Isolation of Symlandine from the Roots of Common Comfrey (*Symphytum officinale*) Using Countercurrent Chromatography

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Three pyrrolizidine alkaloids, symlandine, symphytine, and echimidine (**1**−**3**), were isolated from the roots of *Symphytum officinale* using a one-step countercurrent chromatography procedure. The structures of **1**−**3** were confirmed by several spectroscopic techniques including 2D NMR methods. This is the first description of the separation of symlandine (**1**) from its stereoisomer, symphytine (**2**).

The term comfrey has been applied to several species in the genus *Symphytum* (Boraginaceae), and this is used most often for *Symphytum officinale* L. (common comfrey), but may include *S. asperum* Lepech. (prickly comfrey) and *S. x uplandicum* Nyman (Russian comfrey, the hybrid of *S. officinale* and *S. asperum*).[1,2] Comfrey has been used in folk medicine in the form of an externally applied poultice to aid in the healing of wounds.[1] This practice has been extended to include internal consumption, via teas and capsules, to treat stomach ulcers[1] and other diseases of the digestive tract.[3] Pyrrolizidine alkaloids (PAs), which are suspected to be hepatotoxic,[2,3] have been isolated from several different species of *Symphytum*.[3−10] Of these, echimidine has been reported as being particularly hepatotoxic.[10]

As is often the case in the investigation of herbal drugs, reference standard compounds were unavailable from any commercial source. Thus, we have developed a preparatory scale, one-step, high-speed countercurrent chromatography (CCC) procedure to isolate PAs from an alkaloid extract of the roots of *S. officinale*; this plant part is reported to have much higher concentrations of PAs than the leaf.[2,9] Previously, symlandine (**1**) has been described only as a mixture with its stereoisomer, symphytine (**2**).[7,11] Herein, we report the use of CCC to separate symlandine (**1**) from symphytine (**2**) and also from another pyrrolizidine alkaloid, echimidine (**3**). (See Chart 1 for structures.) As this is the first description of the isolation of pure **1**, the complete physical and spectral data are included.

The crude alkaloid extract was prepared by acid/base partitioning of the methanol extract of the roots of *S. officinale*. TLC analysis with Dragendorff's spray of this crude alkaloid extract indicated the presence of several alkaloids, and CCC was used to isolate **1**−**3**. The purity of each isolate was confirmed by HPLC with t_R 42.9, 41.5, and 31.7 min for compounds **1**, **2**, and **3**, respectively.

The molecular ion peak at *m*/*z* 404 $[M + Na]^+$ in the ESMS of **1** was consistent with an elemental formula $C_{20}H_{31}NO_6$. In the ^{13}C NMR spectrum, carbon signals at δ_C 34.5 (C-6), 73.3 (C-7), and 75.8 (C-8) and two olefinic carbons at δ_C 133.0 (C-1) and 127.7 (C-2) represented the retronecine core (**4**) of a pyrrolizidine alkaloid.[12] Two olefinic proton signals at δ_H 5.83 and 6.12 were observed in the ^1H NMR spectrum, and one of them (δ_H 5.83) was correlated with the olefinic carbon signal at δ_C 127.7 of the retronecine skeleton from an HMQC experiment. The retronecine structure was confirmed by several HMBC correlations (Table 1). Two side chain groups, a viridifloryl and an angelyl group, esterified to this core were identified via NMR signals. A viridifloryl group was observed from the ^1H NMR signals at δ_H 4.00 (H-12), 1.23 (H-13), 2.17 (H-14), 0.87 (H-15), and 0.92 (H-16).[5−7] The ^1H NMR signals at δ_H 6.12 (H-19), 1.96 (H-20), and 1.81 (H-21) and ^{13}C NMR signals at δ_C 166.7 (C-17), 127.2 (C-18), 139.7 (C-19), 15.8 (C-20), and 20.5 (C-21) suggested the presence of an angelyl group.[12,13] The points of connection for the viridifloryl and angelyl groups to the retronecine core were identified via HMBC correlations (Table 1). The H-9 protons (δ_H 4.80 and 4.68) in the retronecine skeleton were long-range correlated with the carbonyl carbon of the viridifloryl group at δ_C 174.4 (C-10), and the H-7 proton of the retronecine skeleton was correlated with the α,β-unsaturated carbonyl carbon signal (δ_C 166.7, C-17) of the angelyl group. Thus, the structure of **1** was identified as symlandine.

The molecular ion peak at *m*/*z* 404 $[M + Na]^+$ in **2** was consistent with the elemental formula $C_{20}H_{31}NO_6$. In the ^1H and ^{13}C NMR spectra, a retronecine core with two esterified side chains was identified. Analogous to **1**, one of these side chains was a viridifloryl group that was esterified to C-9 of retronecine. The olefinic proton (H-19) was observed at δ_H 6.78, which is 0.66 ppm downfield of the analogous signal in **1**. This chemical shift difference indicated that the other side chain could be a tiglyl group for **2**, rather than an angelyl group as in **1**.[7] This observation was confirmed by a theoretical calculation of chemical shifts of the different olefinic protons.[15] According to the equation $\delta_{C=CH} = 5.25 + Z_{gem}\ (0.45) + Z_{cis}\ (0.46) + Z_{trans}\ (-0.22)$, the predicted chemical shift for H-19 in **1** was 5.94 ppm, and from the equation $\delta_{C=CH} = 5.25 + Z_{gem}\ (0.45) + Z_{cis}\ (1.01) + Z_{trans}\ (-0.24)$, the predicted chemical shift for H-19 in **2** was 6.43 ppm. Thus, the chemical shift of proton H-19 in the angelyl moiety (*Z* isomer) in **1** is predicted to be upfield by approximately 0.5 ppm relative to the position of H-19 in the corresponding tiglyl moiety (*E* isomer) in **2**. In addition to these chemical shift differences at position H-19, the ^1H NMR signals of H-20 (δ_H 1.79) and H-21 (δ_H 1.77) in the tiglyl group of **2** nearly overlapped, while signals for H-20 (δ_H 1.96) and H-21 (δ_H 1.81) were separated in the angelyl group in **1**. The carbon signal of C-21 (δ_C 11.9) in **2** appeared upfield of the corresponding signal in **1** (δ_C 20.5). The ^1H and ^{13}C NMR signals were in

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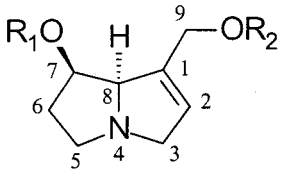
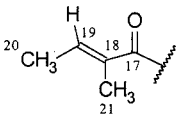
Chart 1



	R_1	R_2
Symlandine (**1**)	Angelyl	Viridifloryl
Symphytine (**2**)	Tiglyl	Viridifloryl
Echimidine (**3**)	Angelyl	Echimidinyl
Retronecine (**4**)	H	H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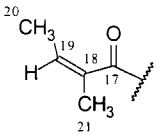
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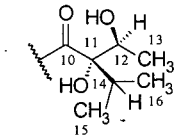
Viridifloryl

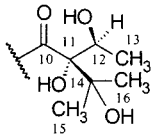
Echimidinyl

Table 1. NMR Data for Symlandine (**1**) and Symphytine (**2**) (125 and 500 HMz, $CDCl_3$)

	symlandine (**1**)			symphytine (**2**)	
position	δ_C[a]	δ_H mult. (*J* in Hz)	HMBC (H→C)	δ_C[a]	δ_H mult. (*J* in Hz)
1	133.0 s			133.0 s	
2	127.7 d	5.83 bs	1, 3, 8	127.2 d	5.85 bs
3	62.4 t	4.05 m, 3.41 m	1, 2	62.2 t	4.10 m, 3.44 m
5	53.8 t	3.48 m, 2.73 q (9.6)	3, 6, 7, 8	53.7 t	3.52 m, 2.77 q (9.6)
6	34.5 t	2.17 m	7, 8	34.3 t	2.16 m
7	73.3 d	5.47 m	5, 8, 17	73.3 d	5.44 m
8	75.8 d	4.53 bs	1, 2, 7	75.7 d	4.57 bs
9	62.2 t	4.80, 4.68 AB d (13.5)	1, 2, 8, 10	62.0 t	4.73 d (13.3)
10	174.4 s			174.3 s	
11	83.5 s			83.5 s	
12	70.8 d	4.00 q (6.6)	10, 11	70.8 d	3.97 q (6.6)
13	17.2 q	1.23 d (6.6)	11, 12	17.2 q	1.23 d (6.6)
14	32.2 d	2.17 m	11, 15, 16	32.2 d	2.16 m
15[b]	16.0 q	0.87 d (6.6)	11, 14, 16	15.9 q	0.86 d (6.9)
16[b]	17.7 q	0.92 d (6.6)	11, 14, 15	17.7 q	0.92 d (6.9)
17	166.7 s			166.8 s	
18	127.2 s			128.4 s	
19	139.7 d	6.12 q (8.7)	17, 18, 20, 21	138.1 d	6.78 q (8.7)
20	15.8 q	1.96 dd (7.2, 1.5)	18	14.4 q	1.79 d (3.0)
21	20.5 q	1.81 m	17, 19	11.9 q	1.77 s

[a] Multiplicity was determined from the APT ^{13}C NMR spectrum. [b] Signals may be interchangeable.

agreement with the chemical shifts for a tiglyl group.[7,12,14] Therefore, the structure of **2** was identified as symphytine.

The ion peak at *m/z* 420 $[M + Na]^+$ in **3** was consistent with the elemental formula $C_{20}H_{31}NO_7$. The NMR signals of the retronecine core were similar to those observed for **1** and **2**. The presence of the angelyl group in **3** was suggested by similarities in the NMR data between **3** and **1** and via the theoretical calculation of chemical shifts discussed above. The presence of the echimidinyl side chain was indicated by the hydroxyl moiety attached to the quaternary carbon, C-14 (δ_C 73.7). The ^1H and ^{13}C NMR signals for C-15 (δ_H 1.24 and δ_C 25.9) and C-16 (δ_H 1.31 and δ_C 24.8) were further downfield than those in the viridifloryl group as observed in **1** and **2**. Comparison of the spectroscopic data with those in the literature[7,10,13,16] enabled the identification of **3** as echimidine.

Experimental Section

General Experimental Procedures. HPLC was carried out using a Waters 600E controller attached to a MetaChem Inertsil ODS 3 (250 × 4.6 mm i.d., 5 μm) column and a MetaChem 0396-MG guard column. HP ChemStation for LC was used as a controlling system, and a HP-Series 1100 PDA detector was used to monitor the separation. The mobile phase was MeOH−H_2O (0.05% TFA) (15:85→45:55, gradient mixtures for 30 min), and the flow rate was 1 mL/min. IR spectra were measured using a Nicolet Avatar 360 FT-IR spectrometer, and UV spectra were recorded on a Varian Cary 3G spectrophotometer. NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 500 NMR spectrometer. Electrospray mass spectrometry (ESMS) was performed on a Finnigan LCQ mass spectrometer.

Plant Material. Air-dried ground roots of *Symphytum officinale* L. (Boraginaceae) were purchased from Richters (Ontario, Canada), in 1998. A voucher specimen (NCU No. 566522) has been deposited in the Herbarium of the University of North Carolina, Chapel Hill.

Extraction and Isolation. The air-dried ground roots of *S. officinale* (4 kg) were extracted with hot MeOH three times. The crude MeOH extract was concentrated under vacuum and made acidic to a 2.0 N solution with concentrated H_2SO_4 and stirred with an excess of Zn dust (20 g) to reduce *N*-oxides. The Zn dust was removed by filtering, and the acidic filtrate was washed with $CHCl_3$. The aqueous layer was made basic (pH 11) with NH_4OH and partitioned with $CHCl_3$. The organic layer was concentrated under vacuum to give the concentrated crude alkaloid fraction (4.6 g).

Countercurrent Chromatography. The concentrated alkaloid fraction was purified using a P.C. Inc. high-speed countercurrent chromatograph. A biphasic solvent system of 5:5:5:5 hexanes−EtOAc−MeOH−H_2O (containing 0.05% trifluoroacetic acid) was allowed to equilibrate overnight. The column was loaded with the upper phase. The system was brought up to speed (75 psi), and then the lower phase was pumped slowly through the column. Once the pressure had increased to 100 psi, the sample was injected, and the fractions were collected (5 mL each). Pure echimidine (**3**), symphytine

(**2**), and symlandine (**1**) were present in fractions 21, 22−24, and 27, respectively.

Symlandine (1): gum (6 mg); $[\alpha]_D$ +4.4° (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (4.01), 287 (2.88) nm; IR (film) ν_{max} 3580, 3521, 3013, 2976, 2936, 2876, 1719, 1454, 1381, 1234, 1154, 1078 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESMS (positive-ion mode) *m*/*z* 404 $[M + Na]^+$ (100), 382 $[M + 1]^+$ (68), 218 (50), 120 (11).

Symphytine (2): gum (16 mg); $[\alpha]_D$ −4.5° (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 216 (4.03) nm; IR (film) ν_{max} 3580, 3576, 3011, 2973, 2926, 1712, 1450, 1443, 1252, 1147, 1073 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESMS (positive-ion mode) *m*/*z* 404 $[M + Na]^+$ (100), 382 $[M + 1]^+$ (57), 218 (10), 120 (14).

Echimidine (3): gum (15 mg); $[\alpha]_D$ +6.6° (*c* 0.3, $CHCl_3$) [lit.[7] +11.5°]; UV (MeOH) λ_{max} (log ϵ) 217 (3.92), 287 (2.91) nm; IR (film) ν_{max} 3572, 3494, 3007, 2938, 2875, 1723, 1455, 1377, 1229, 1155, 1077 cm^{-1}; ^1H and ^{13}C NMR data are consistent with literature values;[10,13,16] ESMS (positive-ion mode) *m*/*z* 420 $[M + Na]^+$ (100), 398 $[M + 1]^+$ (13), 364 (7), 220 (2).

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