Palmatine and Berberine Isolation Artifacts†

Radek Marek,*,‡ Pavlína Sečkářová,‡ Dagmar Hulová,‡ Jaromír Marek,§ Jiří Dostál,⊥ and Vladimír Sklenář‡

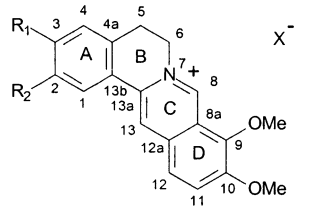
National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic, Laboratory of Functional Genomics and Proteomics, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic, and Department of Biochemistry, Faculty of Medicine, Masaryk University, Komenského náměstí 2, CZ-662 43 Brno, Czech Republic

Received November 4, 2002

Some 8-substituted derivatives of the protoberberine alkaloids palmatine (**1a**) and berberine (**1b**) have been prepared and investigated by 1D and 2D NMR spectroscopy (H-1, C-13, N-15). Complete NMR data for the 8-hydroxy (**2**), 8-methoxy (**3**), 8-ethoxy (**4**), and 8-trichloromethyl (**5**) 7,8-dihydro derivatives of **1a** and **1b**, as well as X-ray data for 8-methoxydihydroberberine (**3b**), 8-trichloromethyldihydropalmatine (**5a**), and 8-trichloromethyldihydroberberine (**5b**), are presented. The physicochemical data for all of these compounds are reviewed and compared with previously published values.

Palmatine (**1a**) and berberine (**1b**) are quaternary protoberberine alkaloids. Their salts (**1**) are typically yellow and are widely distributed in many species of the Berberidaceae, Fumariaceae, Papaveraceae, and other plant families.[1,2] Berberine displays a great variety of biological and pharmacological activities (e.g., antimicrobial,[3] antiplasmodial,[4] antidiarrheal,[5] cardiovascular[6]). Both alkaloids contribute to the chemical defense of plants by complex actions with several molecular targets.[7]

The quaternary protoberberines **1** are iminium cations derived from the 5,6-dihydrodibenzo[*a*,*g*]quinolizinium system. They are characterized by the sensitivity of the polar $C{=}N^+$ bond to nucleophilic attack followed by the formation of the corresponding 8-adduct.[8,9] A number of adducts with, for example, cyanide,[10,11] methoxide,[12] alkaline chloroform,[13−15] and liquid ammonia[16] have been described. Palmatine (**1a**) and berberine (**1b**) are frequently converted into their free bases (**2**), called pseudobases, during the isolation and purification process. In the presence of common solvents such as MeOH, EtOH, and $CHCl_3$, the free bases **2** easily produce adducts **3**, **4**, and **5**. In general, since the pseudobases **2** do not occur in plants, compounds **3**−**5** must be considered to be isolation artifacts. In this contribution we present complete ^1H and ^{13}C NMR data for these free bases **2**, and for the artifacts **3**−**5**, and compare them with the previously published values.



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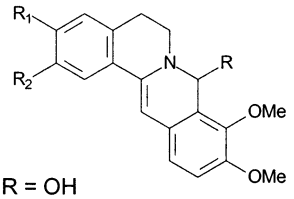


2: R = OH
3: R = OMe
4: R = OEt
5: R = CCl_3

a: palmatine $R_1 = R_2 = OMe$
b: berberine $R_1 + R_2 = OCH_2O$

Results and Discussion

The free bases of palmatine (**2a**) and berberine (**2b**) (the 8-hydroxy-7,8-dihydro derivatives), prepared by alkalizing the quaternary alkaloids (**1**) with sodium hydroxide (see Experimental Section), were rather unstable. Over time (∼12 h), **2a** and **2b** spontaneously decomposed and formed several disproportionation products (probably 7,8-dihydro and 8-oxo-7,8-dihydro derivatives). The 8-hydroxy adducts **2a** and **2b** are less stable than the analogous hydroxy derivatives of the benzophenanthridine alkaloids.[17] One of the reasons for this lower stability may be the higher p*K* values of protoberberines. For comparison, the p*K* values of benzophenanthridines are roughly 7.5−9.0 (in H_2O[18]), the p*K* of berberine is 15.4, and the p*K* of palmatine is 15.7 (in MeOH[19]). Despite this lower stability, we managed to determine the chemical shifts of both **2a** and **2b** in CD_2Cl_2 and C_6D_6 (Tables 1−4). $CDCl_3$ was not used for measurement since preparing the pseudobases **2a** and **2b** by alkalizing the alkaloids with NaOH in $CHCl_3$ solvent could lead to the formation of dichlorocarbene, followed by its subsequent reaction with the alkaloid skeleton.

We also detected in the CD_2Cl_2 solution of berberine free base **2b** minor amounts of bimolecular species, which are well known to exist in solutions of benzophenanthridine alkaloids.[20−22] Investigation of the formation and structure relations of these minor components is currently in progress.

The 8-methoxy adduct of palmatine (**3a**) and 8-methoxy (**3b**) and 8-ethoxy (**4b**) adducts of berberine were prepared by reacting the respective quaternary alkaloid with a solution of sodium methoxide or sodium ethoxide in an appropriate alcohol. The NMR samples of methoxy adducts **3a** and **3b** dissolved in $CDCl_3$ were rather unstable. In the presence of residual H_2O these compounds reacted with $CDCl_3$ to form the 8-trichloromethyl adducts **5a** and **5b**, respectively. The reaction mechanism expected for this conversion is shown in Scheme 1. Protonation of the

* To whom correspondence should be addressed. Tel: +420-541129634. Fax: +420-541211214. E-mail: rmarek@chemi.muni.cz.
† Dedicated to Professor Jiří Slavík on the occasion of his 80th birthday.
‡ National Center for Biomolecular Research.
§ Laboratory of Functional Genomics and Proteomics.
⊥ Department of Biochemistry.

10.1021/np0204996 CCC: $25.00 © 2003 American Chemical Society and American Society of Pharmacognosy
Published on Web 03/21/2003

Table 1. ^{1}H NMR Chemical Shifts (δ) of Some 8-Substituted Derivatives of Dihydropalmatine in CD_2Cl_2, C_6D_6, and $CDCl_3$ at 303 K

	2a	**3a**	**5a**	**2a**	**3a**	**5a**	**3a**	**5a**
H atom	CD_2Cl_2	CD_2Cl_2	CD_2Cl_2	C_6D_6	C_6D_6	C_6D_6	$CDCl_3$	$CDCl_3$
1	7.19	7.20	7.18	7.29	7.28	7.28	7.21	7.18
4	6.66	6.67	6.66	6.34	6.36	6.35	6.65	6.63
5	2.89 m	2.91 m	2.74 m	2.45 m	2.47 m	2.34 m	2.91 m	2.74 m
	2.93 m	2.91 m	3.39 m	2.67 m	2.65 m	3.14 m	2.95 m	3.41 m
6	3.54 m	3.54 m	3.72 m	3.19 m	3.24 m	3.39 m	3.56 m	3.74 m
	3.75 m	3.64 m	3.84 m	3.71 m	3.60 m	3.59 m	3.71 m	3.86 m
8	6.20	6.12	5.64	6.45	6.56	5.92	6.22	5.64
11	6.95 d	6.97 d	6.99 d	6.75 d	6.78 d	6.73 d	6.96 d	6.98 d
12	6.92 d	6.90 d	6.88 d	6.96 d	6.97 d	6.89 d	6.92 d	6.89 d
13	6.15	6.07	6.17	6.34	6.28	6.38	6.06	6.15
2(OCH_3)	3.88	3.88	3.87	3.51	3.47	3.46	3.94	3.93
3(OCH_3)	3.84	3.85	3.84	3.42	3.43	3.43	3.90	3.88
8(OCH_3)		3.05			3.13		3.06	
9(OCH_3)	3.92	3.89	3.92	3.98	4.01	3.88	3.94	3.95
10(OCH_3)	3.86	3.87	3.86	3.45	3.45	3.38	3.89	3.89

Table 2. ^{13}C NMR Chemical Shifts (δ) of Some 8-Substituted Derivatives of Dihydropalmatine in CD_2Cl_2, C_6D_6, and $CDCl_3$ at 303 K

	2a	**3a**	**5a**	**2a**	**3a**	**5a**	**3a**	**5a**
C atom	CD_2Cl_2	CD_2Cl_2	CD_2Cl_2	C_6D_6	C_6D_6	C_6D_6	$CDCl_3$	$CDCl_3$
1	108.05	108.18	107.75	109.06	109.09	108.63	107.58	107.12
2	148.52	148.54	148.49	149.18	149.26	149.15	147.88	147.85
3	149.85	149.83	149.96	150.40	150.40	150.48	149.12	149.23
4	111.30	111.33	111.73	111.73	111.77	112.09	110.60	111.00
4a	128.38	128.28	127.89	128.40	128.15	127.65	127.70	127.34
5	30.25	30.21	30.28	30.02	30.07	30.15	29.87	29.98
6	47.07	47.66	52.21	46.91	47.23	51.93	47.10	51.72
8	79.49	85.42	74.02	79.67	85.10	74.45	84.83	73.63
8a	122.63	119.53	115.17	123.48	119.82	115.40	118.83	114.86
9	145.48	146.25	147.01	146.26	147.16	147.29	145.87	146.48
10	150.13	150.02	150.12	150.27	150.24	150.03	149.59	149.63
11	114.36	114.46	115.17	114.64	114.89	115.61	114.13	114.70
12	119.68	119.16	118.70	119.54	119.02	118.72	119.00	118.48
12a	127.11	129.09	129.98	127.48	130.13	130.46	128.73	129.51
13	94.58	94.30	97.41	94.83	94.57	97.96	94.01	97.01
13a	136.66	137.97	137.97	136.99	138.53	138.18	137.62	137.56
13b	124.28	124.09	123.84	124.79	124.46	124.23	123.76	123.58
2(OCH_3)	56.44	56.43	56.46	55.94	55.85	55.86	56.07	56.11
3(OCH_3)	56.21	56.22	56.25	55.54	55.59	55.58	55.92	55.93
8(OCH_3)		52.47			51.07		51.80	
8(CCl_3)			106.22			106.51		105.60
9(OCH_3)	61.45	61.13	61.18	61.03	60.81	60.69	60.99	60.95
10(OCH_3)	56.56	56.54	56.70	56.07	56.04	56.18	56.26	56.38

Table 3. ^{1}H NMR Chemical Shifts (δ) of Some 8-Substituted Derivatives of Dihydroberberine in CD_2Cl_2, C_6D_6, and $CDCl_3$ at 303 K

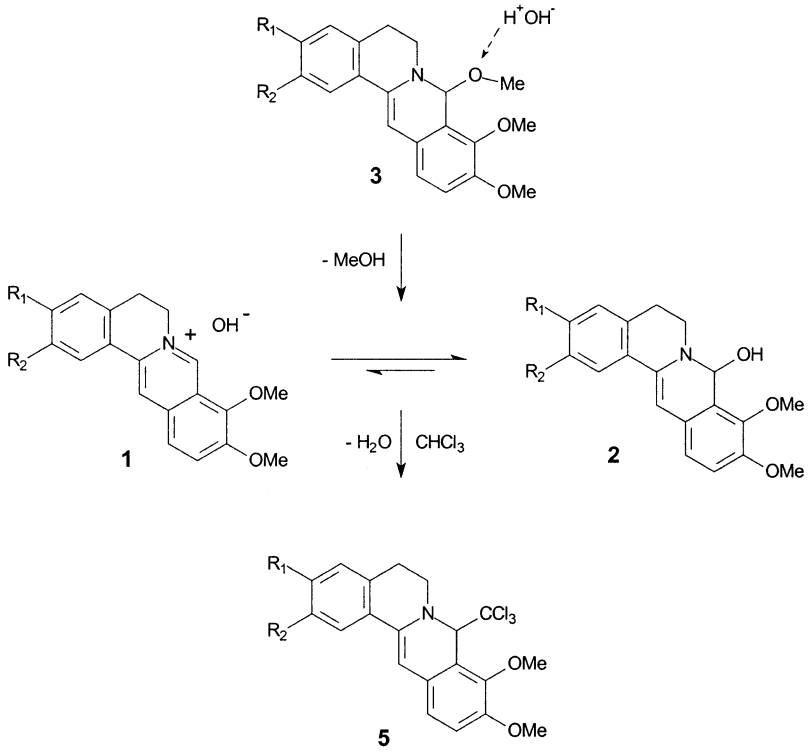
	2b	**3b**	**4b**	**5b**	**2b**	**3b**	**3b**	**5b**
H atom	CD_2Cl_2	CD_2Cl_2	CD_2Cl_2	CD_2Cl_2	C_6D_6	C_6D_6	$CDCl_3$	$CDCl_3$
1	7.15	7.17	7.17	7.16	7.23	7.22	7.19	7.16
4	6.62	6.64	6.63	6.63	6.36	6.37	6.63	6.61
5	2.86 m	2.88 m	2.87 m	2.72 m	2.28 m	2.31 m	2.91 m	2.72 m
	2.89 m	2.88 m	2.87 m	3.32 m	2.50 m	2.48 m	2.91 m	3.34 m
6	3.51 m	3.52 m	3.51 m	3.68 m	3.05 m	3.09 m	3.54 m	3.70 m
	3.72 m	3.64 m	3.60 m	3.86 m	3.57 m	3.44 m	3.70 m	3.87 m
8	6.18	6.11	6.13	5.65	6.36	6.48	6.22	5.64
11	6.94 d	6.96 d	6.95 d	6.99 d	6.73 d	6.76 d	6.92 d	6.97 d
12	6.90 d	6.89 d	6.86 d	6.86 d	6.87 d	6.87 d	6.97 d	6.86 d
13	6.08	6.01	6.00	6.10	6.11	6.05	6.03	6.10
2(OCH_2O)	5.93 d	5.94d	5.94 d	5.93 d	5.34 d	5.32 d	5.94 d	5.94 d
	5.95 d	5.96 d	5.95 d	5.94 d	5.35 d	5.34 d	5.96 d	5.95 d
8(OCH_3)		3.03				3.03	3.06	
9(OCH_3)	3.91	3.88	3.88	3.91	3.95	3.98	3.95	3.94
10(OCH_3)	3.85	3.86	3.85	3.86	3.44	3.44	3.89	3.87
8(OCH_2−)			3.28					
8(−CH_3)			1.00					

methoxy group is probably the first reaction step. We assume that following the elimination of MeOH, two compounds coexist in equilibrium: the 8-hydroxy adduct **2** and the hydroxide of the quaternary alkaloid **1**. The OH^- anion can extract the proton from chloroform (by eliminating H_2O), and the CCl_3^- anion released then attacks the C-8 atom of **1** to form the corresponding 8-trichloromethyl derivative **5**. We expect that the palmatine and berberine pseudobases **2a** and **2b**, dissolved in $CDCl_3$, behave similarly and spontaneously produce the CCl_3 adducts **5a** and **5b**. To determine the physicochemical data unequivocally, we prepared the 8-trichloromethyl adducts **5a** and **5b**

Table 4. ^{13}C NMR Chemical Shifts (δ) of Some 8-Substituted Derivatives of Dihydroberberine in CD_2Cl_2, C_6D_6, and $CDCl_3$ at 303 K

	2b	**3b**	**4b**	**5b**	**2b**	**3b**	**3b**	**5b**
C atom	CD_2Cl_2	CD_2Cl_2	CD_2Cl_2	CD_2Cl_2	C_6D_6	C_6D_6	$CDCl_3$	$CDCl_3$
1	104.96	104.72	104.70	104.31	104.60	105.14	104.66	104.10
2	147.29	147.25	147.22	147.25	147.24	147.85	146.76	146.71
3	147.82	147.92	147.88	148.01	147.93	147.33	147.49	147.44
4	108.16	108.20	108.18	108.49	108.21	108.13	107.86	108.12
4a	129.73	129.75	129.73	129.31	129.85	129.60	129.16	128.69
5	30.48	30.76	30.73	30.83	30.80	30.75	30.42	30.48
6	46.66	47.56	47.53	52.07	46.96	46.96	47.06	51.57
8	79.50	85.32	84.53	73.95	79.39	84.93	84.79	73.47
8a	123.46	119.55	120.18	115.23	122.70	119.79	118.89	114.83
9	146.17	146.16	146.01	146.95	145.46	147.03	146.42	146.34
10	150.33	150.10	150.06	150.19	150.20	150.29	149.69	149.65
11	114.58	114.38	114.18	115.16	114.34	114.76	114.14	114.53
12	119.72	119.31	119.30	118.83	119.82	119.20	119.17	118.58
12a	127.68	128.94	128.85	129.81	127.03	129.96	128.66	129.30
13	95.34	94.78	94.83	97.93	95.06	95.03	94.52	97.46
13a	136.72	137.95	137.90	137.99	136.67	138.29	137.64	137.55
13b	126.21	125.56	125.60	125.43	125.78	125.85	125.19	125.00
2(OCH_2O)	100.99	101.68	101.66	101.70	101.68	100.98	101.22	101.03
8(OCH_3)		52.48				50.93	51.86	
8(CCl_3)				106.18				105.50
9(OCH_3)	61.01	61.13	61.12	61.17	61.44	60.77	61.01	60.95
10(OCH_3)	56.07	56.51	56.45	56.69	56.56	55.99	56.28	56.30
8(OCH_2-)			60.51					
8($-CH_3$)			15.79					

Scheme 1



directly by reacting the alkaloid (**1**) with $CHCl_3$ in the presence of aqueous ammonia.

The elucidation of the structures of compounds **2**−**5** was based primarily on the NMR data. The structures of compounds **3b**, **5a**, and **5b** were also obtained by using X-ray diffraction analysis. The NMR signals were assigned by using the 2D correlation experiments, GHMBC[23,24] (gradient-selected heteronuclear multiple-bond correlation) and GSQMBC[25] (gradient-selected single-quantum multiple-bond correlation) (see Supporting Information). The NMR chemical shifts of compounds **2**−**5** are summarized in Tables 1−4. The complete assignments of the ^{13}C NMR signals of the protoberberine derivatives **2**−**5** in various solvents are reported here for the first time. The portions of the ^{13}C NMR spectra of compounds **2a**, **3a**, and **5a** that indicate unequivocal assignments of the C-8 signals are shown in Figure 1. The influence of substitution at C-8 is clearly demonstrated in notable changes of the C-8 and C-8a chemical shifts. The characteristic ^{13}C NMR shifts of the C-8 atom agree very well with values of analogous −OH, −OMe, and −OEt derivatives of benzophenanthridine alkaloids.[17]

To confirm the structures of 8-methoxydihydroberberine (**3b**) and 8-trichloromethylpalmatine (**5a**) unambiguously, the respective crystals were investigated by X-ray diffraction analysis (see X-ray discussion). The crystal structure

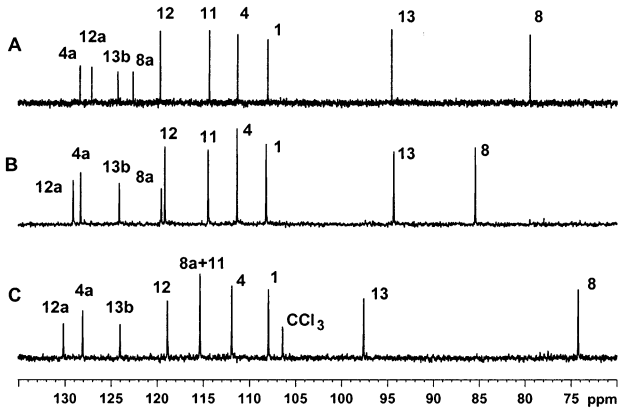


Figure 1. Portions of the ^{13}C NMR spectra of three palmatine derivatives in CD_2Cl_2. Trace A: 8-hydroxydihydropalmatine (**2a**). Trace B: 8-methoxydihydropalmatine (**3a**). Trace C: 8-trichloromethyldihydropalmatine (**5a**). The influence of substitution at C-8 is clearly demonstrated in the notable changes of the C-8, C-8a, C-12a, and C-13 chemical shifts.

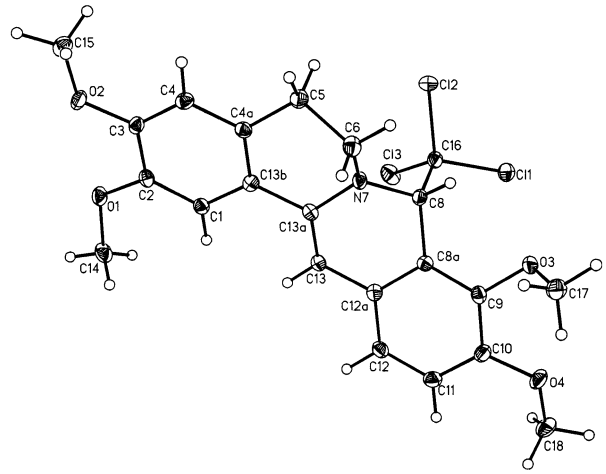


Figure 2. Perspective view of the X-ray structure of compound **5a**.

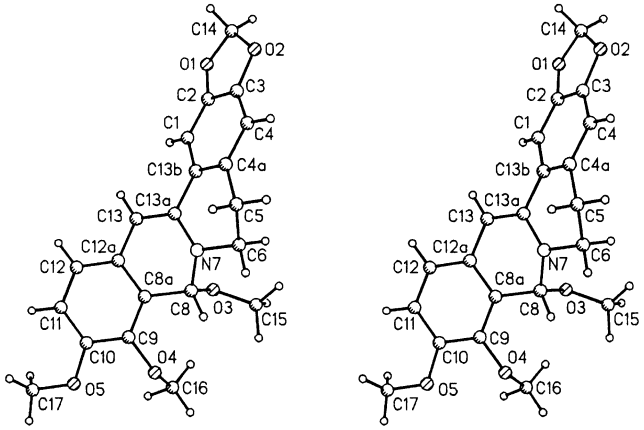


Figure 3. Stereodrawing of the X-ray structure of compound **3b**. Only one of the two symmetry-independent molecules is shown.

of 8-trichloromethyldihydroberberine (**5b**) has already been solved,[26] and it is in good agreement with our new and more precise data (see Experimental Section). ORTEP plots of the 3D structures of compounds **5a** and **3b** are shown in Figures 2 and 3, respectively. The structure of 7,8-dimethoxy-2-methyl-3-(4′,5′-methylenedioxy-2′-vinylphenyl)-1-trichloromethyl-1,2-dihydroisoquinoline has been previously solved using X-ray analysis by Ishii et al.[27] The chemical shift of the CH−CCl_3 proton of this derivative[27] at δ 5.64 is consistent with our data for the 8-trichloromethyl adducts **5a** and **5b**.

Table 5. ^{15}N NMR Chemical Shifts (δ) of Some 8-Substituted Derivatives of Dihydroberberine at 303 K

compound	^{15}N shift[a]	solvent
2b	88.5	CD_2Cl_2
3b	83.6	CD_2Cl_2
5b	64.9	CD_2Cl_2
5b	65.0	$CDCl_3$
5b	64.6	DMSO-d_6

[a] Referenced to 1 M urea in DMSO-d_6 (77.0 ppm)[39] and liquid CH_3NO_2 (381.7 ppm),[39] and reported relative to liquid NH_3.

Systematic comparison of the NMR chemical shifts of the derivatives obtained in our study with the values reported previously[28] reveals that the data presented by Wafo and co-workers as belonging to "7,8-dihydro-8-hydroxypalmatine" isolated from *Enantia chlorantha*, correspond unambiguously to 8-trichloromethyldihydropalmatine (**5a**). The ^1H NMR chemical shifts and melting points are also remarkably consistent with the data for 8-trichloromethyldihydropalmatine (**5a**) published earlier.[13−15] As described, during the procedure[28−30] for isolating this compound from plant material, the sample was in touch with $CHCl_3$ in the presence of aqueous ammonia, and the 8-trichloromethyl adduct (**5**) was probably formed (cf. Scheme 1 and Experimental Section). Similarly, on the basis of our data the compound reported as 2,3,9,10-dimethylenedioxy-1,8-dihydroxy-7,8-dihydroprotoberberine,[31] isolated from *Thalictrum delavayi*, can be identified as the 8-trichloromethyl derivative due to the characteristic chemical shifts of H-8 and C-8. These are approximately δ 6.1−6.2 for H-8 in the 8-O adducts (**2**, **3**, **4**) and δ 5.6 for H-8 in the CCl_3 adducts (**5**) dissolved in $CDCl_3$ or CD_2Cl_2. For C-8 we measured chemical shifts of δ 79 (**2**), 85 (**3**), and 74 (**5**). Previously reported values were δ 5.65 (^1H) and 73.6 (^{13}C) for the derivative of palmatine[28] and δ 5.38 (^1H) and 74.0 (^{13}C) for the derivative of 2,3,9,10-dimethylenedioxy-1-hydroxyprotoberberine.[31]

Moreover, in the two cases discussed above,[28,31] a crude alkaloid extract was treated with 2−10% HCl in the early steps of the extraction procedure. If any 8-hydroxy derivatives (**2**) had occurred in the plants as natural alkaloids, this strong acid would have converted them to the corresponding quaternary cations (**1**). The products were further processed in $CHCl_3$, even in the presence of aqueous ammonia.

Similarly, the recent data[32] reported for synthetically prepared 8-hydroxydihydroberberine (**2b**) do not agree with our data and probably indicate another derivative of berberine (cf. NMR data).

^{15}N NMR spectral parameters of compounds **2b**, **3b**, and **5b**, determined at the natural ^{15}N abundance level,[33,34] are summarized in Table 5. A comparison of the influence of the aromatic and dihydroprotoberberine skeleton on the ^{15}N chemical shifts has recently been published.[35,36] The electron properties of the substitution group are clearly demonstrated in the ^{15}N chemical shift.[37] The nitrogen resonance of the 8-methoxy derivative **3** is more shielded (by ~5 ppm) than the nitrogen of the 8-hydroxy adduct **2**. A substantial upfield shift of ~20 ppm is further detected for the 8-trichloromethyl derivative **5**. An intense response was observed between N-7 and H-13 in the $^1H-^{15}N$ chemical shift multiple bond correlation spectra of the above-specified dihydroprotoberberines in CD_2Cl_2 solution. Other correlations with N-7 were observed for the signals of H-8 and H-5.

Single-Crystal X-ray Diffraction Analysis.[38] Compounds **3b**, **5a**, and **5b** were also investigated by X-ray diffraction analysis (Figures 2, 3, and Supporting Information). Several structural features of 8-methoxydihydroberberine (**3b**) can be highlighted. The hetero ring C is in a flattened half-chair conformation. The C8−OMe group takes a pseudoaxial position, probably being forced to this by the conformational preference of the polycyclic skeleton rather than by the anomeric effect. There are two slightly different conformations of the C-8 methoxy group in the crystal, one of them with a shorter C8−O3 distance, 1.388(8) vs 1.532(8) Å. Ring B is the most puckered ($q_2 = 0.510$ Å, $q_3 = -0.184$ Å, $\phi = -91.4°$). The angle between the two aromatic rings A and C is 40.61(6)°. In the 8-trichloromethyl adducts **5a** and **5b**, the CCl_3 group is equally in the pseudoaxial position (Supporting Information). The C8−CCl_3 bond in compounds **5** is somewhat longer as compared with standard C−C length (1.577(3) Å in **5a**; 1.584(3) Å in **5b**), probably due to the steric repulsion of the CCl_3 group. The angles between the two aromatic rings in **5a** and **5b** are 21.04(7)° and 45.30(6)°, respectively. In all three compounds, the C-10 methoxy group lies almost in the plane of the parent aromatic ring, whereas the neighboring C-9 methoxyl is nearly perpendicular to this plane. These orientations of the C9−OMe and C10−OMe groups are characteristic for the structural arrangements of other protoberberines in the crystal state.[11,16,26] The C2−OMe and C3−OMe groups in the palmatine-chloroform adduct **5a** are more or less in-plane. Each of the compounds **3b**, **5a**, and **5b** contains one stereogenic center (C-8). Owing to their synthetic origin, these compounds are obviously racemic.

To conclude, several findings should be summarized. The derivative of palmatine reported[28−30,32] as being the pseudobase **2a** isolated from *Enantia chlorantha* corresponds unequivocally to 8-trichloromethyldihydropalmatine (**5a**) and must be classified as an artifact of the isolation. Similarly, the reported 2,3,9,10-dimethylenedioxy-1,8-dihydroxy-7,8-dihydroprotoberberine[31] obtained from *Thalictrum delavayi* is very probably 2,3,9,10-dimethylenedioxy-1-hydroxy-8-trichloromethyl-7,8-dihydroprotoberberine. The discrepancies between our data for 8-hydroxydihydroberberine (**2b**) and those reported by Nyasse et al.[32] are likewise substantial. Although the solvent used for the NMR measurements is not specified in the paper,[32] the reported data probably indicate another derivative of berberine. In light of the evidence presented in this paper, as well as in numerous previous contributions, $CHCl_3$ should not be used when isolating protoberberines under basic conditions. $CHCl_3$ can be replaced advantageously by other organic solvents (e.g., CH_2Cl_2), as already recommended by Shamma and Rahimizadeh.[14] We believe that the data summarized in this contribution will clarify the structure−reactivity relationships of the protoberberine alkaloids palmatine and berberine.

Experimental Section

General Experimental Procedures. Melting points were determined on a Mettler FP 51 apparatus and are uncorrected. Mass spectra were measured on a Fisons TRIO 1000 quadrupole mass spectrometer using electron ionization (EI, 70 eV). NMR spectra were recorded using a Bruker Avance DRX 500 spectrometer operating at frequencies of 500.13 MHz (^{1}H) and 125.77 MHz (^{13}C), a Bruker Avance 300 spectrometer operating at frequencies of 300.13 MHz (^{1}H), 75.48 MHz (^{13}C), and 30.41 MHz (^{15}N), and a Bruker Avance 600 spectrometer operating at frequencies of 600.15 MHz (^{1}H) and 150.92 MHz (^{13}C). All NMR spectra were measured at 303 K. The ^{1}H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the signals of the solvents [7.16 (^{1}H) and 128.00 (^{13}C) for C_6D_6; 5.31 (^{1}H) and 53.80 (^{13}C) for CD_2Cl_2; 7.26 (^{1}H) and 77.00 (^{13}C) for $CDCl_3$]. The ^{15}N chemical shifts were referenced to liquid CH_3NO_2 (381.7 ppm)[39] and are reported relative to liquid NH_3. The 2D NMR experiments GHMBC and GSQMBC were used for assigning the individual ^{13}C and ^{1}H resonances.[40]

The diffraction data were collected with 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 reflections. Cell parameters were refined from all the strong (stronger than 1000 pulses) reflections. Data reduction was carried out using the program CrysAlis RED. Direct methods in SHELXS-97[41] were used to solve the structures, and the structures were refined by using SHELXL-97.[42] The tables were prepared for publication by using SHELXL and PARST,[43] and the figures were generated with ORTEP-III.[44]

Alkaloids. Palmatine chloride (**1a**, X = Cl) and berberine chloride (**1b**, X = Cl), isolated from *Jatrorrhiza palmata* Miers. and *Berberis vulgaris* L., respectively, were kindly donated by Professor Jiří Slavík (Masaryk University, Brno, Czech Republic). The melting points (**1a** 205−210 °C, **1b** 209−210 °C) and spectral characteristics of these samples agree well with previously published data.

Spectroscopic Studies of Compounds 2a and 2b. (i) Alkaloid **1** (10−30 mg) was dissolved in 1 mL of D_2O, and 1 mL of benzene-d_6 was added. After alkalization with 20% NaOH (100 μL) and gentle shaking, the C_6D_6 phase was separated and measured. (ii) Alkaloid **1** (10−30 mg) was suspended in 1 mL of CD_2Cl_2, and 100 μL of 20% NaOH was added. The CD_2Cl_2 phase was separated, dried with anhydrous Na_2CO_3, and measured. For ^{1}H and ^{13}C NMR data see Tables 1-4, ^{15}N NMR, see Table 5. **2a**: EIMS *m/z* 369 $[M]^+$ (3), 368 (19), 367 (54), 352 $[M - OH]^+$ (100). **2b**: EIMS (30 eV) *m/z* 353 $[M]^+$ (3), 352 (17), 351 (51), 336 $[M - OH]^+$ (100).

8-Methoxy-7,8-dihydropalmatine (3a). Palmatine chloride (**1a**, 20 mg) was stirred in 5 mL of a 20% solution of MeONa in MeOH in an argon atmosphere. After 24 h the solvent was evaporated and H_2O (alkalized with NaOH) was added. The product was extracted into diethyl ether and dried with a mixture of NaOH and anhydrous Na_2SO_4. Evaporation of the solvent yielded the yellow crystals of **3a** (90%), mp 168−172 °C (dec); for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 383 $[M]^+$ (0.4), 353 (100), 352 $[M - OMe]^+$ (61), 351 (54).

8-Methoxy-7,8-dihydroberberine (3b). This was prepared like **3a**, but from berberine chloride (**1b**). Evaporation of the solvent yielded yellow crystals of **3b**, mp 137−139 °C (dec); for ^{1}H and ^{13}C NMR data, see Tables 3 and 4; for ^{15}N NMR data, see Table 5; EIMS *m/z* 367 $[M]^+$ (0.8), 338 (1.4), 336 $[M - OMe]^+$ (0.4), 57 (100).

Crystal Data for 3b. CCDC ref. No. 193920. Crystallized from diethyl ether, $C_{21}H_{21}NO_5$, $M_{rel} = 367.39$, $T = 120(2)$ K, monoclinic, $\lambda = 0.71073$ Å, space group $P2_1/n$, $a = 7.4433(4)$ Å, $b = 11.0224(6)$ Å, $c = 21.3225(11)$ Å, $\beta = 100.04(3)°$, $V = 1722.57(16)$ Å^3, $Z = 4$, $D_{calc} = 1.417$ Mg/m^3, crystal size 0.55 × 0.50 × 0.30 mm, $R = 0.052$.

8-Ethoxy-7,8-dihydroberberine (4b). Berberine chloride (**1b**, 15 mg) was stirred in 5 mL of a solution of EtONa in EtOH in an argon atmosphere. After 24 h the solvent was evaporated and H_2O (alkalized with NaOH) was added. The product was extracted into diethyl ether and dried with a mixture of NaOH and anhydrous Na_2SO_4, and the solvent was evaporated; for ^{1}H and ^{13}C NMR data, see Tables 3 and 4.

8-Trichloromethyl-7,8-dihydropalmatine (5a). Palmatine chloride (**1a**, 80 mg) was stirred with 10 mL of $CHCl_3$ and 0.5 mL of concentrated aqueous ammonia. After 24 h the phases were separated and the organic phase was dried with a mixture of NaOH and anhydrous Na_2SO_4. Evaporation of the solvent yielded yellow crystals of **5a** quantitatively, mp 194−196 °C (dec); for ^{1}H NMR data, see Table 1; for ^{13}C NMR,

see Table 2; EIMS *m*/*z* 471 $[M]^+$ (0.1), 436 (2.4), 435 $[M - Cl]^+$ (0.5), 434 (3.6), 352 $[M - CCl_3]^+$ (100).

Crystal Data for 5a. CCDC ref. No. 193921. Crystallized from benzene, $C_{22}H_{22}Cl_3NO_4$, $M_{rel} = 470.76$, $T = 120(2)$ K, triclinic, $\lambda = 0.71073$ Å, space group $P1$, $a = 7.3910(6)$ Å, $b = 12.2514(10)$ Å, $c = 12.8568(10)$ Å, $\alpha = 63.240(8)°$, $\beta = 87.858(7)°$, $\gamma = 77.204(7)°$, $V = 1011.08(14)$ Å^3, $Z = 2$, $D_{calc} = 1.546$ Mg/m^3, crystal size $0.50 \times 0.35 \times 0.30$ mm, $R = 0.035$.

8-Trichloromethyl-7,8-dihydroberberine (5b). This was prepared like **5a**, but from berberine chloride (**1b**). Evaporation of the solvent yielded yellow crystals of **5b**, mp 186−188 °C (dec); for ^{1}H NMR data, see Table 3, for ^{13}C NMR, see Table 4; for ^{15}N NMR, see Table 5; EIMS *m*/*z* 455 $[M]^+$ (0.2), 420 (2.5), 419 $[M - Cl]^+$ (0.4), 418 (3.8), 336 $[M - CCl_3]^+$ (100).

Crystal Data for 5b. CCDC ref. No. 193922. Crystallized from diethyl ether, $C_{21}H_{18}Cl_3NO_4$, $M_{rel} = 454.71$, $T = 153(2)$ K, triclinic, $\lambda = 0.71073$ Å, space group $P1$, $a = 8.6994(6)$ Å, $b = 10.7566(8)$ Å, $c = 11.0433(8)$ Å, $\alpha = 69.321(7)°$, $\beta = 87.403(6)°$, $\gamma = 83.486(6)°$, $V = 960.55(12)$ Å^3, $Z = 2$, $D_{calc} = 1.572$ Mg/m^3, crystal size $0.50 \times 0.50 \times 0.40$ mm, $R = 0.032$.

Acknowledgment. The authors thank Radovan Kareš for measuring the EI mass spectra. The financial support of this work by the Ministry of Education of the Czech Republic (LN00A016) is gratefully acknowledged.

Supporting Information Available: GSQMBC spectrum of **2b** and perspective views of **3b** and **5b**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Bhakuni, D. S.; Jain, S. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1986; Vol. 28, Chapter 2, pp 95−181.
(2) Preininger, V. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1986; Vol. 29, Chapter 1, pp 1−98.
(3) Hu, J. P.; Takahashi, N.; Yamada, T. *Oral Dis.* **2000**, *6*, 297−302.
(4) Wright, C. W.; Marshall, S. J.; Russell, P. F.; Anderson, M. M.; Phillipson, J. D.; Kirby, G. C.; Warhurst, D. C.; Schiff, P. L., Jr. *J. Nat. Prod.* **2000**, *63*, 1638−1640.
(5) Baird, A. W.; Taylor, C. T.; Brayden D. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 111−120.
(6) Lau, C.-W.; Yao, X.-Q.; Chen, Z.-Y.; Ko, W.-H.; Huang, Y. *Cardiovasc. Drug Rev.* **2001**, *19*, 234−244.
(7) Schmeller, T.; Latz-Brüning, B.; Wink, M. *Phytochemistry* **1997**, *44*, 257−266.
(8) Shamma, M. *The Isoquinoline Alkaloids*; Academic Press: New York, 1972; pp 268−314.
(9) Bentley, K. W. *The Isoquinoline Alkaloids*; Harwood Academic Publishers: Amsterdam, 1998; pp 219−263.
(10) Vennerstrom, J. L.; Klayman, D. L. *J. Med. Chem.* **1988**, *31*, 1084−1087.
(11) Man, S.; Potáček, M.; Nečas, M.; Žák, Z.; Dostál, J. *Molecules* **2001**, *6*, 433−441.
(12) Contreras, M. L.; Rivas, S.; Rozas, R. *Heterocycles* **1984**, *22*, 101−106.
(13) Miana, G. A. *Phytochemistry* **1973**, *12*, 1822−1823.
(14) Shamma, M.; Rahimizadeh, M. *J. Nat. Prod.* **1986**, *49*, 398−405.
(15) Slavík, J.; Slavíková, L. *Collect. Czech. Chem. Commun.* **1989**, *54*, 2009−2020.
(16) Man, S.; Dostál, J.; Nečas, M.; Žák, Z.; Potáček, M. *Heterocycl. Commun.* **2001**, *7*, 243−248.
(17) Sečkářová, P.; Marek, R.; Dostál, J.; Dommisse, R.; Esmans, E. L. *Magn. Reson. Chem.* **2002**, *40*, 147−152.
(18) Kovář, J.; Stejskal, J.; Paulová, H.; Slavík, J. *Collect. Czech. Chem. Commun.* **1986**, *51*, 2626−2634.
(19) Šimánek, V.; Preininger, V.; Lasovský, J. *Collect. Czech. Chem. Commun.* **1976**, *41*, 1050−1055.
(20) Marek, R.; Sklenář, V.; Dostál, J.; Slavík, J. *Tetrahedron Lett.* **1996**, *37*, 1655−1658.
(21) Marek, R.; Toušek, J.; Králík, L.; Dostál, J.; Sklenář, V. *Chem. Lett.* **1997**, 369−370.
(22) Toušek, J.; Dommisse, R.; Dostál, J.; Žák, Z.; Pieters, L.; Marek, R. *J. Mol. Struct.* **2002**, *613*, 103−113.
(23) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.
(24) Willker, W.; Leibfritz, D.; Kerssebaum, R.; Bermel, W. *Magn. Reson. Chem.* **1993**, *31*, 287−292.
(25) Marek, R.; Králík, L.; Sklenář, V. *Tetrahedron Lett.* **1997**, *38*, 665−668.
(26) Khamidov, I. I.; Tashkhodzhaev, B.; Aripova, S. F.; Telezhenetskaya, M. V.; Karimov, A. K. *Khim. Prir. Soedin.* **1996**, 889−893.
(27) Ishii, H.; Takeda, S.; Ogata, K.; Hanaoka, M.; Harayama, T. *Chem. Pharm. Bull.* **1991**, *39*, 2712−2714.
(28) Wafo, P.; Nyasse, B.; Fontaine, C. *Phytochemistry* **1999**, *50*, 279−281.
(29) Tan, P. V.; Nyasse, B.; Enow-Orock, G. E.; Wafo, P., Forcha, E. A. *Phytomedicine* **2000**, *7*, 291−296.
(30) Tan, P. V.; Nyasse, B.; Dimo, T.; Wafo, P., Akahkub, B. T. *Pharmazie* **2002**, *57*, 409−412.
(31) Li, M.; Chen, X.; Tang, Q.-M.; Zhang, J.-S. *Planta Med.* **2001**, *67*, 189−190.
(32) Nyasse, B.; Nkwengoua, E.; Sondengam, B.; Denier, C.; Willson, M. *Pharmazie* **2002**, *57*, 358−361.
(33) Marek, R.; Lyčka, A. *Curr. Org. Chem.* **2002**, *6*, 35−66.
(34) Martin, G. E.; Hadden, C. E. *J. Nat. Prod.* **2000**, *63*, 543−585.
(35) Marek, R.; Humpa, O.; Dostál, J.; Slavík, J.; Sklenář, V. *Magn. Reson. Chem.* **1999**, *37*, 195−202.
(36) Marek, R.; Marek, J., Dostál, J.; Táborská, E.; Slavík, J.; Dommisse, R. *Magn. Reson. Chem.* **2002**, *40*, 687−692.
(37) Dostál, J.; Marek, R.; Slavík, J.; Táborská, E.; Potáček, M.; Sklenář, V. *Magn. Reson. Chem.* **1998**, *36*, 869−872.
(38) Crystallographic data for the structures 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).
(39) Wishart, D. S.; Bigam, C. G.; Yao, J.; Abildgaard, F.; Dyson, H. J.; Oldfield, E.; Markley, J. L.; Sykes, B. D. *J. Biomol. NMR* **1995**, *6*, 135−140.
(40) Marek, R.; Toušek, J.; Dostál, J.; Slavík, J.; Dommisse, R.; Sklenář, V. *Magn. Reson. Chem.* **1999**, *37*, 781−787.
(41) Sheldrick, G. M. *SHELXS97, Program for the Solution of Crystal Structures;* University of Göttingen: Germany, 1997.
(42) Sheldrick, G. M. *SHELXL97, Program for the Refinement of Crystal Structures*; University of Göttingen: Germany, 1997.
(43) Nardelli, M. *Comput. Chem.* **1983**, *7*, 95−98.
(44) Burnett, M. N.; Johnson, C. K. *ORTEP-III: Oak Ridge Thermal Ellipsoid Plot Program for Crystal Structure Illustrations*; Oak Ridge National Laboratory Report ORNL-6895, 1996.

NP0204996