1560, 1480, 1360. Uv λ_{\max} (MeOH) nm (log ϵ): 223(4.42), 248(4.13), 266(4.21), 283(4.18), 301(3.94), 434(3.92). Elms (75ev) m/z (%): 284(100, [M]⁺), 283(4, [M-H]⁺), 256(4, [M-CO]⁺), 255(9, [M-CHO]⁺), 241(6, [M-MeCO]⁺), 142(3, [M]²⁺). ¹H-Nmr δ (ppm) in CDCl₃: 12.30(s, -OH), 12.11 (s, -OH), 7.62(d, =1.0 Hz, 4-H), 7.36(d, J=2.4 Hz, 5-H), 7.08(d, J=1.0 Hz, 2-H), 6.68(d, J=2.4 Hz, 7-H), 3.94(s, 6-OMe), 2.45(s, 3-Me). ¹³C-Nmr δ (ppm) in CDCl₃: 190.6(C=O), 181.9(C=O), 166.4(C), 164.8(C), 162.1(C), 148.3(C), 135.1(C), 133.1(C), 124.3(CH), 121.2(CH), 113.5(C), 110.1(C), 108.1(CH), 106.6(CH), 56.0 (OMe), 22.0 (Me).

Compound (II) (Emodin, 1,6,8-trihydroxy-3-methylantraquinone)

Orange color needles (82 mg) from CHCl₃-C₆H₁₄, mp 266-268°C (lit.,¹⁰ mp 264-265°C). Ir ν_{\max} (KBr) cm⁻¹: 3450, 1620, 1470, 1330. Uv λ_{\max} (MeOH) nm (log ϵ): 221(4.57), 254(4.33), 266(4.32), 289(4.36), 436(4.07). Elms (75ev) m/z(%): 270(100, [M]⁺), 253(4, [M-OH]⁺), 242(13, [M-CO]⁺), 241(10, [M-CHO]⁺), 213(11), 139(10), 135(8, [M]²⁺), 121(10, [M-CO]²⁺). ¹H-Nmr δ (ppm) in (CD₃)₂CO: 12.15(s, -OH), 12.03 (s, -OH), 11-9.5(br., -OH), 7.52(d, J=1.3 Hz, 4-H), 7.21(d, J= 2.4 Hz, 5-H), 7.10(d, J=1.3 Hz, 2-H), 6.64(d, J=2.4H, 7-H), 2.45(s, 3-Me). ¹³C-Nmr δ (ppm) in (CD₃)₂CO: 190.0(C=O), 181.6(C=O), 165.8 (C), 164.7(C), 161.7(C), 148.4(C), 135.2 (C), 132.9 (C), 124.2(CH), 120.7(CH), 113.4(C), 109.2(CH), 108.9 (C), 108.1(CH), 21.6(Me).

Compound (III) (Physcion 8-O- β -D-glucopyranoside)

Orange color needles (30 mg) from CHCl_3 -MeOH, mp 246-248°C (lit.,¹⁶ mp 237-239°C). $^1\text{H-Nmr}$ δ (ppm) in DMSO- d_6 : 13.08(s, -OH), 7.49(br., 4-H), 7.36(d, $J=2.3$ Hz, 5-H), 7.19(br., 2-H), 7.18(d, $J=2.3$ Hz, 7-H), 5.17(d, $J=8.0$ Hz, anomeric H), 5.11(m, -OH), 4.68(m, -OH), 3.96(s, 6-OMe), 2.41(s, 3-Me).

Compound (IV) (Cepharanone B)

Yellow color needles (6 mg) from CHCl_3 -MeOH, mp 261-263°C (lit.,¹⁷ mp 264-265°C).

$\text{C}_{17}\text{H}_{13}\text{NO}_3$ (high resolution ms m/z 279.0879, calcd 279.0896). $\text{Uv } \lambda_{\text{max}}$ (MeOH) nm (log ϵ): 232(4.68), 264(4.61), 276(4.67), 287(4.66), 318(4.03), 384(4.01). Elms (75ev) m/z (%): 279(100, $[\text{M}]^+$), 264(21, $[\text{M-Me}]^+$), 236(19, $[\text{M-MeCO}]^+$), 221(15, $[\text{M-2Me-CO}]^+$), 218(10), 209(11), 193(16), 181(12), 165(14), 164(12), 139.5(14, $[\text{M}]^{2+}$), 132(6, $[\text{M-Me}]^{2+}$), 82.5(16). $^1\text{H-Nmr}$ δ (ppm) in $(\text{CD}_3)_2\text{CO}$: 9.75(br., NH), 9.26(m, 5-H), 7.91(m, 8-H), 7.83(s, 2-H), 7.6-7.5(m, 6-, 7-H), 7.15(s, 9-H), 4.14(s, 3, 4-OMe). $^{13}\text{C-Nmr}$ δ (ppm) in $(\text{CD}_3)_2\text{CO}$: 168.82 (C=O), 154.53(C), 150.74(C), 135.25(C), 135.02 (C), 129.34(CH), 127.87(CH), 127.12(CH), 126.18(C), 125.90(CH), 123.55 (C), 121.72(C), 120.18(C), 110.15(CH), 105.12(CH), 60.25(OMe), 57.19(OMe).

Compound (V) (Sauchinone)

Colorless crystals (1.42 g) from CHCl_3 -MeOH, mp 224-226°C, $[\alpha]_D^{25}$ 97.8 (c. 0.0218 in CHCl_3).

$\text{C}_{20}\text{H}_{20}\text{O}_6$ (high resolution ms m/z 356.1244, calcd 356.1260). $\text{Ir } \nu_{\text{max}}$ (KBr) cm^{-1} : 3448, 3072, 3052, 2964, 2956, 2924, 2880, 2784, 1676, 1664, 1540, 1504, 1496, 1480, 1456, 1436, 1408, 1384, 1368, 1344, 1320, 1304, 1284, 1268, 1240, 1220, 1208, 1180, 1160, 1128, 1116, 1100, 1088, 1040. $\text{Uv } \lambda_{\text{max}}$ (MeOH) nm (log ϵ): 242(4.25), 297(3.72). Elms (75ev) m/z (%): 356(100, $[\text{M}]^+$), 326(4), 325 (3), 319(4), 298(12), 297(4), 288(8), 285(6), 283(4), 270(6), 269(7), 257(10), 256(5), 243(10), 241(6), 229(5), 228(6), 218(6), 215(6), 205(19), 203(6), 201(4), 189(6), 188(7), 178(9), 175(26), 151(21), 150(4), 149(4), 147(5), 138(13), 115(6). $^1\text{H-Nmr}$ δ (ppm) in CDCl_3 : 6.82(1H, s), 6.38 (1H, s), 5.91(1H, d, $J=1.3$ Hz), 5.87(1H, d, $J=1.3$ Hz), 5.65(1H, s), 5.60(1H, s), 5.50(1H, s), 3.03 (1H, d, $J=3.9$ Hz), 2.47(3H, m), 1.91(2H, m), 1.64(1H, m), 1.21(3H, d, $J=7.3$ Hz), 0.71 (3H, d, $J=7.4$ Hz). $^{13}\text{C-Nmr}$ δ (ppm) in CDCl_3 : 193.31(C=O), 168.41(C), 146.49(C), 144.76 (C), 143.02 (C), 115.50(C), 106.29(CH), 101.08(CH_2), 100.17(CH), 99.97(C), 99.00(CH), 98.38 (CH_2), 37.34(CH), 37.34(CH), 34.82(CH), 34.58(CH), 33.20(CH), 25.03 (CH_2), 21.02 (CH_3), 20.66 (CH_3). Anal. calcd for $\text{C}_{20}\text{H}_{20}\text{O}_6$: C, 67.41; H, 5.66; O, 26.94. Found: C, 67.18; H, 5.68; O, 27.14.

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