Revision of the Structure of Escholidine

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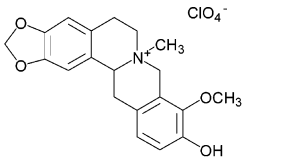
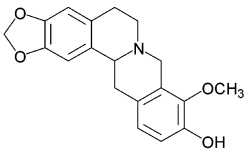
The structure of the quaternary tetrahydroprotoberberine alkaloid escholidine is revised on the basis of 2D NMR spectroscopy and X-ray diffraction. In contrast to the originally reported constitution, escholidine bears an −OH group at C-9 and an $-OCH_3$ group at C-10. The ^1H and ^{13}C NMR data and long-range $^1H-^{13}C$ and NOE interactions of escholidine are compared with those of thalifendine and tetrahydroberberrubine. The ^{15}N NMR data of escholidine obtained by using long-range $^1H-^{15}N$ correlation experiments at natural abundance are also reported.

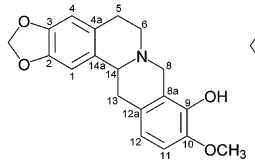
The protoberberines represent a widely distributed class of secondary metabolites belonging to the large group of alkaloids constitutionally derived from isoquinoline.[1] The structure of the alkaloid escholidine,[2] isolated as the principal quaternary base from the roots of *Eschscholtzia californica* CHAM., *E. douglasii* (HOOK. et ARN.) WALP., and *E. glauca* GREENE and from the aerial parts of *Hunnemannia fumariaefolia* SWEET, was originally determined as **1**. This structure was assigned on the basis of the similarity of its IR and MS data to those of the alkaloid tetrahydrothalifendine (**2**) rather than to its regioisomer, tetrahydroberberrubine (**3**).[2]



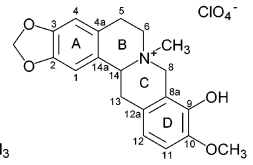
Escholidine perchlorate (**1**, original structure)



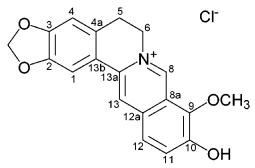
Tetrahydrothalifendine (**2**)



Tetrahydroberberrubine (**3**)



Escholidine perchlorate (**4**, revised structure)



Thalifendine chloride (**5**)

During our systematic NMR study of isoquinoline alkaloids,[3−8] we measured the ^1H and ^{13}C NMR spectra of the alkaloid escholidine. The individual ^1H and ^{13}C resonances were assigned by using gradient-selected (gs-) COSY,[9] NOESY,[10] and $^1H-^{13}C$ gs-HMBC[11] experiments. The NMR data of tetrahydroberberrubine (**3**), escholidine perchlorate (**4**, revised structure), and thalifendine chloride (**5**) are summarized in Table 1.

Table 1. ^1H and ^{13}C NMR Chemical Shifts (δ in ppm) of Alkaloids **3**−**5** in DMSO-d_6 at 303 K

	3		**4**		**5**	
atom	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	6.90	105.59	6.93	106.70	7.76	105.18
2		145.57		146.17		147.53
3		145.26		147.47		149.40
4	6.66	107.92	6.87	108.83	7.07	108.30
4a		127.42		122.53		130.17
5	2.60, 2.91	28.94	3.17	23.02	3.19	26.33
6	2.43, 3.07	50.71	3.61, 3.70	51.77	4.89	54.93
8	3.27, 4.01	53.47	4.62, 4.74	58.50	9.69	143.55
8a		122.08		113.84		122.09
9		141.82		142.52		141.11
10		144.77		145.79		150.88[a]
11	6.78	110.00	6.98	112.05	7.84	123.34
12	6.57	118.37	6.69	118.40	7.84	123.42
12a		127.48		121.75		131.58
13	2.53, 3.27	36.81	3.10, 3.35	32.63	8.83	120.17
14/13a	3.37	59.00	4.74	64.55		136.01
14a/13b		130.92		124.85		120.62
$-OCH_2O-$	5.94	100.38	6.01, 6.03	101.28	6.16	101.89
$-OCH_3$	3.76	55.87	3.80	56.03	4.06	60.96
$-NCH_3$			3.20	49.69		
−OH	8.57		9.38		*b*	

[a] Chemical shift from $^1H-^{13}C$ gs-HMBC spectrum (interaction with ^1H signal at 7.84 ppm). [b] No data obtained.

The ^1H NMR resonance at $\delta = 3.80$ ppm (3H) was assigned to the protons of the $-OCH_3$ group. The doublets of the aromatic H-11 ($\delta = 6.98$ ppm) and H-12 ($\delta = 6.69$) were readily distinguished from the singlets of the H-1 and H-4 atoms. Mutual interaction of the protons of the $-OCH_3$ group and H-11 was obtained in a gs-COSY experiment. Similarly, the interaction of the $-OCH_3$ protons with C-11 was detected in an $^1H-^{13}C$ gs-HMBC experiment adjusted for a long-range coupling of 7.5 Hz (Supporting Information).

These interactions indicated that the C-9, C-10 substitution pattern of escholidine (**4**) could be the reverse of that originally published. If the $-OCH_3$ group were located at C-9, long-range $^1H-^{13}C$ interactions of $-OCH_3$ with C-9 and of H-8 and H-13 with C-9 should be observed (compare with thalifendine, vide infra). A NOESY experiment with a mixing time of 800 ms was used to confirm the constitution of escholidine (Supporting Information). The cross-peak obtained between $-OCH_3$ and H-11 unequivocally

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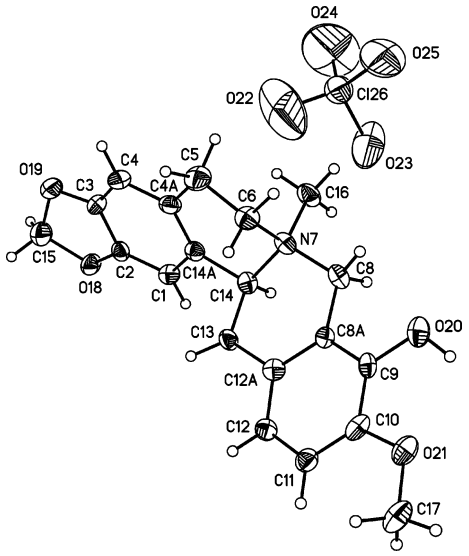

Figure 1. Perspective view of the X-ray structure of compound **4**. Thermal ellipsoids are plotted at the 50% probability level. Hydrogen atoms are shown as small spheres of arbitrary size.

confirmed their spatial proximity and thus the C-9-OH, C-10-OCH_3 substitution pattern of escholidine.

Crystallization of escholidine perchlorate from MeOH afforded crystals suitable for single-crystal X-ray diffraction. The details of data collection and structure refinement[12] are summarized in the Experimental Section, and the molecular structure is shown in Figure 1. X-ray analysis confirmed the constitution of the molecule based on the NMR measurements and unambiguously confirmed the C-9-OH, C-10-OCH_3 substitution pattern. The relative orientation of the *N*-CH_3 group and H-14 is *cis* with a dihedral angle H14−C14−N7−C16 of 51.2°. The aromatic rings A and D are twisted to form a dihedral angle of 60.2(1)°. The hydroxy group at C-9 and the methoxy group at C-10 are located almost in the plane of aromatic ring D. The hydroxy group is connected to the oxygen atom of the perchlorate anion via a hydrogen bond with a donor−acceptor (O···O) distance of 305.7(8) pm.

In the original article,[2] the structure of escholidine was determined by comparing the spectroscopic data with those of tetrahydrothalifendine (**2**) and tetrahydroberberrubine (**3**), both substituted at positions C-9 and C-10. Therefore, we reinvestigated the structures of tetrahydroberberrubine (**3**) and thalifendine (**5**) in order to confirm their substitution patterns at positions C-9 and C-10. 2D NMR spectroscopy was used for this purpose.

Tetrahydroberberrubine (**3**) was prepared by heating berberine with urea according to the procedure published previously (see Experimental Section).[2] Thalifendine (**5**) is a quaternary berberine type of alkaloid that possesses a substitution pattern identical with tetrahydrothalifendine (**2**) because **2** is formed from **5** upon reduction with Adam's catalyst.[13] The constitution of the structurally related alkaloid 8-oxotetrahydrothalifendine has recently been characterized by single-crystal X-ray diffraction.[14] The ^1H and ^{13}C NMR resonances of tetrahydroberberrubine (**3**) and thalifendine (**5**) were assigned by using $^1H-^{13}C$ g-HMBC experiments (see Table 1).

In the case of thalifendine chloride (**5**), long-range $^1H-^{13}C$ interactions of H-8 with C-8a (122.09 ppm), C-9 (141.11), C-12a (131.58), and C-13a (136.01) were clearly detected in a gs-HMBC experiment (J_{HC} adjusted for 7.5 Hz). The interactions of H-11 with C-9 and H-13 with C-9 were also observed. Finally, the interaction of C-9 with the protons of the $-OCH_3$ group proved the C-9 position of the methoxy group in **5** (and consequently **2**) unequivocally.

$^1H-^{13}C$ HMBC of tetrahydroberberrubine (**3**) identified the interaction of H-8 with C-9 (141.82 ppm). However, the protons of the $-OCH_3$ group are coupled with another carbon (144.77 ppm), suggesting the C-10 position for the methoxy group. The constitution of **3** was unambiguously confirmed by a NOESY experiment (mixing time 800 ms) showing interaction between H-11 and $-OCH_3$ (Supporting Information). The observed NMR interactions clearly confirmed the C-9, C-10 substitution patterns of the alkaloids tetrahydroberberrubine (**3**) and thalifendine (**5**).

The ^{15}N NMR chemical shifts were determined by using a $^1H-^{15}N$ GSQMBC experiment. Interaction of the hydrogen atoms of the *N*-CH_3 group with N-7 (δ = 52.7 ppm) was observed for escholidine perchlorate (**4**). However, the intensity of the corresponding cross-peak was relatively weak, and the value of $^2J_{HN}$, ~3.5 Hz, was extracted from the antiphase splitting. Interactions with other hydrogen atoms at 3.17 ppm (H-5, cross-peak observed in the GSQMBC with $^nJ_{HN}$ = 3.6 Hz) and 4.74 ppm (H-8 or H-14, in the GSQMBC with $^nJ_{HN}$ = 8 Hz) resulted in very weak signals. For comparison, the ^{15}N chemical shift of the related *N*-methyltetrahydroprotoberberine alkaloid cyclanoline iodide is 52.6 ppm (DMSO-d_6).[5]

In conclusion, escholidine (**4**) is C-9-OH, C-10-OCH_3 substituted quaternary *N*-methyltetrahydroprotoberberine, and tetrahydroberberrubine (**3**) is its *N*-demethyl analogue.

Experimental Section

General Experimental Procedures. Melting points were determined on a Nagema PHMK 05 apparatus. Infrared spectra were measured on a Nicolet Impact 410 spectrometer. NMR spectra were recorded using a Bruker Avance DRX 500 spectrometer operating at frequencies of 500.13 MHz (^1H) and 125.77 MHz (^{13}C), a Bruker Avance 300 spectrometer operating at frequencies of 300.13 MHz (^1H), 75.48 MHz (^{13}C), and 30.41 MHz (^{15}N), and a Bruker Avance 600 spectrometer operating at frequencies of 600.15 MHz (^1H) and 150.92 MHz (^{13}C). All NMR spectra were measured at 303 K. The ^1H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the signal of the solvent [2.50 (^1H) and 39.43 (^{13}C) for DMSO-d_6]. The 2D NMR experiments $^1H-^{13}C$ gs-HMBC, COSY, and NOESY were used to assign the individual ^{13}C and ^1H resonances.[9−11] The homospoil gradient in NOESY was applied during a mixing time of 800 ms, and the spectra were processed using the STATES-TPPI protocol.[15] The gradient ratio for the $^1H-^{13}C$ HMBC experiment was 30:18:24 G cm^{-1}, and the experiment was adjusted for long-range couplings of 3.6 and 7.5 Hz. The ^{15}N NMR chemical shifts were referenced[16] relative to 1 M urea in DMSO-d_6 (77.0 ppm). The $^1H-^{15}N$ GSQMBC[17] was adjusted for couplings of 3.6 and 8 Hz with settings published previously.[3,5]

The diffraction data were collected on a KM4CCD four-circle area-detector diffractometer (Oxford Diffraction, UK) equipped with an Oxford Cryostream Cooler (Oxford Cryosystems, UK). We performed the ω-scan technique with different κ and φ offsets in order to cover the entire independent part of the reflection set up to 25° θ. The crystallographic package ShelXTL[18] was used to solve and refine the structure and to prepare the figure.

Tetrahydroberberrubine (3).[2] Berberine chloride (1 g) and urea (2.5 g) were heated at 205 °C for 30 min, the red melt was dissolved in aqueous AcOH, and the hot solution was reduced with Zn and HCl until colorless. The filtrate was made strongly alkaline with aqueous NaOH, and the residual nonphenolic bases were removed by extraction with Et_2O. The aqueous layer was acidified with AcOH, made alkaline with aqueous NH_3, and extracted with Et_2O, and the extract was evaporated. The residual solid was recrystallized twice from MeOH to afford 0.19 g of **3**: mp 180−181 °C; IR ν_{max} 3520 cm^{-1} (OH); for the ^1H and ^{13}C NMR data, see Table 1.

Escholidine perchlorate (4). Escholidine was isolated as the perchlorate from the roots of *E. glauca* GREENE:[2] mp 281−282 °C (lit. 281−283 °C);[19] for the ^1H and ^{13}C NMR data, see Table 1; ^{15}N NMR (DMSO-d_6) δ 52.7 ppm (N-7).

Crystal Data for 4. CCDC ref no. 298724. Crystallized from MeOH, $C_{20}H_{21}ClNO_8$, M_{rel} = 438.83, T = 120(2) K, orthorhombic, λ = 0.71073 Å, space group $P2_12_12_1$, a = 6.649(1) Å, b = 12.092(2) Å, c = 24.058(5) Å, V = 1934.3(6) Å^3, Z = 4, D_{calc} = 1.507 Mg/m^3, crystal size 0.45 × 0.40 × 0.40 mm, θ range for data collection 3.28−25.00°, completeness to 25.00° 99.7%, refinement full-matrix least-squares on

F^2, data/parameters = 3395/272, hydrogens treated as riding, slight disorder around the perchlorate anion unresolved (instead absorbed in its higher thermal motion), $R = 0.0549/0.0690$ ($I > 2\sigma(I)$/all data), $\rho_{max}/\rho_{min} = 0.769/-0.491$ e·Å^{-3}.

Thalifendine Chloride (5). The sample of thalifendine chloride (~1 mg), isolated from *Thalictrum javanicum* Bl.,[20] was kindly donated by Professor Paul L. Schiff, Jr., Department of Pharmaceutical Sciences, The School of Pharmacy, University of Pittsburgh. The NMR sample was prepared by dissolving thalifendine chloride in 260 μL of DMSO-d_6, and the solution was injected into a 5 mm Shigemi tube. For the ^{1}H and ^{13}C NMR data, see Table 1.

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Supporting Information Available: ^{1}H−^{1}H NOESY (mixing time 800 ms) and ^{1}H−^{13}C gs-HMBC spectrum of escholidine perchlorate (**4**) and ^{1}H−^{1}H NOESY of tetrahydroberberrubine (**3**) in DMSO-d_6 at 303 K. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center (CCDC). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(0)1223-336033 or e-mail deposit@ccdc.cam.ac.uk).
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