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A 7,8-dihydro-8-hydroxypalmatine from *Enantia chlorantha*

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Abstract

7,8-dihydro-8-hydroxypalmatine, a novel protoberberine-type alkaloid, along with palmatine, has been isolated and characterized from an anti-HIV active extract from *Enantia chlorantha*. The structures of the two compounds were elucidated by spectroscopic analyses and from chemical evidence.

Keywords: *Enantia chlorantha*; Annonaceae; Alkaloids; Palmatine; 7,8-Dihydro-8-hydroxypalmatine

1. Introduction

As part of our programme aiming at the isolation of antiprotozoal and antiviral compounds from medicinal plants locally used in the treatment of hepatic disorders, we have focussed our attention on the minor constituents of *Enantia chlorantha*, the roots of which are used in the healing of various diseases, including tuberculosis, hepatic infections and some forms of ulcers (Hamonnière, Leboeuf, & Paris, 1975). Some years ago, it was shown that protoberberines (Jalander, Sjöholm, & Virtanen, 1990), namely palmatine, columbarnine, jatrorrhizine from the stem bark of *E. chlorantha* synergically had preventive and curative effects on artificially-provoked liver injury (Virtanen, Lassila, Njimi, & Ekotto Mengata, 1988a,b). During the biological screening of this species for antiviral compounds, we have observed that certain alcoholic fractions of its bark exhibited moderate to significant anti-HIV activity. Fractionation of one of the active fractions has led to the isolation of a new compound, 7,8-dihydro-8-hydroxypalmatine (**1**) along with the main palmatine (**2**). We report herein the structural elucidation of the novel alkaloid **1**.

2. Results and discussion

The new compound **1**, obtained as yellow crystals from MeOH (m.p. 188–190°C), showed a strong yellow fluorescence under UV light (254 and 366 nm) and positive reactions with alkaloid-precipitating reagents, such as Dragendorff's. The IR spectrum of **1** revealed the presence of bands at 3420, 1605, 1511, 974 and 851 cm^{-1} suggestive of an *hydroxyl group* and of *aromatic nuclei*. Many compounds have these moieties. The ^1H spectrum (1D and H/H COSY version was more significant with proton signals for two methylene groups, respectively, at δ 2.76 (1H, dt, $J = 4.4$ and 15.5 Hz/δ 3.42 (1H, ddd, $J = 4.4$; 9.9 and 15.5 Hz) and δ 3.75 1H, ddd, $J = 3.8$; 9.9 and 13.7 Hz)/δ 3.87 (overlapping with the large methoxyl signals). These methylene protons, together with the last non-aromatic signal at δ 5.65 (1H, s) attributed to a methine group bearing an oxygen atom, were very suggestive of a dihydroprotoberberine system containing the four methoxyl groups at δ 3.89, 3.90, 3.95 and 3.96, respectively. Furthermore, the aromatic part of the ^1H spectrum exhibited two singlets at δ 7.20 (1H, s) and 6.65 (1H, s) ascribable to two isolated protons situated *para* with respect to each other in a 2,3,5,6-tetrasubstituted benzene ring. Another singlet was observed at δ 6.20 (1H, s). An AB-system at δ 7,0 (1H, d, $J = 8.4$ Hz) and

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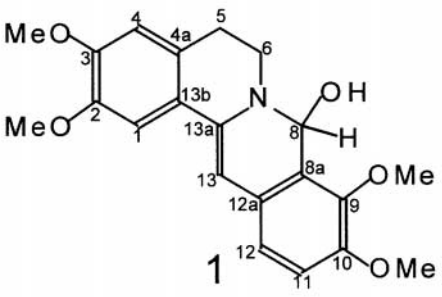

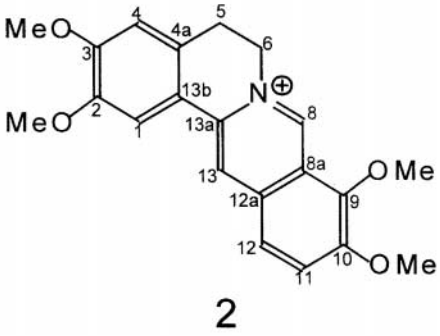

6.90 (1H, d, $J = 8.4$ Hz) of another tetrasubstituted benzene ring was also observed.

In order to establish the substitution pattern of the suspected dihydroprotoberberine skeleton, the HMQC and HMBC spectra of **1** were recorded and analysed accordingly (Table 1). At first glance, these data coupled to those described above suggested a close structural relationship with palmatine **2**. For example, the proton at δ 5.65 carried by the carbon atom at δ 73.6 was correlated through three bonds both to the quaternary aromatic carbon atom at δ 129.5 and to the one at δ 146.6 carrying the methoxyl group, appearing itself at δ 61.1. Likewise, one of the AB-system protons, i.e. the one at δ 7.0 was also correlated to the carbon atoms at δ 129.5 and 146.6 whilst the isolated proton at δ 6.20 showed long-range couplings with the carbon atoms at δ 118.6 bearing the second AB-system proton. The above correlations sustained by the observed NOE effects between the proton at δ 6.20 and those at δ 7.20 (1H, s) and 6.90 (1H, d, $J = 8.4$ Hz) belonging, respectively, to two different aromatic nuclei are consistent with an 7,8-dihydro-8-hydroxypalmatine skeleton for **1**.

Such a proposition was in good agreement with the mass spectrum of **1** which showed the predominent fragment ion at m/z 352 resulting from the loss of an hydroxyl group from the parent ion. Another supporting argument to structure **1** was obtained from its chemical conversion to palmatine **2**. When **1** in ethanol was refluxed under basic conditions for 3 h, 20% of palmatine **2** could be isolated in pure form.

Though the novel alkaloid **1** and palmatine **2** were isolated from an anti-HIV active fraction from *E. chlorantha*, both of them failed to exhibit any interesting effect on the corresponding virus when submitted to the NCI screening programme in their pure forms.

3. Experimental

3.1. General

M.p.'s are uncor. ^{1}H NMR were recorded at 400 MHz, ^{13}C NMR at 100 MHz in $CDCl_3$ or DMSO-d_6 using TMS as int. standard. EIMS were obtained at 70 eV. Silica gel 60 (Fluka, 230–400 mesh) was used for CC and precoated silica plates (Merck, silica gel 60 F_{254}, 0.25 mm) were used for TLC.

3.2. Plant material

E. chlorantha Oliver was collected at Edéa (Littoral, Cameroon) in March 1995. A voucher specimen is deposited in the National Herbarium, Yaoundé, Cameroon.

3.3. Extraction and isolation

Air-dried and finely powdered stem bark (2 kg) was macerated in MeOH (8 l) for 72 h. After filtration and evapn at pres., the crude material (200 g) was treated with 10% aq. HCl (2 l) to form a precipitate, which upon filtration, afforded a yellow solid mixt. (30 g). The filtrate was, in turn, extracted several times with $CHCl_3$ and lyophilized to a fluffy solid (80 g). Half of this solid was chromatographed on silica gel (600 g) using hexane–$CHCl_3$ mixts of increasing polarity and collecting 200 ml frs. Identical frs by TLC obtained upon elution with hexane–$CHCl_3$ (7:3) were pooled and the solvent removed. The semi-solid thereby obtained was further purified on a smaller silica gel column using the same eluting system as above to give a solid (2 g) which, in turn, was recrystallized from hot MeOH to yield yellow crystals of **1** (1.2 g). Further elution of the column with $CHCl_3$–MeOH (19:1) 5 g of solid palmatine **2**.

3.4. 7,8-Dihydro-8-hydroxypalmatine (*1*)

Yellow crystals (1.2 g), m.p. 188–190°C. $[\alpha]_D$ −35.0 (c 0.08 $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 3421, 1606, 1511, 1490, 1343, 1316, 1190, 1036, 1001, 975, 830, 807. EIMS m/z: 352 $[M - 17]^+$. NMR: Table 1.

Table 1. ^{1}H and ^{13}C NMR data for compound **1**

Atom	Assignments		Observed correlations	
	C	H	HBMC	NOE
1	105.60	7.20(s)	3, 4a, 13a	H-13, MeO-2
2	147.90	–	–	
3	149.25	–	–	
4	111.90	6.65 (s)	2, 5, 13a	MeO-3
4a	127.40	–	–	
5	30.00	2.76 (dt, 4.4 and 15.5 Hz) 3.42 (ddd, 4.4, 9.9 and 15.5 Hz)		
6	51.80	3.75 (ddd, 3.8, 9.9 and 13.7 Hz) 3.87 (overlapping)		H-8
8	73.60	5.65 (s)	6, 8a, 9, 12a, 13a	H-1, H-12
8a	114.90	–	–	
9	146.59	–		
10	149.77	–		
11	114.60	7.00 (d, 8.4 Hz)	9, 12a	MeO-10
12	118.60	6.90 (d, 8.4 Hz)	8a, 10	H-13
12a	129.50			
13	97.10			
13a	137.60			
13b	123.50			
OMe	61.10	3.95 s		H-1
Ome	56.40	3.89 s		H-11
Ome	56.10	3.96 s		–
Ome	56.10	3.90 s		H-4

3.5. Palmatine (**2**)

Yellow solid (5 g), m.p. 206–208°C. IR ν_{max} (KBr) cm^{-1}: 3606, 3367, 1636, 1605, 1567, 1524, 1511, 1365, 968. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.95 (1H, s, H-8), 9.15 (1H, s, H-13), 8.15 (1H, d, $J = 9.2$ Hz, H-11), 8.10 (1H, d, $J = 9.2$ Hz, H-10), 7.75 (1H, s, H-1), 7.15 (1H, s, H-4), 4.95 (2H, t, $J = 6.2$ Hz, H-6), 4.15 (3H, s, C-9-OCH_3), 4.00 (3H, s, C-10-OCH_3), 3.90 (3H, s, C-2-OCH_3), 3.88 (3H, s, C-3-OCH_3), 3.25 (2H, t, $J = 6.2$ Hz, H-5). ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.7 (C-3), 151.5 (C-10), 150.8 (C-2), 146.2 (C-8), 145.6 (C-9), 139.9 (C-13a), 135.4 (C-12a), 129.8 (C-4a), 128.0 (C-11), 124.3 (C-8a), 121.1 (C-13), 120.4 (C-13b), 112.0 (C-4), 109.7 (C-1), 62.4 (C-9-OCH_3), 57.4 (C-10-OCH_3), 57.3 (C-2-OCH_3), 56.8 (C-3-OCH_3), 56.5 (C-6), 27.8 (C-5).

3.6. Conversion of **1** (**2**)

7,8-Dihydro-8-hydroxypalmatine (**1**) (200 mg) was suspended in a mixt. of $CHCl_3$ (20 ml), EtOH (20 ml) and 30% aq. NaOH (20 ml) and refluxed for 4 h. The reaction mixt. was then acidified to pH 6 with 10% HCl and extracted ×2 with $CHCl_3$ (2 × 100 ml). The resulting organic phase was dried ($MgSO_4$), filtered and the solvent removed to leave a residue which upon purification over silica gel using hexane–$CHCl_3$ mixts of increasing polarity afforded 38 mg (20%) of palmatine (**2**).

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