CYPHOPLECTINE, A NORDITERPENE ALKALOID FROM *DELPHINIUM CYPHOPLECTRUM*

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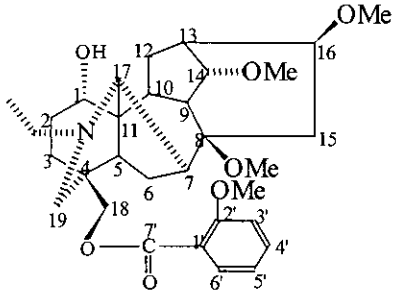
Abstract- From the aerial Parts of *Delphinium cyphoplectrum* Boiss.,a new norditerpene alkaloid named cyphoplectine has been isolated along with the known norditerpene alkaloids browniine and delphatine. The structure of cyphoplectine was established on the basis of ^{1}H, ^{13}C, APT, homonuclear ^{1}H COSY, HETCOR and NOESY NMR studies. The alkaloidal mixture showed slight antifeedant and insect repellent activities.

In continuation of our investigations of Turkish *Delphinium* species[1-3] for the diterpene alkaloids we have now studied *D. cyphoplectrum* Boiss. chemically, and have also investigated its insect repellent, antifeedant and insecticidal activities. The chemical investigation of the aerial parts of *D. cyphoplectrum* led to the isolation of a norditerpene alkaloid cyphoplectine together with two known alkaloids browniine [4] and delphatine.[5] The crude alkaloid, isolated from the plant material at pH 8-10, was purified on an Al_2O_3 column by VLC. Fractions obtained from this column were combined to give Fractions A-C. Chromatographic separations of the fractions A-C were performed on Al_2O_3 rotors of a Chromatotron. Fraction A yielded the two known compounds browniine and delphatine. Cyphoplectine (**1**) was obtained from Fraction B as an amorphous, homogenous alkaloid. Fraction C didn't yield any alkaloid. The EIMS and HRMS of the new compound (**1**) indicated the molecular ion at *m/z* 555 suggesting the formula $C_{32}H_{45}NO_7$.The NMR spectra of cyphoplectine (**1**) showed the presence of N-ethyl group [δ_C 13.9 q, δ_H 1.10 (3H, t, J=7 Hz); δ_C 51.8 t, δ_H 2.48, 2.62 (2H, m)], as well as three methoxy groups [δ_C 59.3q, δ_H 3.42 (3H, s); δ_C 57.5q, δ_H 3.37 (3H, s); δ_C 56.1q, δ_H 3.34 (3H, s)]. These signals and biogenetic considerations indicated that cyphoplectine is a norditerpenoid alkaloid. The downfield signals [δ_C 166.8 s, carboxyl C; δ_C 129.9 d, δ_H 7.10 (1H, br d, J=8.5 Hz); δ_C 127.7 d, δ_H 7.20 (1H, br d, J=8.5 Hz); δ_C 115.3 d, δ_H 6.80 (4H, m)] and an aromatic methoxy group [δ_C 55.3q, δ_H 3.74 (3H, s)] together with COSY experiment (Table 1) showed the presence of a methoxybenzoate moiety attached to the alkaloid. The MS

degradation signals correlated the presence of this moiety having the base peak at *m/z* 420 $[M-C_8H_7O_3]^+$ and a strong peak at *m/z* 404 $[M-C_8H_7O_2]^+$. The ^{13}C NMR (APT) spectrum indicated the presence of five methyl, eight methylene, thirteen methine and six quaternary carbons for 32 C atoms of cyphoplectine. Besides the *O*-methoxybenzoate group the alkaloid should contain three methoxy and one hydroxyl groups. From the NMR signals (δ_C 72.1 d, δ_H 3.85 m), the hydroxyl placed at C-1, the correlation of H-1 with the protons at C-2 (δ_C 26.5 t, δ_H 1.25 m , H-2α; 2.00, m, H-2β) was observed in the COSY spectrum. NOESY experiment showed H-1β was on the same side with H-10, thus indicating the hydroxyl at C-1 is α.



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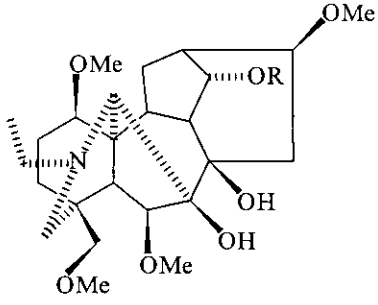

R=H Brownine
R=OMe Delphatine

The first of the three methoxy groups was placed at C-16 due to biogenetic reasons and from the NMR shifts (δ_C 84.6 d, δ_H 3.8, 1H, dd, J=7 and 14 Hz). H-16 showed correlation with the protons at C-15 [δ_C 45.0 t, δ_H 2.65 (1H, dd, J=12, 14, H-15 α), 2.90 (1H, dd, J=7,12 Hz, H-15 β)] in the COSY spectrum, the second methoxyl was situated at C-14 as decided from the typical signal at δ_H 3.65 (1H, t, J=5 Hz) and δ_C 82.1 d, H-14 showed correlations with H-13 [δ_C 42.0 d, δ_H 1.20 (1H, m)] in the COSY and with the protons H-9, H-10 and H-12β in the NOESY spectra. The third methoxy group was placed at C-8 (δ_C 78.5 s). The only plausible place for the *O*-methoxybenzoate seems to be at C-18 which was also shown from the NMR data [δ_C 68.4 t, δ_H 4.20 (1H, d, J=10 Hz) and 4.22 (1H, d, J=10 Hz)]. The structure of cyphoplectine was established from its ^1H-^1H COSY, ^1H-^{13}C HETCOR and from NOESY experiments (Table 1).

Using 10% ethanolic stock solution of the crude alkaloidal mixture, antifeedant, insect repellent and insecticidal tests were carried out. Although no insecticidal activity was found against *Spodoptera littoralis* Boiss., slight activity was established in antifeedant and insect repellent tests. These results were shown in Table 2.

EXPERIMENTAL

General Experimental Procedures. - ^1H NMR spectra were recorded on 200 MHz and 500 MHz instruments, and ^{13}C NMR spectra on 50.0 MHz and 125.0 MHz instruments in $CDCl_3$. HRMS were measured at 70 eV. IR spectra were recorded in $CHCl_3$. Chromatographic separations were carried out on a Chromatotron instrument using rotors coated with 1mm thick layer of neutral Al_2O_3.

Table 1. NMR data of Cyphoplectine (**1**)

Position	^1H	^{13}C	COSY ^1H-^1H	NOESY
1β	3.85 m	72.1 d	H-2α, H-2β	H-3β, H-10
2α	1.25 m	26.5 t	H-1β, H-2β, H-3β	
2β	2.00 m		H-1β, H-2α	
3α	1.30 m	31.7 t	H-2β, H-3β	
3β	2.40 m		H-3α	H-1β
4	-	38.1 s		
5	1.80 m	41.6 d	H-6	H-6
6	1.60 m	25.0 t	H-5	H-5
7	2.47 m	46.9 d	H- 6	H-6
8	-	78.5 s		
9	1.75 m	42.3 d	H-10, H-14	H-5
10	1.85 m	35.7 d	H-9	
11	-	49.4 s		
12α	1.70 m	29.5 t	H-12 β	
12β	2.30 m		H-12 α	
13	1.20 m	42.0 d	H-12 α, H-14	
14	3.65 t (5)	82.1 d	H-13	H-9, H-10, H-12β
15α	2.65 dd (12,14)	45.0 t	H-15 β, H-16	H-16
15β	2.90 dd (7,12)		H-15 α, H-16	
16	3.80 dd (7,14)	84.6 d	H-15 α, H-15 β	
17	2.70 brs	65.6 d		
18 α	4.20 d (10)	68.4 t		
18 β	4.22 d (10)			
19 α	1.80 m	60.1 t		
19 β	3.30 m			
N-CH_2	2.48 (m); 2.62 (m)	51.8 t		
CH_3	1.10 t (7)	13.9 q		
OMe-8	3.42 s	59.3 q		
OMe-14	3.37 s	57.5 q		
OMe-16	3.34 s	56.1 q		
1'	-	123.2 s		
2'	-	145.0 s		
3'	7.20 brd (8.5)	127.7 d	H-4'	
4'	6.80 m	115.3 d	H-3', H-5'	
5'	6.80 m	115.3 d	H-4', H-6'	
6'	7.10 brd (8.5)	129.9 d	H-5'	
7'	-	166.8 s		
OMe-2'	3.74 s	55.3 q		

Plant material. - *Delphinium cyphoplectrum* Boiss. was collected from Van-Erek mountain of Eastern Turkey at an altitude 2000 m in July 1993. A voucher (H.Ö. 6345) specimen is deposited in the Herbarium of the Faculty of Sciences, University of Süleyman Demirel (Isparta).

Extraction of Crude Alkaloids. - Dried and powdered aerial parts (200 g) of the plant were macerated in MeOH (600 mL) for 24 h at 22°C, filtered and evaporated *in vacuo* to yield 9.5 g of a residue. The residue was acidified to pH 1.5 by 5% H_2SO_4 and extracted with CH_2Cl_2 to obtain the nonalkaloidal mixture (7 g). The acidic aqueous solution was basified (pH 8-10) by using 10% NaOH and extracted with CH_2Cl_2 (25x100 mL) to yield 150 mg of crude alkaloidal mixture, which was fractionated into three main fractions A-C in a VLC column eluting with ethyl acetate followed by a gradient of methanol up to 10 %. Fractions A-C were separated on neutral Al_2O_3 rotors of a Chromatotron respectively, eluting with ethyl acetate and methanol to yield alkaloids. Browniine (15 mg) and delphatine (8 mg) were isolated from fraction A, while cyphoplectine (**1**) (20 mg) was isolated from fraction B. Fraction C didn't yield any alkaloid.

Cyphoplectine (**1**) - $[\alpha]_D$ =+0° ($CHCl_3$, 0.1). IR $\nu_{max}^{CHCl_3}$cm^{-1}: 3430, 3050, 2980, 2850, 1705, 1680, 1580, 1520, 1460, 1350, 1205, 1110, 1050, 980, 850. ^{1}H and ^{13}C NMR spectra ($CDCl_3$) given in Table 1. HRMS*m/z* (rel.int.): 555.3188 $[M]^+$. MS *m/z* (rel.int.) 555 (2), 524 $[M\text{-}OMe]^+$ (2), 420 $[M\text{-}C_8H_7O_2]^+$ (100), 404 $[M\text{-}C_8H_7O_3]^+$ (45), 388 (22), 350 (20), 330 (35), 282 (15), 178 (25), 107 (22), 82 (55), 71 (33).

Biological Tests. - Insect: *Spodoptera littoralis* Boisd. (Lep., Noctuidae) was reared on parsley leaves, under long-day photoperiod (16L:8D) at 25±1°C and 65% relative humidity. Larvae used for mortality test were newly hached first instar, for antifeedant test were 115±5 mg of weighed larvae, and for repellency test were newly hached first instar and 115±5 mg of weighed larvae.

In all tests, extract was applied as freshly prepared emulsions of their ethanolic solutions in water. Known amount of particular extract was dissolved in ethanol to obtain 10% (w/v) stock solutions. Various concentrations of extract emulsions (2.5 and 5g dried extracts in 100 mL distilled water are referred to in the text as 2.5 and 5%) were prepared by diluting appropriate volumes of stock solutions in a small volume of ethanol and then the diluted solutions were emulsified in distilled water containing Triton X-100 0.1% as an emulsifier.

Leaves of parsley were submersed in particular extract emulsion for 3-5 seconds, the excess of liquid was blotted on towel paper, and then the leaves were air-dried. Control leaves were dipped in water containing ethanol and aforementioned surfactant at the same concentration as in the extract emulsions.To determine the effectiveness of extract on the basis of mortality, completely dried leave particules were placed in petri dishes (11 cm diameter). The lower end of each stem part was wrapped up with moist cotton wool to maintain freshness and prevent drying of the foliage during the time to exposure. Larvae were deposited in the groups of 10 first instar exactly onto the treated leaves and were allowed to feed for 48 h. After this time, larvae were transferred to untreated, daily renewed parsley leaves and observed for larval mortality until death of larvae.[6] There were five replications.

To obtain information on a possible repellent effect of these extracts on larvae of *S.littoralis*, an aqueous extract of plant extracts was prepared as described above. Parsley leaves were divided into two groups, one group being dipped into the extracts, and the other one into distilled water. After drying the leaves from both groups were placed in a petri dish (15 cm diameter), without touching each other in an appropriate way so that their cut ends showed periphery. Ten first instar larvae were then released exactly in the middle of each petri dish. Possible repellent effect was determined after 1st and 18th hour counting of larval distribution onto both sides.[7] This test was performed five replications.

To determine antifeedant effect of extracts to larvae, larvae weighing 115 ±5 mg were allowed to feed for 48 h on the treated parsley. The parsley was kept in plastic containers, covered with cloth held in place with rubber bands. At the end of exposure period, larvae were weighed. Larval weight were compared with control.[8,9] This test was performed ten replications, each replication consisting of one larva.

The insecticidal effectiveness of the plant extract used were found not enough to use in practice (Table 2). From the antifeedant test results, it could be concluded that all the extracts for 48 h test period caused insects not to feed on it. Repellency tests showed that larval stages of *S.littoralis* were affected by the application of the extracts used.

Table 2. Biological test results

Mortality test results

		% Mortality	
Stage of larvae	Concentration	*D. cyphoplectrum*	Control
First instar	(%) 5	40.0	13.3

Repellency test results

		% Repellency			
		D. cyphoplectrum		Control	
Stage or weight of larvae	Concentration (%)	1.h	18.h	1.h	18.h
First instar	2.5	27.3	22.2	72.7	77.8
115±5.0 mg	5.0	50.0	33.3	50.0	66.7

Weight gain (antifeedant) test results

		Weight change of larvae (%) after 48 h	
Initial weight of larvae	Concentration (%)	*D. cyphoplectrum*	Control
115±5.0 mg	1.0	-10.0	+28.8

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