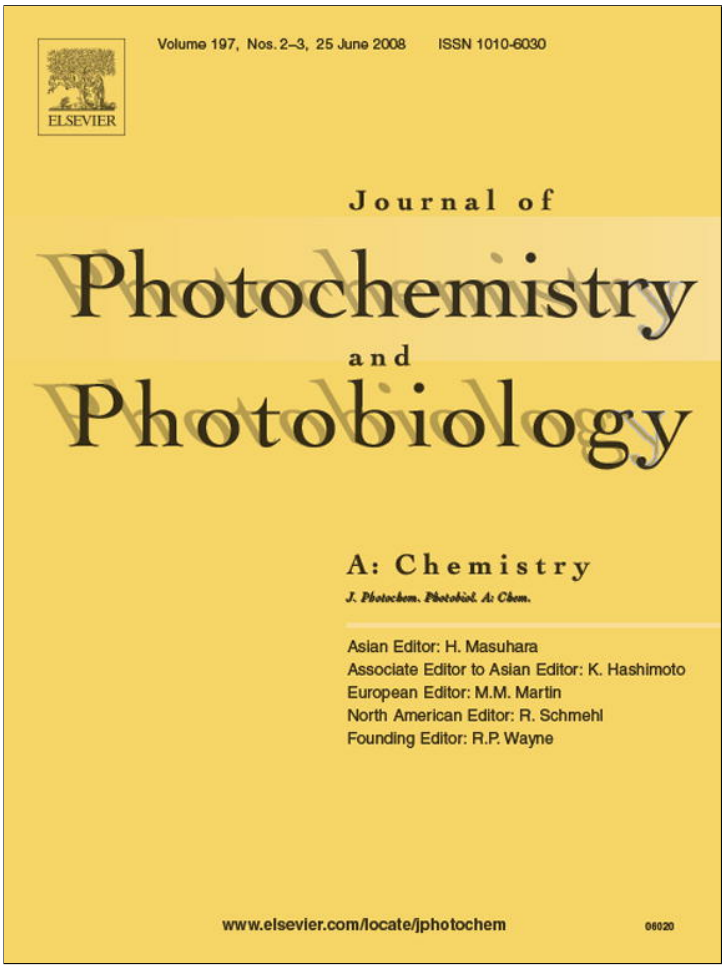

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Phototransformation products of the alkaloid lappaconitine: Multinuclear NMR study

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Abstract

The NMR technique has been applied to characterize the phototransformation products of the natural alkaloid lappaconitine. It was demonstrated that the photolysis of lappaconitine **1** results in cleavage of the ester bond with elimination of *N*-acetylanthranilic acid. The final reaction product was found to be an immonium salt **4** of *N*-acetylanthranilic acid and enamine **3**. An equilibrium between the imine cation and the enamine **3** was detected.

Keywords: Lappaconitine; Phototransformation; *N*-acetylanthranilic acid; NMR

1. Introduction

Lappaconitine (**1**) – a diterpene alkaloid extracted from roots of *Aconitum septentrionale* Koelle – is known to demonstrate bradycardic and hypotensive activity [1,2]. However, due to toxicity and side effects in humans, its clinical applications are significantly restricted [2–4]. Another drawback impeding the use of **1** for clinical treatment is its high photochemical sensitivity [5]. Its phototransformations might be the reason for the decreased therapeutic effect. From this point of view, investigation of the photodegradation pathways and structural study of the final products of the transformations are of great importance. Lappaconitine molecule consists of two chemically different parts: diterpene alkaloid nucleus and 2-acetylanthranilic acid moiety with ester linkage in between. Our recent study of the mechanism of photo-induced interaction of lappaconitine with biological targets (amino acids) [5] showed that in the presence of electron donors, such as, tyrosine and tryptophan, the reaction occurs via the formation of an intermediate lappaconitine radical anion followed by proton transfer and cleavage of the ester bond to form *N*-acetylanthranilic acid as the final product of the transformation of the aromatic moiety of the starting compound. Additionally, lappaconitine was found to be photounstable even in the absence of the additives. In the present study we have applied multinuclear (^{1}H, ^{13}C and ^{15}N) high-field NMR technique to elucidate the structure of the products resulted from diterpene nucleus of the lappaconitine molecule.

2. Experimental

NMR spectra were recorded using a Bruker DRX 500 spectrometer (500.13 MHz for ^{1}H, 125.75 MHz for ^{13}C and 36.15 MHz for ^{15}N) for solutions in methanol-d_4 (20 mg/mL) at +30 °C with the signals of the solvent as internal standard (δ_H 3.30 ppm, δ_C 49.00 ppm, δ_N 14.0 ppm of N_2). Spectral assignments were made based on: (1) 1D *J*-modulated carbon-13 spectrum (J = 140 Hz, broad band decoupling); (2) 2D ^{1}H–^{1}H shift correlation; (3) 2D ^{1}H–^{13}C shift correlation (J = 140 Hz); and (4) 2D ^{1}H *J*-resolved spectrum. Standard NMR Software System was used to record the spectra. The photolysis was carried out using a 1000 W high-pressure mercury lamp (irradiation time 10 min). Product formation was monitored by the ^{1}H

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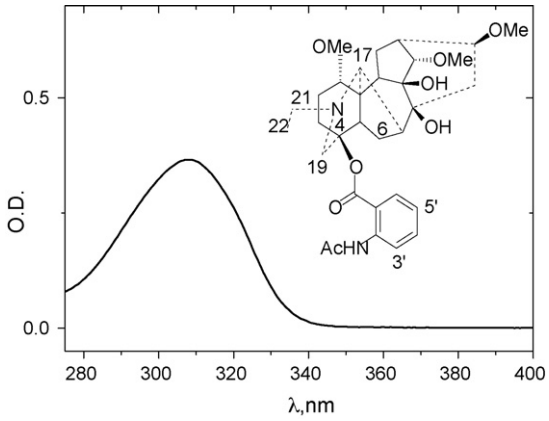


Fig. 1. Absorption spectrum of a 0.1 mM methanol solution of lappaconitine.

NMR during irradiation. LC-MS experiments were carried out using an Agilent 1100 Series LC/MSD (Nucleosil C18 column, 5 μm, 0.1 M Tris in CH_3CN as eluent). Crystalline lappaconitine (purity >90% according to the ^1H NMR) was isolated from dried roots of *Aconitum Septentrionale* Koelle. Semi-empirical quantum chemical calculations (AM1, PM3, MNDO, MINDO/3) were carried out by the MOPAC 2000 program (RMS gradient = 0.01) to locate local energy minima.

3. Results and discussion

Photophysical properties of **1** are fully determined by the anthranilic function [6–8], and the UV–vis spectra of lappaconitine show an absorption maximum around 310 nm (see Fig. 1).

In our hands irradiation of a methanolic solution of **1** with a laser beam (308 nm) resulted in rapid photodegradation of the alkaloid to produce two main products (LC/MS) with $m/z = 179$ and $m/z = 405$, respectively. The component with $m/z = 179$ is *N*-acetylanthranilic acid, whose formation is also proved by monitoring the reaction by the ^1H NMR: irradiation of a solution of **1** in CD_3OD results in appearing a characteristic set of the signals of *N*-acetylanthranilic acid [5] (see Fig. 2).

Elimination of *N*-acetylanthranilic acid from the lappaconitine molecule results in non-aromatic product with $m/z = 405$, whose structure might be drawn as unsaturated compound **3** with newly formed C=C bond at the location of the cleavage. The molecule **3** should be strained enough and so might be quite unstable. Moreover, the set of up-field ^1H NMR signals (1.0–4.0 ppm) of the product are not in agreement with the structural formula **3**. To elucidate the structure of the new product, we have applied multinuclear high-field NMR. NMR ^1H and ^{13}C data are summarized in Table 1.

Precise analysis of the NMR spectra for the non-aromatic reaction product (1D ^1H, 2D ^1H *J*-resolved spectrum, 1D *J*-modulated ^{13}C, 2D ^1H–^1H shift correlation, and 2D ^1H–^{13}C shift correlation spectra) indicates the product to have the same carbon frame and the same topology of the azaheterocyclic ring system as the diterpene nuclei of the starting compound had. At the same time, as compared to the starting lappaconitine, ^1H and ^{13}C spectra of the photolysis product have the following features: (1) low-field shifts of the signals C-19 and C-21; (2) up-field shifts of the signals C-2 and C-3; (3) a significant up-field shift of one of the H-12 protons; (4) a long-range spin–spin coupling $J_{(H\text{-}19)\text{–}(H\text{-}21)}$; and (5) significant changes in the vicinal couplings $J_{(H\text{-}1)\text{–}(H\text{-}2)}$ and $J_{(H\text{-}2)\text{–}(H\text{-}3)}$ (see Figs. 2 and 3); (6) when the photolysis is carried out in CD_3OD, no signals of the atoms H-4 and C-4 occur (see Fig. 2 and Table 1), while the photolysis in CH_3OH results in the product with visible signals H-4 and C-4, which are slowly disappearing when the product is stored as a solution in CD_3OD.

All the features listed can be explained by Scheme 1.

According to this scheme, the immonium salt **4** is the final photolysis product of lappaconitine **1**. The immonium moiety of the product **4** is stabilized by intramolecular Coulomb interactions between the positively charged immonium nitrogen and the partially negatively charged oxygen of the methoxyl

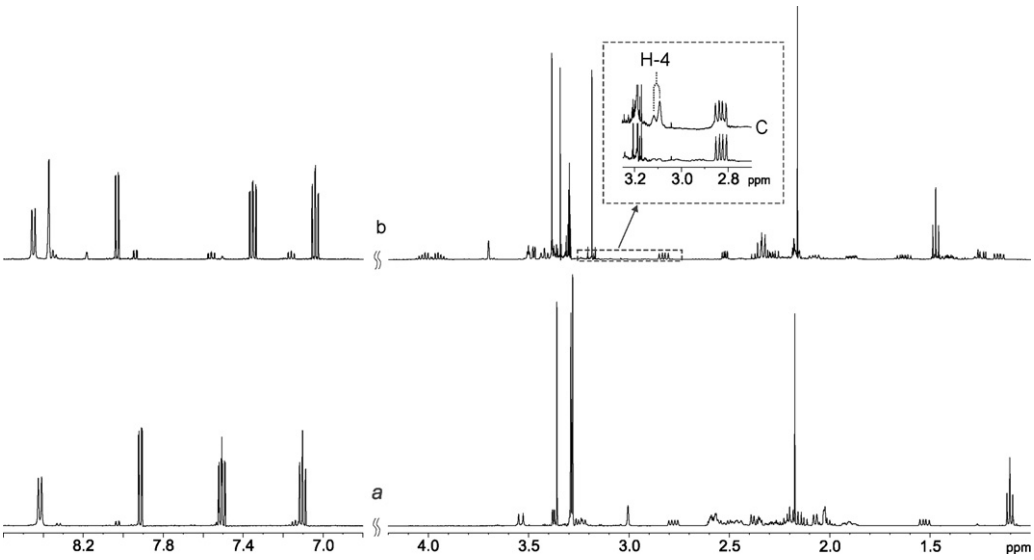
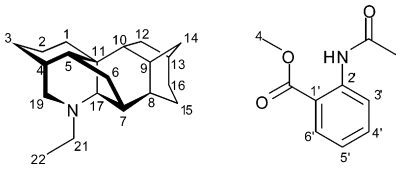


Fig. 2. ^1H NMR spectra (500 MHz, 20 mg/mL in CD_3OD at +30 °C) of lappaconitine (a), the photolysis products in CD_3OD (b), and the photolysis products in CH_3OH (c).

Table 1
NMR ^{1}H and ^{13}C data from lappaconitine and its phototransformation products measured in methanol solution at +30 °C



i	**1**		**4**		
	δC_i (ppm)	δH_i (ppm) (*J*, Hz)	δC_i (ppm)	δH_i (ppm)	$J(H_i–H_i)$ (Hz)
1	85.41	3.26 dd (10.1, 7.2)	81.81	3.51	3.2 (2b), 2.7 (2a), 0.5 (3b)
2	28.00	[2.20, 2.28]	19.27	1.42 (a)	15.0 (2b), 11.8 (3a), 8.4 (3b), 2.7 (1)
				1.90 (b)	15.0 (2a), 7.4 (3a), 3.2 (1), 0.7 (3b)
3	32.88	1.92 dddd (12.9, 12.4, 3.7, 2.0)	19.87	1.64 (a)	14.4 (3b), 11.8 (2a), 7.4 (2b), 1.5 (4)
		2.60 ddd (12.2, 4.7, 3.1)		2.09 (b)	14.4 (3a), 11.5 (4), 8.4, 0.7 (2b), 0.5 (1)
4	86.12	–	44.64	3.10	11.5 (3b), 3.0 (19), 3.0 (5), 1.5 (3a)
5	49.40	2.09 d (8.4)	34.71	2.172	8.0 (6b), 3.0 (4), 1.5 (6a), 1.4 (19), 1.0 (17)
6	25.16	1.54 dd (15.0, 8.4)	29.20	1.17 (a)	14.8 (6b), 8.0 (5), 1.5 (7)
		2.79 dd (15.0, 7.5)		2.84 (b)	14.8 (6a), 8.0 (7), 0.7 (5), 0.7 (17)
7	49.45	2.40 d (7.5)	56.98	2.33	8.0 (6a), 0.5 (17)
8	76.02	–	74.65	–	
9	80.05	–	77.42	–	
10	52.30	[2.05]	48.73	2.177	12.5 (12b), 5.1 (12a), 0.5 (17)
11	52.47	–	52.54	–	
12	27.44	[2.03, 2.45]	28.06	1.25 (a)	14.3 (12b), 5.1 (10)
				2.31 (b)	14.3 (12a), 12.5 (10), 7.9 (13)
13	37.70	2.37 dd (7.9, 4.7)	37.44	2.54	7.9 (12b), 4.7 (13)
14	91.27	3.39 dd (4.8, 1.1)	90.50	3.49	4.7 (13), 1.2 (16)
15	44.48	2.15 dd (14.4, 8.2)	43.65	2.33 (a)	14.8 (15b), 8.3 (16)
		2.22 dd (14.4, 8.2)		2.37 (b)	14.8 (15a), 8.3 (16)
16	84.68	[3.29]	83.69	3.43	8.3 (15a), 8.3 (15b), 1.2 (14)
17	62.20	3.02 br.s	68.91	3.71	0.7 (6b), 1.0 (5), 0.5 (7)
19	55.87	2.45 d (11.4)	178.93	8.34	1.2 (21a), 1.2 (21b), 1.4 (5) 3.0 (4)
		3.56 d (11.4)			
21	49.88	2.52 dq (13.0, 7.0)	56.49	3.96 (a)	14.3 (21b), 7.2 (22), 1.2 (19)
		[2.57]		4.03 (b)	14.3 (21a), 7.2 (22), 1.2 (19)
22	13.72	1.12 t (7.0)	13.43	1.48	7.2 (21a), 7.2 (21b)
$\underline{C}H_3$–O-1	56.73	3.305	56.81	3.20	
$\underline{C}H_3$–O-14	58.20	3.37	58.31	3.40	
$\underline{C}H_3$–O-16	56.35	3.295	56.33	3.36	
1′	118.69	–	124.81	–	
2′	141.88	–	141.29	–	
3′	122.03	8.42 ddd (8.8, 1.2, 0.3)	120.46	8.46	8.3 (4′), 1.2 (5′), 0.4 (6′)
4′	135.09	7.52 ddd (8.4, 7.3, 1.7)	132.21	7.35	8.3 (3′), 7.3 (5′), 1.7 (6′)
5′	124.23	7.12 ddd (8.0, 7.3, 1.2)	123.47	7.04	7.8 (6′), 7.3 (4′), 1.2 (3′)
6′	132.13	7.93 ddd (8.0, 1.7, 0.3)	132.40	8.03	7.8 (5′), 1.7 (4′), 0.4 (3′)
1′-$\underline{C}$=O	168.37	–	174.34	–	
2′-NH–$\underline{C}$=O	171.42	–	171.16	–	
$\underline{C}H_3$–CONH-2′	24.96	2.19	25.09	2.17	

Notes: (1) chemical shifts in square brackets were taken from the 2D $^{1}H–^{13}C$ shift correlation spectrum; (2) photolysis in CD_3OD resulted in the product with no detectable signals of the atoms C-4 and H-4.

group at C-1. Analysis of $^{1}H–^{1}H$ vicinal spin–spin couplings of the protons H-1, ^{2}H-2, and ^{2}H-3 shows the carbocycle C1–C2–C3–C4–C5–C11 to be a partially-distorted chair in lappaconitine **1** and the twist-form in the immonium salt **4**. The chair → twist conversion seems to be a result of the Coulomb interaction which forces the methoxyl to come closer to the immonium moiety. Semi-empirical quantum chemical calculations (AM1, PM3) show that the twist-form should be more stable than the chair ($\Delta\Delta G^{\circ} = 1–3$ kcal/mol) with the estimated $O\cdots N^{+}$ distance of 2.74–2.75 Å for the twist-form and 2.94–3.02 Å for the chair (Note: the calculations by MNDO and MINDO/3 methods do not fix the chair-like form as stable conformation and geometry optimization invariably transform the molecule to the twist-form).

In addition, the chemical shifts of the H-12 protons are determined by the anisotropy of the magnetic susceptibility of the C(1)–O bond, so the chair → twist conversion changes the orientation of the methoxyl C-1 relative to the two H-12 protons

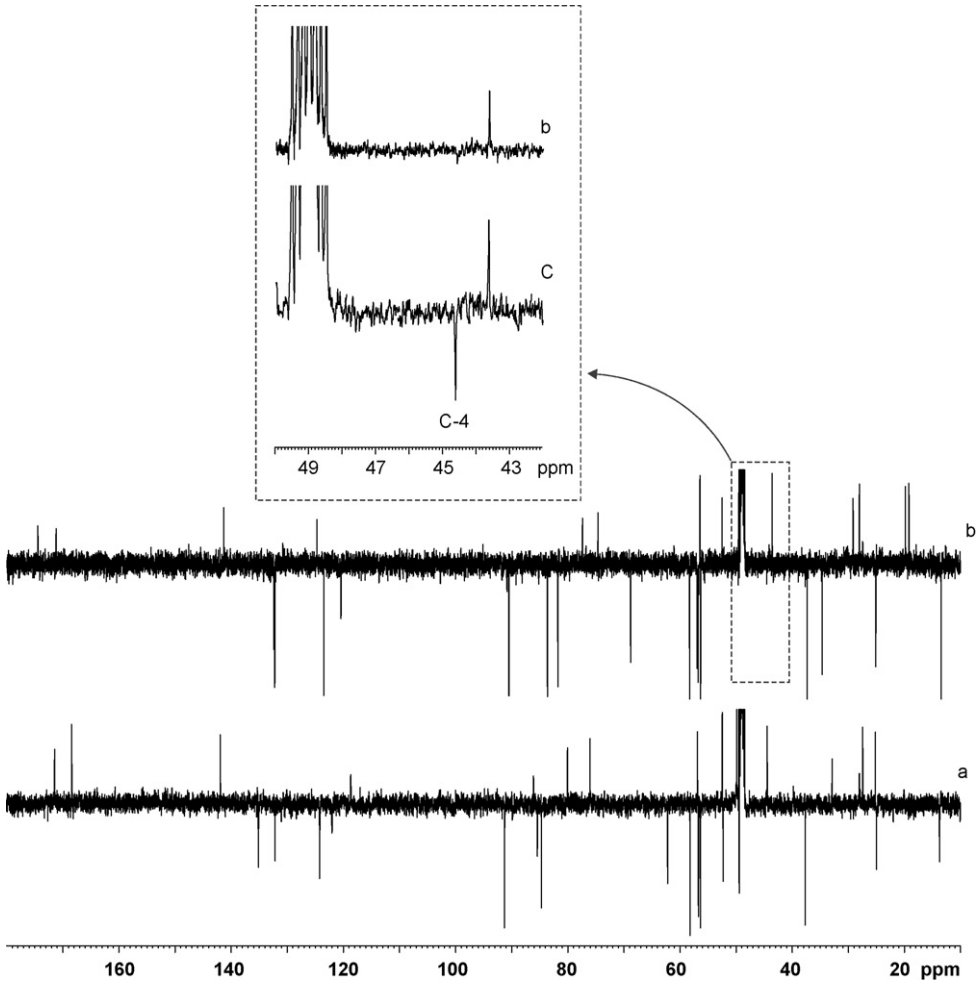
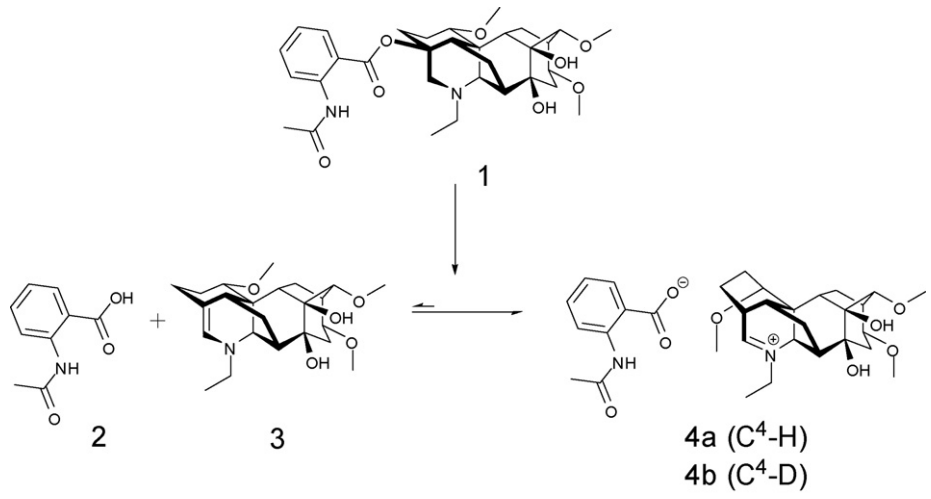


Fig. 3. 1D *J*-modulated ^{13}C spectrum (125.75 MHz, $J = 140$ Hz, broad band decoupling), of lappaconitine (a); the photolysis products in CD_3OD (b); and the photolysis products in CH_3OH (c).

and thus results in a significant up-field shift of one of the H-12 protons.

Immonium compounds of the type **4** are known in the series of diterpene alkaloids. For example, cyclic *N*-methylimmonium salt was prepared by methylation of the corresponding cyclic diterpene imine and shows the following set of signals of the group N^+=CH– in the NMR spectra ($CDCl_3$): δ_H 7.5 ppm, δ_C 164.6 ppm [9]. The immonium salt **4** is nothing but the protonated form of the enamine **3**, so the salt **4** exists in equilibrium with the neutral pair of free tertiary amine **3** and *N*-



Scheme 1. Photo-induced decomposition of lappaconitine in solution.

acetylanthranilic acid **2**. Although, the later compound is quite a weak acid and normally does not form salts with amines, enamine **3** is a very strained compound because of the presence of the carbon–carbon double bond at the head of the bridge, so protonation of enamine **3** at C-4 takes away the molecular strain. Enamine **3** is not observable because chemical shifts of the carbons C-4 (δ 44.64 ppm) and C-19 (178.93 ppm) indicate the equilibrium to be shifted completely towards immonium salt **4**, but the equilibrium is proved by isotope exchange at C-4 when compound **4a** is placed in a deuterated medium (CD_3OD): the photolysis in CD_3OD results in the [4-^{2}H]-derivative, while the photolysis in CH_3OH gives rise the non-deuterated product which is being transformed slowly to the [4-^{2}H]-derivative when dissolved in CD_3OD.

^{15}N NMR spectrum of the product contains only two signals at 191 and 287 ppm which correspond to the *N*-acetylanthranilic and the immonium fragment of **4**.

4. Conclusions

The results obtained show that the biologically active alkaloid lappaconitine is very sensitive to UV irradiation, so its therapeutic activity may decrease because of rapid photodegradation due to daylight exposure even in the absence of any additive. Chemical structure of the photodegradation products was determined by multinuclear NMR techniques: UV-induced photodecomposition of lappaconitine results in cleavage of the complex starting molecule to liberate *N*-acetylanthranilic acid **2** and diterpene nuclei in the form of enamine **3**, which clenches the liberating *N*-acetylanthranilic acid to form the corresponding immonium salt **4.** Identification of the new products of the natural alkaloid photolysis might be helpful in discovering the biogenesis of the aconitum-type diterpene alkaloids.

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