

IDENTIFICATION OF SHIKONIN FROM THE ROOTS OF *ECHIUM PYCNANTHUM POMEL.*TARIK CHAOUCHE^{Med*}, FARAH HADDOUCHI, FAWZIA ATIK BEKKARA

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ABSTRACT

Qualitative analysis of quinones by TLC and ¹H and ¹³C NMR, allowed us to purify and determine the structure of quinones responsible for the color red roots. It is a derivative of shikonin, the angelylshikonine. This result confirms that our plant belongs unequivocally to the genus *Echium*.

Keywords: angelylshikonine,¹H and ¹³C NMR, medicinal plants, quinones, TLC.

INTRODUCTION

Echium pycnanthum (family Boraginaceae) is a wild plant, endemic to the arid and desert¹. It is well known among traditional healers, is traditionally used to treat hepatitis. It is mostly red roots that are used and sold in markets. The need to discover the nature of that color, led us to a study by ¹H and ¹³C NMR.

Shikonin, its enantiomer alkannin and their ester derivatives (shikonins and alkannins) are well known secondary metabolites most commonly found in the root epidermis of many traditional medicinal plants of the Boraginaceae family,^{2,3}(Fig. 1).

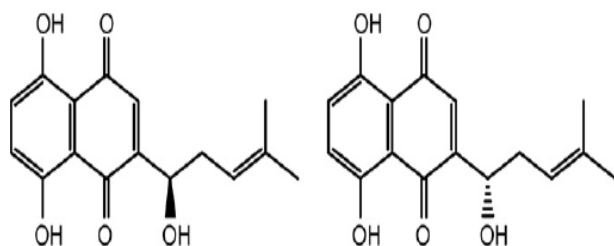
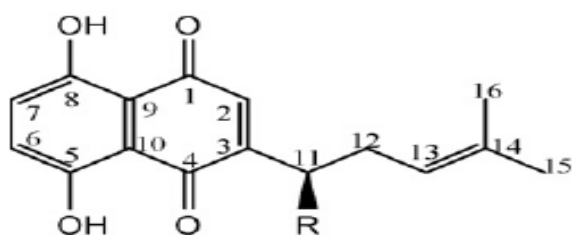


Figure 1: Enantiomers shikonin (left) and alkannin (right).

These naphthoquinones are characteristically red and were originally used as colorants for foods, cosmetics and fabrics. Subsequently they found applications in traditional medicine⁴. Shikonins and alkannins possess the isohexenylnaphthazarin structure and have anti-inflammatory^{5,6}, antioxidant^{7,8}, antitumor⁹, wound healing¹⁰, antibacterial¹¹, antithrombotic¹² and antifungal properties¹³.

There are nine shikonin derivatives: shikonin (S), acetylshikonine (AS), propionylshikonine (PS), isobutyrylshikonine (IBS), tiglylshikonine (TS), 3,3-dimethylacrylshikonine (DAS), angelylshikonine (ANS), 2-methyl-nbutyrylshikonine (MBS) and isovalerylshikonine (IVS). MBS / IVS and TS / DAS / ANS are positional isomers and TS / ANS are geometric isomers (Fig. 2)¹⁴.



To know the nature of quinones responsible for the color red roots of *Echium pycnanthum*, we did a chemical analysis from an analytical technique ¹H and ¹³C NMR.

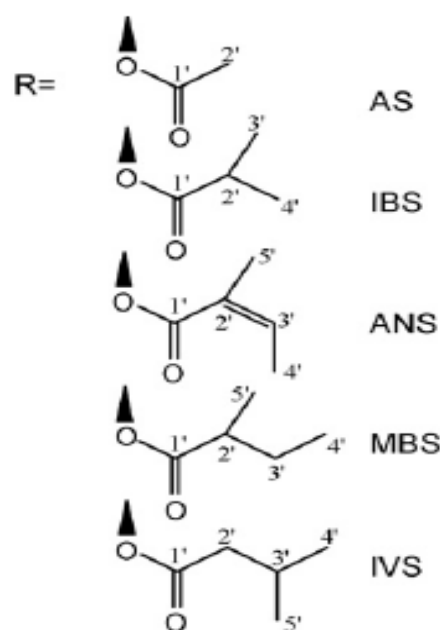


Figure 2: Shikonin pigments isolated from *Echium italicum* L. and subjected to NMR analysis.

MATERIAL AND METHODS

Phytochemical screening of quinone

The color red more or less the chloroform extract (1 mL) supplemented with NH₄OH diluted by half, indicating the presence of free anthraquinones¹⁵.

Extraction and isolation of quinones

5 g of plant powder are moistened with a few drops of hydrochloric acid in 1 / 5 and are placed in a stoppered conical flask with 30 mL of ether-chloroform. After 24 hours of soaking, the solvent is filtered and then evaporated to dryness¹⁴.

ATLC was chosen to characterize the extracted quinones

The solvent use dihexane-ethyl acetate (80/20). The filing was in the form of a band, deposited over the entire width of the plate. After developing the chromatogram, the plates were dried at room temperature and then examined in the UV ($\lambda=365\text{nm}$)¹⁴. The appearance of several bands of Rf and color, was observed. We scraped the red band with Rf 0.75 and we have dissolved in methanol. The extract is filtered to get rid of the silicagel. We established a second chromatography of the extract obtained in the

same manner as the first, to purify it. Achieving a single band of the same Rf confirmed the purity of our sample.

¹³C-NMR analysis

NMR spectra were recorded on a Bruker AC 200 Fourier transform spectrometer operating at 50.323 MHz for ¹³C, equipped with a 10 mm (or 5 mm) probe, in deuterated chloroform (CDCl₃), with all shifts referred to internal tetramethylsilane (TMS). ¹³C-NMR spectra were recorded with the following parameters: pulse width (PW), 5 μs (or 3 μs) (flip angle 45°); acquisition time, 1.3 s for 32 K data table with a spectral width (SW) of 12 500 Hz (250 ppm); CPD mode decoupling; digital resolution 0.763 Hz per pt. In a typical procedure, 280 mg of extract quinone were used in 2 mL (or 0.5 mL) of CDCl₃. The number of accumulated scans ranged between 2000 and 10 000 for sample, depending on the amount of oil available. Exponential line broadening multiplication (LB = 1 Hz) of the free induction decay (FID) was applied before Fourier transformation.

RESULTS AND DISCUSSION

The test result indicates the presence of phytochemical quinones in the roots. The extraction yield is 1.51%. The identification of compound sis based on comparison of chemical shifts and ¹H-¹³C NMR of four samples (Table 1) with those of naphthoquinones present in the roots of *Echiumpyrcanthum* compounds mentioned in the literature (Table 2).¹⁶ The chemical shifts of ¹H and ¹³C NMR of four samples correspond to the values of angelylshikonine (ANS) shown in Table 2.¹⁶ Chemical shifts for carbon atoms 1, 3, 4, 5, 8, 9, 10, 14 and 1' do not

appear in the spectrum of four samples. It is a quaternary carbon whose intensity is slow compared to other carbons (about 1/3).

Table 1: Spectra of ¹H and ¹³C for the sample of quinones extracted from *Echiumpyrcanthum*.

	Chemical shifts (δ) in ppm	
	¹ H	¹³ C
1	-	-
2	6.96 d	131.5
3	-	-
4	-	-
5	12.60 s	-
6	7.19 s	132.6
7	7.19 s	132.7
8	12.43 s	-
9	-	-
10	-	-
11	6.08 ddd	69.2
12	2.51m, 2.66 m	32.9
13	5.15 m	117.9
14	-	-
15	1.58d	25.8
16	1.69 d	17.9
1'	-	-
2'	-	139.9
3'	6.17 qq	-
4'	2.01 dq	15.8
5'	1.96 m	20.5

Table 2: ¹H and ¹³C spectra of five shikonins.

Atom number	Chemical shifts δ (ppm) ^a									
	AS		IBS		ANS		MBS		IVS	
	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C
1	-	176.6	-	176.8	-	177.0	-	176.7	-	176.7g
2	6.99	131.4	6.98 d	131.3	6.96 d	131.5	6.98 d	131.3	6.99d	131.4
3	-	148.1	-	148.5	-	148.7	-	148.6	-	148.5
4	-	178.1	-	178.3	-	178.5	-	178.2	-	178.2g
5	12.58 s	166.9	12.59 s	166.8	12.60 s	166.7	12.59 s	166.9	12.59 S ^b	166.9f
6	7.18 s	132.6	7.19 s	132.7	7.19 s	132.6	7.19 s	132.7	7.18s	132.7e
7	7.18 s	132.8	7.19 s	132.8	7.19 s	132.7	7.19 s	132.8	7.18s	132.8e
8	12.42 s	167.4	12.43 s	167.4	12.43 s	167.2	12.42 s	164.4	12.42 S ^b	167.4f
9	-	111.5	-	111.6	-	111.6	-	111.6	-	111.6d
10	-	111.7	-	111.8	-	111.8	-	111.8	-	111.8d
11	6.03 ddd	69.4	6.02 ddd	69.0	6.08 ddd	69.2	6.04 ddd	69.0	6.04 ddd	69.2
12	2.47 m	32.8	2.47	32.9	2.52	32.9	2.47m, 2.62m,	33.0	2.47m, 2.61m,	33.0
13	2.62 m	117.6	m, 2.69m	117.6	m, 2.66m	117.9	5.13 m	117.8	5.12m	117.8
14	5.12 m	136.0	5.12 m	136.0	5.15 m	135.9	-	135.9	-	136.0
15	-	25.7	-	25.7	-	25.8	1.59 d	25.7	1.58 d ^c	25.7
16	1.58 d	17.9	1.58 d	17.9	1.58 d	17.9	1.69 d	17.9	1.68 d ^c	17.9
1'	1.69 d	169.7	1.3.69 d	175.8	1.69 d	166.5	-	175.4	-	171.8
2'	-	20.9	-	34.0	-	139.9	2.47 m	41.2	2.27	43.4
3'	2.14 s	-	2.64 m	18.8	-	127.1	1.50 m	26.6	m, 2.28m,	25.8
4'	-	-	1.21 d	19	6.17 qq	15.8	0.92 t	11.6	2.13m	22.3 ^l
5'	-	-	1.20 d	-	2.01 dq	20.5	1.20 d	18.4	0.98d	22.4 ^l
					1.96 m				0.98d	

^a relative to TMS

CONCLUSION

Quinones present in the roots of *Echiumpyrcanthum* are derivative of shikonin. This is angelylshikonine responsible for the red color of the roots of this plant.

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