HETEROCYCLES, Vol. 60, No. 7, 2003, pp. 1645 - 1652

Received, 31st March, 2003, Accepted, 8th May, 2003, Published online, 12th May, 2003

CHEMICAL CONSTITUENTS OF THE STEM OF *SARGENTODOXA CUNEATA*

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Abstract – A new phenylpropanoid glycoside, sargentol (**1**), was isolated and characterized from the traditional Chinese crude drug "Daxueteng", the stem of *Sargentodoxa cuneata*, together with twenty seven known compounds. The structures of the isolated compounds were elucidated by means of various NMR techniques and MS spectral analysis.

INTRODUCTION

"Daxueteng", the stem of *Sargentodoxa cuneata* (also referred as hongteng or xueteng or sargento-doxavine or sargentogloryvine), is an important crude drug in the Chinese traditional medicine prescribed to remove toxins (detoxicant), and furuncles, clear heat, invigorate blood circulation, promote the flow of channels, dispel wind, kill intestinal parasites, reduce swelling and alleviate pain.[1,2] This herbal drug has also been used in the treatment of acute appendicitis, abdominal pain, amenorrhea, dysmenorrhea, rheumatic arthritis, trauma and painful menstruation.[1,2] Recent pharmacological studies have revealed antiinflammatory, antiviral, antihypoxia, hemolytic, cardiovascular, gastrointestinal, vasodilating and myocardial ischemia inhibitory effects of the crude extracts or isolated constituents from this drug.[3-8] *S. cuneata* Rehd. Et Wils (Sargentodoxaceae) is a deciduous climber, widely spread over open forests on mountainous regions from east Asia to central China.[9] According to dictionary of Chinese drugs the mashed leaves have been plastered onto sores, the root was antirheumatic, whereas the stem was used as

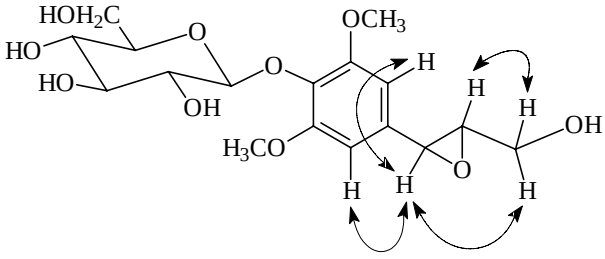
anthelmintic, antibacterial, antirheumatic, carminative, diuretic, tonic, insecticide, and as a blood stimulant. A decoction or tincture was used in the treatment of amenorrhea, metrorrhagia, anemia, traumatic injuries, hookwarm disease, round warm, and fylariasis. In previous reports on chemical investigations of this plant, sterols, triterpene saponins, anthraquinones, lignans, phenolic acids, polycyclic phenolic compounds, acetophenone glycosides, sugars and polysaccharides have been found as active ingradients.[3-8] As a part of a continuing project to study the chemical composition of Chinese herbal drugs, we have examined the stem of *S. cuneata*. This paper deals with the isolation and structural elucidation of a new phenylpropanoid glycoside (**1**).

RESULTS AND DISCUSSION

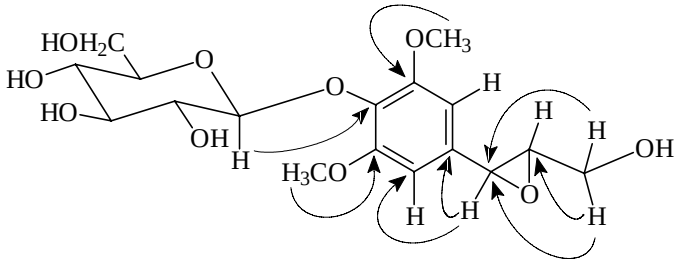
Dried stems of *S. cuneata* were extracted with ethanol and dried. The dry extract was suspended in water and partitioned with $CHCl_3$ and *n*-BuOH, successively. The $CHCl_3$ extract was applied to silica gel column to provide compounds (**2**-**4**). The *n*-BuOH solubles were fractionated by reversed-phase column chromatography using Diaion HP-20. Selected fractions were rechromatographed on silica gel using different solvent combinations to yield compounds (**1**) and (**5**-**28**).

Compound (**1**) was obtained as colorless crystals from methanol. It responded positively to the Molisch test indicated it to be a glycoside. The positive-ion HRFAB MS spectrum showed pseudomolecular ion at *m/z* 389.1445 consistent with the molecular formula $C_{17}H_{24}O_{10}$ and a prominent fragment ion at *m/z* 226 $[M+H-163]^+$ in FABMS indicating the loss of an hexose moiety. The UV absorption maxima at 230, 276, and 358 nm suggested the presence of an aromatic ring in **1**. The IR spectrum contained bands at 1594, 1507, and 1463 cm^{-1} consistent with the presence of an aromatic ring in addition to strong hydroxyl and epoxide stretching bands at 3390 and 1235 cm^{-1}, respectively. The carbon resonances observed in the ^{13}C NMR spectrum were established to be two methoxyls, two oxygen bearing methylenes, two aromatic methines, seven oxygenated methines, and four aromatic quartenary carbons through DEPT and HMQC experiments. The ^1H NMR spectrum displayed a shielded aromatic singlet with the intensity of two protons at δ 6.64 and a singlet integrating for six protons of two equivalent methoxyl groups at δ 3.74.

The integration of these two signals demonstrated that the aromatic ring is symmetrically substituted as shown. The corresponding carbon signals were assigned with the aid of HMQC spectrum. Oxirane protons appeared as a doublet at δ 4.64 (*J* = 2.8 Hz, H-1') and a multiplet at δ 3.08 (H-2'). The relative stereochemistry was determined from the magnitude of the vicinal coupling of 2.8 Hz between H-1' and H-2', exactly as expected for a *trans*-disubstituted epoxide[10] and the results of NOESY experiment. The presence of a hydroxymethyl group on C-2' was evidenced by the ^{1}H NMR signals of a hydroxyl group at δ 4.94 coupled with methylene protons at δ 4.18 (1H, dd, *J* = 9.2, 6.4 Hz, H-3'a) and 3.80 (1H, dd, *J* = 9.2, 3.2 Hz, H-3'b). A doublet at δ 4.85 with the coupling constant 7.2 Hz assignable to anomeric proton combined with carbon signals at δ 102.8, 70.1, 76.6, 74.3, 77.3 and 61.0 showed that **1** contained *β*-D-glucosyl moiety. The position of the glucosyl linkage in **1** was investigated by HMBC NMR spectroscopy. The long range coupling between H-1" (δ 4.85) and C-1 (δ 133.8) suggested that the glucose was attached to the phenylpropanoid moiety at C-1. The ^{3}J cross peaks in the HMBC spectrum between methoxyl protons (δ 3.74) and carbon at δ 152.8 established the placement of methoxyl groups on C-2 and C-6. Consequently, the structure of **1** was defined as [4-(1', 2'-epoxy-3'-hydroxypropyl)-2, 6-dimethoxyphenyl]-*O*-*β*-D-glucopyranoside, for which the trivial name sargentol is proposed.



Key NOESY correlations of **1**



Key HMBC correlations of **1**

Glucoside, sargentol seems to be included in the plant as protected and stored form of synapyl alcohol, which is susceptible to oxidation and is regarded as biosynthetic precursors of lignans.[11] Using a combination of radio- and stable-isotopically labeled precursor administration experiments, Davin *et al.* proved that furofuran lignans were resulted from stereoselective coupling of phenylpropenyl alcohols, such as *E*-conyferyl or *E*-synapyl alcohols.[12] Therefore, compound **1** has biogenetic significance because it is a plausible intermediate of the furofuran lignan such as liriodendrin[8] isolated from this plant.

The known compounds, *β*-sitosterol (**2**),[13] *β*-sitosterylglucoside (**3**),[13] *β*-sitosterone (**4**),[13] tyrosol (**5**),[14]

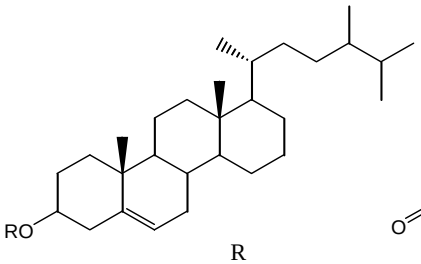
salidroside (**6**),[15] 2-(3,4-dihydroxyphenyl)ethyl glucoside (**7**),[16] methylprotocatechuate (**8**),[17] vanillic acid (**9**),[13] vanillic acid glucoside (**10**),[18] vanilloyl-*β*-D-glucoside (**11**),[19] *p*-methoxyphenylacetic acid (**12**),[20] *p*-hydroxyphenylacetic acid (**13**),[21] *p*-hydroxyphenylacetone (**14**),[22] acetovanilone (**15**),[23] 3,4,5-trimethoxyphenyl-*β*-D-glucoside (**16**),[24] syringic acid (**17**),[13] erigeside C (**18**),[25] *p*-hydroxybenzoic acid (**19**),[13] androsin (**20**),[26] methylchlorogenate (**21**),[27] ferulic acid (**22**),[28] *p*-hydroxycinnamic acid (**23**),[29] 2,2-dimethylchromane-6-carboxylic acid (**24**),[30] secoisolariciresinol (**25**),[31] (+)-*epi*-syringaresinol di-*O*-*β*-D-glucoside (**26**),[32] liriodendrin (**27**),[32] and quadranoside IV (**28**)[33] were also isolated and identified by comparison of their spectroscopic data with the values in literature. Among the known compounds, twenty one (**4**, **5**, **7**-**16**, **18**-**25**, **28**) of the minor compounds were isolated for the first time from this plant.

EXPERIMENTAL

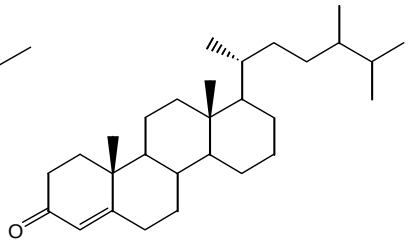
General Experimental Procedures. Melting points were recorded on Yanaco MP-S3 micro melting point apparatus without correction. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on a Hitachi UV-3210 spectrophotometer in MeOH. IR spectra were recorded on a Shimadzu FT-IR DR-8011 spectrophotometer. ^{1}H and ^{13}C NMR spectra were determined on Bruker AMX-400, AVANCE-300 and Varian Unity Plus 400 spectrometers. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard. EI, FAB, and HRFAB mass spectra were measured on a VG70-250S spectrometer by a direct inlet system.

Plant Material. The stems of *S. cuneata* were collected in September 1999 from Shanghai, and authenticated by Prof. C. Q. Hu. A voucher specimen (TSWu 199900031) is deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The dried and pulverized stems of *S. cuneata* (2.0 kg) were extracted with ethanol (5 L × 8) under reflux for 8 h and the extract was concentrated under reduced pressure to give a residue (203 g). The residue was suspended in water and partitioned successively with $CHCl_3$ and *n*-BuOH. The $CHCl_3$ extract (13 g) was chromatographed over silica gel using $CHCl_3$ with increasing



	R
2	H
3	glc



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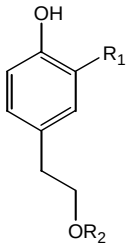
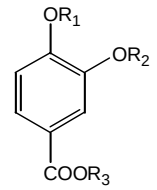
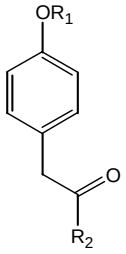


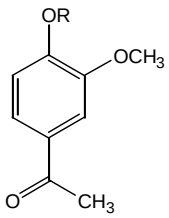
	R_1	R_2
5	H	H
6	H	glc
7	OH	glc



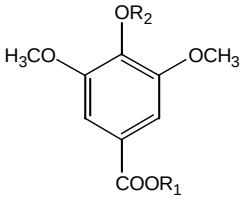
	R_1	R_2	R_3
8	H	H	CH_3
9	H	CH_3	H
10	glc	CH_3	H
11	H	CH_3	glc



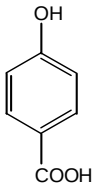
	R_1	R_2
12	CH_3	OH
13	H	OH
14	H	CH_3



	R
15	H
20	glc



	R_1	R_2
16	glc	CH_3
17	H	H
18	glc	H



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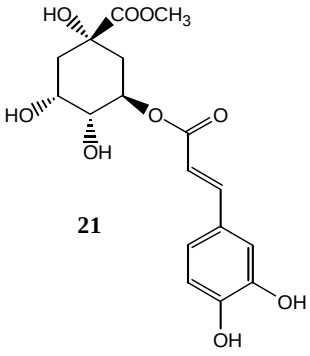

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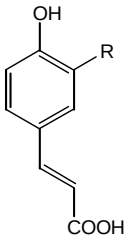
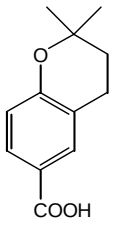


	R
22	OCH_3
23	H



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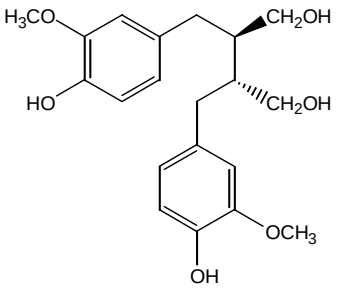

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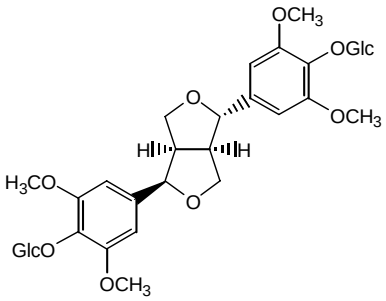

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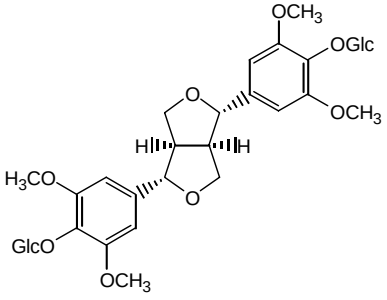

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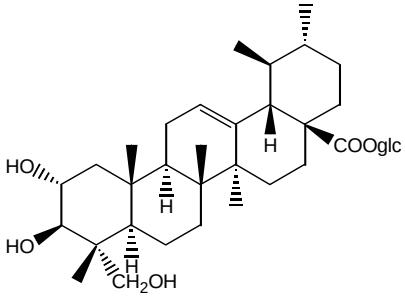

28

amount of MeOH as eluent to afford four fractions. Fraction 1 was rechromatographed on silica gel by eluting with *n*-hexane-EtOAc (19: 1) to obtain **4** (5.0 mg). Fractions 2, 3, and 4 were separately subjected to further column chromatography on silica gel and eluted with a gradient of $CHCl_3$ and MeOH (49: 1) to get **2** (222.0 mg) and **3** (126.0 mg). The *n*-BuOH extract (161 g) was loaded on Diaion HP-20 and eluted with a stepwise gradient of MeOH in H_2O to afford 13 fractions. Fraction 4 on repeated column chromatography over silica gel using $CHCl_3$: MeOH: H_2O (9: 1: 0.1) and EtOAc: MeOH: H_2O (5: 1: 0.1) eluents followed by prep. TLC afforded **5** (6.4 mg), **6** (5.0 mg), **7** (2.5 mg), **8** (7.0 mg), **9** (5.0 mg), **10** (3.2 mg), **11** (2.0 mg) and **12** (1.2 mg). Fractions 5, 6, and 7 were separately subjected to column chromatography over silica gel several times using EtOAc: MeOH: H_2O (9: 1: 0.1) eluent and finally separated by prep. TLC to obtain **5** (2.0 mg), **6** (4.2 mg), **13** (1.0 mg), **14** (1.0 mg), **15** (5.2 mg), **16** (12.0 mg), **18** (1.2 mg), **19** (10.0 mg) and **20** (24.0 mg). Fraction 11 was subjected to further column chromatography over silica gel with $CHCl_3$: MeOH: H_2O (5: 1: 0.1) solvent system to give two subfractions and compound (**1**) (50.0 mg) was crystallized from subfractions 1 and 2 in methanol and prep. TLC of filtrates yielded **9** (1.0 mg), **17** (3.1 mg), **19** (1.0 mg) and **21** (5.0 mg). Fraction 12 on silica gel column chromatography using EtOAc: MeOH: H_2O (9: 1: 0.1) as eluent afforded three subfractions, which were separately rechromatographed on silica gel ($CHCl_3$: EtOAc, 1: 9) followed by prep. TLC with EtOAc: MeOH: H_2O (19: 1: 0.1) to give **1** (3.0 mg), **9** (5.0 mg), **17** (4.5 mg), **19** (0.5 mg), **22** (2.7 mg), **23** (2.4 mg), **24** (1.0 mg), and **25** (2.0 mg). Fraction 13 was subjected to Sephadex LH-20 column chromatography with H_2O: MeOH (3: 1) and finally purified by prep. TLC to obtain **19** (0.5 mg), mixture of **26** and **27** (2.2 mg), and **28** (15.0 mg).

Sargentol (1) $C_{17}H_{24}O_{10}$: Colorless needles; mp 272-273°C(MeOH); $[\alpha]_D^{24}$ -75.2^o (*c* 0.02, MeOH); UV λ_{max}^{MeOH} nm (log ε): 230 (2.43), 276 (1.31), 358 (1.19); IR ν_{max} cm^{-1}: 3390, 2930, 2872, 1594,1507, 1463, 1235; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.01 (1H, m, H-5”), 3.08 (1H, m, H-2’), 3.11 (1H, m, H-2”), 3.13 (1H, m,H-3”), 3.16 (1H, m, H-4”), 3.37 (1H, dd, *J* = 11.6, 5.6 Hz, H-6”b), 3.56 (1H, dd, *J* = 11.6, 5.6 Hz, H-6”a), 3.74 (3H, s, OMe-2, 6), 3.80 (1H, dd, *J* = 9.2, 3.2 Hz, H-3’b), 4.18 (1H, dd, *J* =9.2, 6.4 Hz, H-3’a), 4.27 (1H, t, *J* = 5.6 Hz, OH-6”), 4.64 (1H, d, *J* = 2.8 Hz, H-1’), 4.85 (1H, d, *J* = 7.2 Hz, H-1”), 4.88 (1H, d, *J* = 5.2 Hz, OH-2”), 4.94 (3H, m, OH-3’, 3”, 4”), 6.64 (2H, s, H-3, 5); ^{13}C NMR

(DMSO-d_6, 100 MHz): δ 53.7 (C-2'), 56.6 (OCH_3), 61.0 (C-6"), 70.1 (C-2"), 71.5 (C-3'), 74.3 (C-4"), 76.6 (C-3"), 77.3 (C-5"), 85.2 (C-1'), 102.8 (C-1"), 104.4 (C-3, 5), 133.8 (C-1), 137.2 (C-4), 152.8 (C-2, 6); FABMS (*rel. int.* %): *m/z* 389 ($[M+H]^+$, 16), 226 ($[M+H-163]^+$, 27); HRFABMS *m/z* 389.1445 $[M+H]^+$ (calcd for $C_{17}H_{25}O_{10}$, 389.1448).

ACKNOWLEDGMENTS

The partial financial support of this work by National Science Council, Republic of China, is gratefully acknowledged.

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