Wilforcidine, a New Alkaloid from Tripterygium wilfordii

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WILFORCIDINE, A NEW ALKALOID FROM *TRIPTERYGIUM WILFORDII*

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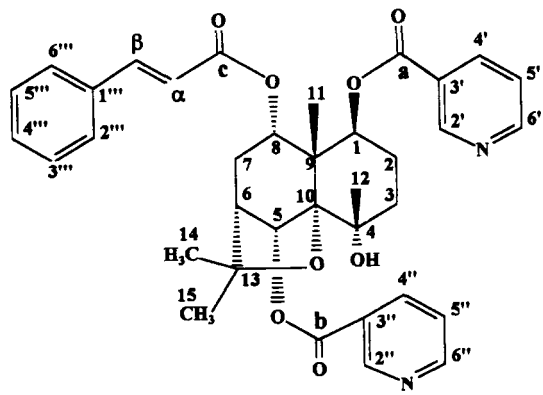
ABSTRACT.—A new dihydroagarofuran sesquiterpene alkaloid with a *trans*-cinnamoyloxy group at the C-8 position was isolated from *Tripterygium wilfordii*. Its structure was elucidated by spectral analysis, including 2D nmr DQF-COSY, HMBC, and NOESY spectra.

Tripterygium wilfordii belongs to the plant family Celastraceae. It is a perennial twining vine growing throughout the southern regions of the People's Republic of China. Crude extracts are widely used to treat cancer and immunological diseases in mainland China. In previous contributions, we have reported the chemical structures of sesquiterpene pyridine macrolide alkaloids (1–3). This paper reports the continued study of *Tripterygium wilfordii*, from which a new dihydroagarofuran sesquiterpene-type alkaloid with a *trans*-cinnamoyloxy group at the C-8 position, named wilforcidine [**1**], has been isolated.

Wilforcidine [**1**] gave a molecular formula, $C_{36}H_{38}N_2O_8$ ([M]$^+$ 626.2628, calcd 626.2628) by hrms and showed ir absorptions at 3400, 1728, 1720, 1580 and 1495 cm^{-1} for a hydroxy group, multicarbonyl esters and an aromatic ring, respectively. The uv spectrum exhibited absorption bonds at 222, 265, 270 and 281 (sh) nm. Fragment peaks at *m/z* 520 [M−C_5H_4N]$^+$, 495 [M−C_6H_5CH=CH-CO]$^+$, 131 [C_6H_5CH=CH-CO]$^+$, 124 [C_5H_4NCO]$^+$, 106 [C_5H_4N]$^+$, and 103 [C_6H_5CH=CH]$^+$ in the eims indicated the presence of cinnamoyl and nicotinoyl ester groups. One cinnamoyl and two nicotinoyl groups were indicated by the low-field signals of 15 protons from δ 6.25 to 9.40 in the ^{1}H-nmr spectrum. The signals of five protons from δ 7.40 to 7.46 were assignable to a cinnamoyl aromatic ring system. A trans- configuration for the cinnamoyl moiety was suggested



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by olefinic signals at δ 6.25 (d, J=16 Hz, α-H) and 7.35 (d, J=16 Hz, β-H). The remaining eight low-field proton signals were divided into two sets and were assigned to protons of two nicotinoyl groups on the basis of DQF-COSY and NOESY spectra. The 21 carbon signals from δ 117.6 to 165.6 in the ^{13}C-nmr spectrum of **1** are compatible with these partial structures and the assignments for each group shown in Table 1 were made using ^{1}H-^{13}C COSY and HMBC spectra. The remainder of the molecule consisted of $C_{15}H_{23}O_5$, for which the ^{1}H-nmr spectrum contained signals assignable to four tertiary methyl groups (δ 1.56, 1.55, 1.55, 1.39) and to protons on the carbon atoms carrying three secondary ester groups (δ 5.70, 5.70, 5.00). The ^{13}C nmr characteristics of the remaining partial

TABLE 1. ^{1}H and ^{13}C-Nmr Chemical Shifts (ppm) of Wilforcidine [**1**] and Protons to Which Long-Range Connectivity Was Observed in the HMBC Experiment.

Position	H (δ, m, J)[a]	C (δ)[b]	HMBC (^{1}H)
1	5.70 dd J=12.5/3.7	73.8	H-11
2	1.66 dddd J=12.5/12.5/12.5/3.7	23.7	
	2.06 dddd J=12.5/3.7/3.7/3.4		
3	1.81 ddd J=13.4/12.5/3.7	38.8	H-12
	2.03 ddd J=3.4/3.7/13.4		
4		70.7	H-12
5	5.70 br s $W_{h/2}$=3.5	81.0	H-6
6	2.38 br dd J=2.7/2.5	49.1	H-8, H-15
7	2.25 dd J=16.4/2.5	32.0	H-8
	2.57 ddd J=16.4/6.9/2.7		
8	5.00 d J=6.9	72.7	H-11
9		51.7	H-11
10		91.6	H-6, H-11, H-12
11	1.56 s	19.9	H-8
12	1.39 s	23.9	
13		84.6	H-14, H-15
14	1.55 s	25.7	
15	1.55 s	29.8	
a		164.2	H-4′
2′	8.99 br s	152.7	H-4′
3′		126.5	H-2′
4′	8.06 br d J=7.9	137.3	H-2′, H-6′
5′	7.30 dd J=7.9/5.0	123.7	H-4′
6′	8.72 br d J=5.0	150.0	H-4′
b		164.7	H-4″
2″	9.40 br s	153.3	H-4″
3″		126.0	H-2″
4″	8.55 br d J=7.8	138.0	H-2″, H-6″
5″	7.46	123.3	H-4″
6″	8.83 br d J=5.0	151.2	H-4″
c		165.6	H-8, H-β
α	6.25 d J=16.0	117.6	
β	7.35 d J=16.0	145.6	
1‴		134.2	H-α
3‴	7.40 dd J=1.4/5.4	128.2	
(5‴)			
2‴	7.46 (overlap)	128.9	H-β
(6‴)			
4‴		130.5	

[a] J values expressed in Hz.
[b] All protonated carbon nuclei were assigned by a ^{1}H-^{13}C COSY nmr experiment.

structure exhibited signals for three carbons carrying secondary esters (δ 73.8, 81.0, 72.7), four tertiary methyl groups (δ 19.9, 23.9, 25.7, 29.8), four quaternary carbons (δ 70.7, 84.6, 91.6, 51.7) and three methylenes (δ 23.7, 32.0, 38.8). These data suggested a tri-substituted dihydroagarofuran skeleton. The positions of the three secondary ester groups were established by a detailed interpretation of the COSY and HMBC nmr spectra. Observation of HMBC connectivities between the H-11 methyl proton signal and the C-1 (δ 73.8) and C-8 (δ 72.7) carbon signals indicated that the two ester groups should be at the C-1 and C-8 positions. The third ester was determined to be at the C-5 position using a DQF-COSY nmr spectrum. Placement of the cinnamoyl group at the C-8 position was deduced by the HMBC connectivity between H-8 (δ 5.00) and the carbonyl carbon (δ 165.6), which further showed HMBC connectivity with an olefinic proton (δ 7.35, β-H). Therefore, the two nicotinoyl groups should be located at the C-1 and C-5 positions.

The relative stereochemistry of the molecule was resolved by analysis of the NOESY spectrum and the *J*-coupling pattern. The nOe observations between CH_3-12 and H-5; CH_3-11 and H-7(β); CH_3-11 and H-5; CH_3-12 and CH_3-11 indicated that they were all β-axial, and that the nicotinoyloxy group at C-5 should be α-equatorial. The α-axial orientation of the cinnamoyloxy group at C-8 was deduced from the nOe cross-peak between H-8, CH_3-11 and H-7(β) in the NOESY spectrum. In addition, the coupling pattern (dd, $J=12.5$, $J=3.7$ Hz) of H-1 indicated that the nicotinoyloxy function at C-1 should be β-equatorial.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp was determined on a Kofler apparatus. ^{1}H-nmr and ^{13}C-nmr spectra were recorded on a Bruker AMX-600 nmr spectrometer with TMS as internal standard and $CDCl_3$ as solvent. A ^{1}H-^{13}C-COSY nmr experiment was run on a JEOL-400 instrument. Uv spectra in MeOH were obtained on a Unican SP-1800 spectrophotometer. Ms were taken on a Varian MAT-711 mass spectrometer. Ir spectra were determined on a Perkin-Elmer 599B instrument.

EXTRACTION AND ISOLATION.—The Et_2O-soluble total alkaloids were dissolved in 2% HCl. The acidic aqueous phase was shaken with $CHCl_3$. The combined $CHCl_3$ extracts were then concentrated to afford an alkaloidal mixture. The alkaloidal mixture (39.57 g) was repeatedly chromatographed over Si gel eluted with $CHCl_3$-Me_2CO (4:1) and (6:1), respectively. Subsequently, the fractions containing wilforcidine [**1**] were subjected to reversed-phase chromatography on Fuji-gel, ODS Q_3 with MeOH-H_2O (20:5), flow rate 2 ml/min, monitored by hplc (column, Waters C_{18}; solvent, CH_3CN-H_2O [39:48]; uv 296 nm; flow rate, 1 ml/min; R_t 9.8 min) to obtain wilforcidine. Recrystallization from EtOH afforded pure wilforcidine (**1**, 12 mg).

Wilforcidine [**1**].—Colorless needles (from EtOH); mp 189°, $[\alpha]^{25}D$ +17.4° (c=0.5, $CHCl_3$); uv λ max (log ε) 281 sh (3.34), 270 (3.41), 265 (3.40), 222 (3.67) nm; ir ν max (KBr) 3530, 3400, 2945, 1728, 1725, 1720, 1638, 1590, 1578, 1560, 1495, 1420, 1332, 1302, 1285, 1265, 1240, 1200, 1165, 1140, 1125, 1095, 1028, 980, 975, 950, 900, 770, 742, 705 cm^{-1}; hrms *m/z* 626.2628 ($[M]^+$, $C_{36}H_{38}N_2O_8$, calcd 626.2628); eims *m/z* 626 $[M]^+$ 520, 495, 464, 463, 462, 436, 285, 235, 232, 231, 229, 216, 211, 191, 185, 181, 132, 131, 125, 124, 106, 105, 103; ^{1}H nmr and ^{13}C nmr, see Table 1.

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