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STRUCTURE OF ENSHICINE FROM *SCHISANDRA HENRYI**

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Key Word Index—*Schisandra henryi*; Schisandraceae; fruit; enshicine; 1-oxy-2*S*,3*S*-dimethyl-4*R*-(3-methoxy-4-hydroxyphenol)-6,7-methylenedioxytetralin; desoxyenshicine; tetralin lignans; absolute configurations; structural determination.

Abstract—A new lignan, enshicine, isolated from the fruit of *Schisandra henryi*, is shown to be 1-oxy-2*S*,3*S*-dimethyl-4*R*-(3-methoxy-4-hydroxyphenol)-6,7-methylenedioxytetralin, by means of spectral analysis and chemical correlations.

INTRODUCTION

In previous papers [1, 2], it was reported that the dibenzocyclooctadiene lignan compounds, schisanhenol, schisanhenrin, schisantherin B, and the triterpenoids, schisanhenric acid and kadsuric acid, were isolated from the fruit of *Schisandra henryi* collected in the district of Xinyang, Henan province of China. Schisanhenrin [2, 3], **1**, possesses the activity of lowering abnormally high serum glutamic–pyruvic transaminase (SGPT) levels (induced by carbon tetrachloride) in mice. A new lignan compound for which the name enshicine is proposed has been isolated from the same species of plant collected in the district of Enshi, Hubei province of China. In this paper we report spectral and chemical data which allows the assignment of structure **2** to enshinine, the first tetralin lignan isolated from plants of the genus *Schisandra*.

RESULTS AND DISCUSSION

Enshicine, **2**, $C_{20}H_{20}O_5$ $[M]^+$ *m/z* 340.1308, mp 146–147°, afforded a significant fragment in the mass spectrum, $[M - C_4H_8]^+$, which is a typical fragmentation of tetralin lignans [4]. The ^{13}C NMR spectrum of **2** (Table 2) indicates the presence of 13 sp^2 carbons, of which three are aromatic carbons bearing carbon, four are aromatic carbons bearing oxygen, five are aromatic carbons bearing hydrogen, and one is a carbonyl carbon (δ199.45; IR spectrum of **2**; 1670 cm^{-1}). These observations are consistent with the tetralin lignan skeleton with four oxygenated substituents. The ^1H NMR spectrum (Table 1) further supports the tetralin lignan skeleton, showing a doubly benzylic methine proton (H-4, δ3.91, *d*, $J_{3,4}$ = 5.80), and two secondary methyls with their related methine protons (H-2, δ2.78, *qd*, $J_{2,11}$ = 7.02, $J_{2,3}$ = 3.05; H-3, δ2.39, *qdd*, $J_{3,12}$ = 7.02, $J_{3,4}$ = 5.80, $J_{2,3}$ = 3.05). ^1H–^1H decoupling experiments support the assignments. Irradiation of H-3 collapses the signals of H-4 and H-12 to a singlet and that of H-2 into a quartet. When H-2 was irradiated, the signal of H-11 collapses to a singlet and that of H-3 into a quartet of doublets. The above decoupling experiments also mean that there is no proton at C-1.

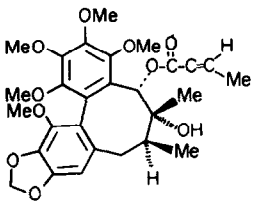

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Table 1. ^1H NMR data* of enshicine

Proton	Chemical shift	Multiplicities	^1H–^1H coupling constants (Hz)
H-2	2.78	*qd*	$J_{2,11}$ = 7.02; $J_{2,3}$ = 3.05
H-3	2.39	*qdd*	$J_{3,12}$ = 7.02; $J_{2,3}$ = 3.05; $J_{3,4}$ = 5.80
H-4	3.91	*d*	$J_{3,4}$ = 5.80
H-5	6.41	*s*	
H-8	7.51	*s*	
H-2′	6.56	*d*	$J_{2',6'}$ = 1.83
H-5′	6.84	*d*	$J_{5',6'}$ = 7.94
H-6′	6.53	*dd*	$J_{5',6'}$ = 7.94; $J_{2',6'}$ = 1.83
H-11	1.12	*d*	$J_{2,11}$ = 7.02
H-12	0.97	*d*	$J_{3,12}$ = 7.02
H-13	3.82	*s*	
H-14A	5.99	*d*	$J_{14A,14B}$ = 1.22
H-14B	5.98	*d*	$J_{14A,14B}$ = 1.22
OH	5.55	*s*	

*Chemical shifts ($CDCl_3$) in δ-values (ppm), relative to internal TMS. The assignments are based on ^1H–^1H decoupling experiments.

The oxygenation pattern of the aromatic ring and the relative configurations about C-2, C-3 and C-4 were elucidated by measuring intramolecular nuclear

*Part 3 in the series "Constituents of *Schisandra henryi*". For part 2, see ref. [2].

Table 2. ^{13}C NMR data* of enshicine

Carbon	Chemical shift	Carbon	Chemical shift
C-1	199.45	C-1′	135.45
C-2	43.10	C-2′	111.25
C-3	42.13	C-3′	144.66
C-4	50.71	C-4′	152.32
C-5	109.60	C-5′	114.42
C-6	146.89	C-6′	121.93
C-7	147.27	C-11	11.72
C-8	105.91	C-12	15.97
C-9	127.14	C-13	56.06
C-10	141.23	C-14	101.68

*In $CDCl_3$ solution. Chemical shifts in δ-values (ppm), relative to internal TMS. The assignments are based on the ^1H–^{13}C selective frequency decoupling experiments and full coupled spectra.

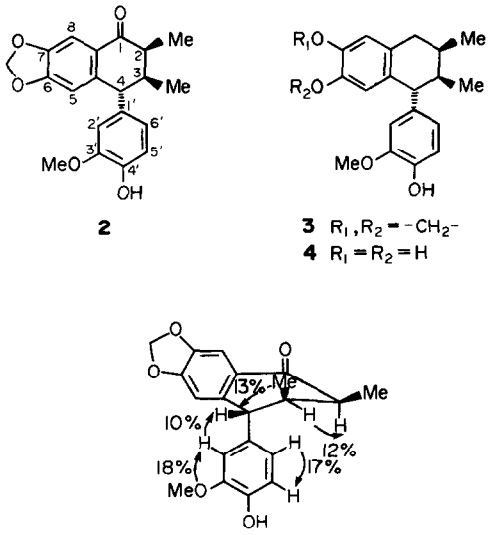

Fig. 1. The NOE enhancements of enshicine.

Overhauser enhancements (NOE) (see Fig. 1). When the methoxyl protons, H-6′ and H-2′ were irradiated an NOE of 18%, 17% and 10% was observed for H-2′, H-5′ and H-4, respectively, which strongly supports the presence of a 4-(3-methoxy-4-hydroxyphenyl) substituent. Irradiation of the methyl protons (H-12) and the methine proton (H-3) yielded an NOE of 13% and 12% to H-4 and H-2, respectively, which is consistent with a *cis* relationship between H-2 and H-3, and a *trans* relationship between H-3 and H-4. In addition, the ^1H NMR spectrum reveals the presence of a pair of *para* protons (H-5, δ6.41, *s* and H-8, δ7.51, *s*; H-8 was assigned the lesser shielding because of the proximity of the carbonyl group) [5] and a methylenedioxy group (H-14A, δ5.99, *d*, $J_{14A,14B}$ = 1.22; H-14B, δ5.98, *d*, $J_{14A,14B}$ = 1.22), which is located at position 6 and 7.

The absolute configuration at the chiral centres was assigned in the following manner. Reduction of enshicine **2** with sodium borohydride in methanol followed by hydrogenolysis in the presence of palladium–charcoal led directly to desoxyenshicine **3**, which on demethylenation in acetic acid–sulfuric acid and phloroglucinol afforded desoxydemethylenechicanine, **4**. Since the absolute configuration of **3** has been established as 2*R*, 3*R*, 4*R*, [6] the absolute configuration of enshicine **2** should be 2*S*, 3*S*, 4*R*. Therefore, all the facts support the conclusion that enshicine possesses structure **2**, namely, 1-oxy-2*S*,3*S*-dimethyl-4*R*-(3-methoxy-4-hydroxyphenyl)-6,7-methylenedioxytetralin.

EXPERIMENTAL

General. The chemical formulas of all reported peaks in MS were determined by high resolution measurements. UV spectra were obtained in MeOH. NMR measurements were determined at 400 MHz on samples dissolved in $CDCl_3$, with TMS as int standard (chemical shift, δ, in ppm, coupling constant, *J* in Hz). NOE measurements were performed by the difference method [7]: four accumulations were collected with the decoupler on resonance for 10 sec and stored on disc, four accumulations were collected off-resonance and stored on disc, and the entire sequence repeated for improved signal-to-noise ratio. The two free induction decays were processed, then subtracted to yield only enhanced resonances plus the transition irradiated. Optical rotations were measured on an automatic polarimeter. ORD and CD curves were recorded at 26°. Mps are uncorr. Skellysolve B refers to petrol, bp 62–70°.

Enshicine (**2**). Powdered fruits of *S. henryi* Clarke collected in the district of Enshi, Hubei province of China, were extracted with C_6H_6 at room temp. The extract was dissolved in petrol (bp 60–90°), and then extracted with 80% MeOH. The MeOH extract was chromatographed over silica gel eluting with petrol, C_6H_6, C_6H_6–EtOAc (10:1), C_6H_6–EtOAc (4:1) and then EtOAc. The fractions of C_6H_6–EtOAc (10:1) were rechromatographed on a silica gel column and the middle fractions gave enshicine (yield 0.07%), which was crystallized as needles from MeOH–H_2O. Mp 146–147°, $[\alpha]_D^{23}$ −52.3° (*c* 0.13, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 210 (4.08), 236 (4.12), 279 (3.73), 320 (3.55). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540 (OH), 1670 (aromatic ketone). MS *m/z* (rel. int.): $C_{20}H_{20}O_5$ $[M]^+$, Calc.: 340.1311, Found: 340.1308 (100), $C_{19}H_{17}O_5$ $[M-Me]^+$ (14), $C_{16}H_{12}O_5$ $[M-C_4H_8]^+$ (78), $C_{16}H_{11}O_5$ $[M-C_4H_9]^+$ (5), $C_{15}H_9O_5$ $[M-C_4H_8-Me]^+$ (11), $C_{14}H_9O_4$ $[M-C_4H_8-Me-CO]^+$ (17), $C_{12}H_{12}O_3$ $[M-C_6H_4(OH)(OMe)\text{-}H]^+$ (44), $C_{12}H_9O_3$ (11). ORD (MeOH): $[\phi]_{330}$ = −10200 tr, $[\phi]_{297}$ = −9520, $a \times 10^{-2}$ = −6.80 $[\phi]_{281}$ = +26520 pk, $[\phi]_{246}$ = +23800 pk. CD (MeOH): $[\phi]_{320}$ = −408, $[\phi]_{290}$ = −1496, $[\phi]_{275}$ = +680, $[\phi]_{240}$ = +3060.

Desoxyenshicine (**3**). Enshicine (**2**, 28 mg) in MeOH (4 ml) was reduced with $NaBH_4$ (100 mg) at room temp overnight. The reaction mixture was diluted with H_2O and HCl, then extracted with $CHCl_3$. The extract in EtOH was hydrogenated in the presence of 5% Pd–C for 3 hr, affording desoxyenshicine **3** (18 mg), which was crystallized as needles from MeOH, mp 121–122°, $[\alpha]_D^{23}$ = −33.3° (*c* 0.09, MeOH). IR ν_{max}^{film} cm^{-1}: 3500 (OH), 1600, 1515, 1485, 1260, 1220, 1200. MS *m/z* (rel. int.): $C_{20}H_{22}O_4$ $[M]^+$, [Calc. 326.1518, Found 326.1512] (100), $C_{16}H_{14}O_4$ $[M-C_4H_8]^+$ (19), $C_{16}H_{13}O_4$ $[M-C_4H_9]^+$ (19), $C_{16}H_{13}O_3$ $[M-C_4H_8OH]^+$ (15), $C_{15}H_{11}O_3$ $[M-C_4H_8-OMe]^+$ (34), $C_{15}H_{10}O_3$ $[M-C_4H_9-OMe]^+$ (10), $C_{13}H_{14}O_2$ $[M-C_6H_4(OH)(OMe)\text{-}H]^+$ (13), $C_{12}H_{11}O_2$ (7). ^1H NMR ($CDCl_3$) δ_{TMS}: 0.89 (6H, *d*, $J_{2,Me}$ = 6.71, $J_{3,Me}$ = 6.71, C-2 Me and C-3 Me), 1.92 (1H, *ddq*, $J_{2,3}$ = 3.05, $J_{3,4}$ = 6.41,

$J_{3,Me}$ = 6.71, H-3), 2.03 (1H, *dddq*, $J_{1e,2}$ = 5.19, $J_{1a,2}$ = 7.32, $J_{2,3}$ = 3.05, $J_{2,Me}$ = 6.71, H-2), 2.44 (1H, *dd*, $J_{1a,2}$ = 7.32, $J_{1a,1e}$ = 16.48, H-1a), 2.87 (1H, *dd*, $J_{1e,2}$ = 5.19, $J_{1a,1e}$ = 16.48, H-1e), 3.60 (1H, *d*, $J_{3,4}$ = 6.41, H-4), 3.82 (3H, *s*, OMe), 5.46 (1H, *s*, OH), 5.85, 5.86 (1H each, *d*, $J_{14A,14B}$ = 1.52, $-OCH_2O-$), 6.31 (1H, *s*, H-5), 6.49 (1H, *dd*, $J_{5',6'}$ = 7.93, $J_{2',6'}$ = 1.83, H-6'), 6.53 (1H, *d*, $J_{2',6'}$ = 1.83, H-2'), 6.57 (1H, *s*, H-8), 6.79 (1H, *d*, $J_{5',6'}$ = 7.93, H-5'); ORD (MeOH): $[\phi]_{303} = +3622$ pk, $[\phi]_{285} = 24269$ tr, a $\times 10^{-2} = +279$. CD (MeOH): $[\phi]_{294} = +1014$, $[\phi]_{278} = -869$, $[\phi]_{247} = -181$.

Desoxydemethylenechicanine (**4**). Desoxyenshicine (**3**, 23 mg) in HOAc (3 ml) and 50% H_2SO_4 (2 ml) with phloroglucinol (100 mg) was heated at 100° under N_2 for 2 hr. Then the reaction mixture was diluted with H_2O (5 ml), and extracted with Et_2O. The extract was chromatographed on a silica gel column with Et_2O–Skellysolve B (2:1). The phenolic fractions, detected as green spots on TLC when sprayed with $FeCl_3$ afforded desoxydemethylenechicanine (**4**, 11 mg), which was crystallized as needles from CH_2Cl_2. This was identical (IR, ^{1}H NMR, MS, ORD, mmp) with an authentic sample [6]. CD (MeOH): $[\phi]_{294} = +1425$, $[\phi]_{297} = -1208$, $[\phi]_{240} = -966$.

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