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Nα-quinaldyl-L-arginine·HCl, a new defensive alkaloid from *Subcoccinella-24-punctata* (Coleoptera, Coccinellidae)

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Abstract. The isolation of Nα-quinaldyl-L-arginine·HCl (**1**) from the Coccinellidae *Subcoccinella-24-punctata* is reported. The structure, first established on the basis of the analysis of the spectral properties of **1**, has been confirmed by synthesis. The alkaloid is of endogenous origin and markedly deterrent to ants.
Key words. Coleoptera; Coccinellidae; *Subcoccinella-24-punctata*; chemical defense; quinoline alkaloid; Nα-quinaldyl-L-arginine·HCl.

Ladybirds have few natural enemies. This has been attributed in part to the fact that when molested, they emit hemolymph droplets at the joints of their legs. This behaviour is known as reflex bleeding[1]. It has been demonstrated that the repellent properties of this fluid from several species of Coccinellidae result from the presence of alkaloids[2,3]. Many types of defensive alkaloids have so far been discovered, including aliphatic and aromatic amines[4,5], azaphenalenes[6,7], homotropanes[3,8,9], piperidines[5,9], pyrrolidines[5], azamacrolides[10] and polycyclic dimeric alkaloids[11,12].

The object of this paper is to report the isolation and characterization of Nα-quinaldyl-L-arginine·HCl (**1**) from the phytophagous coccinellid *Subcoccinella-24-punctata*. This alkaloid represents a new type of ladybird defensive substance.

Materials and methods

Extraction and isolation. Three hundred and twenty eight adults of *Subcoccinella-24-punctata* (Coleoptera, Coccinellidae) were collected on *Saponaria officinalis* in a meadow close to Petrich (Bulgaria) and preserved in methanol. Exhaustive extraction of the insects with methanol gave a crude extract (436 mg) that was partitioned between the two phases of the mixture MeOH/H_2O/hexane (5:5:5). The aqueous layer was evaporated to dryness under reduced pressure and the residue (380 mg) was chromatographed over a column of Sephadex LH20 (eluent: MeOH). The separation was monitored by thin layer chromatography (TLC) (SiO_2; CH_2Cl_2/MeOH/NH_4OH 6:4:0.3; Dragendorff reagent). This led to the isolation of a main Dragendorff positive fraction that was further purified by chromatography over a column of Sil RP-18 eluting with a solvent gradient system from H_2O/MeOH 85:15 to 70:30 to give compound **1** (8 mg) homogenous in TLC and high pressure liquid chromatography (HPLC).

Spectroscopic and chromatographic methods. Ultraviolet (UV) spectra were taken on a Philips PU 8700 UV-VIS spectrophotometer in MeOH. Electron ionization mass spectrometry (EIMS) measurements were performed on a VG Micromass 7070F, and Electron spray mass spectrometry (ESMS) on a Fisons VG Quatro II. Infrared (IR) spectra were recorded on a Bruker IFS 25 instrument as KBr pellets. The ^1H and ^{13}C NMR spectra were recorded in CD_3OD at 600 and 150.87 MHz (Varian Unity 600 instrument). The chemical shifts are reported in ppm (δ) and the coupling constants in Hertz. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 589 nm (Na D line) in a 1 dm cell. Gel permeation chromatographies were performed over Sephadex LH-20, reversed-phase chromatographies over Sil RP-18 prepared according to Kuhler and Lindsten[13] and TLC analyses on Polygram SilG/UV_{254} precoated plates (0.25 mm). HPLC were performed using a Waters LC Model 1 instrument equipped with a Lichrospher 100 RP-18 column (5μ; 250 mm × 4 mm i.d.).

Synthesis of compound 1. A mixture of quinaldic acid (**2**, 686 mg), thionyl chloride (1.6 ml) and 1 drop of DMF was heated under reflux for 2 h. The excess of reagent was eliminated by evaporation under reduced pressure to give crude quinaldic acid chloride (**3**). Part of the crude acid chloride (95.5 mg) was dissolved in CH_2Cl_2 (2 ml), then L-arginine (53 mg) and sodium bicarbonate (126 mg) dissolved in water (2 ml), were added to the solution. The resulting mixture was stirred overnight at room temperature. After decantation and

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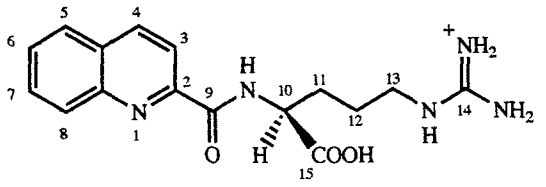

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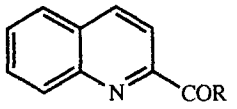
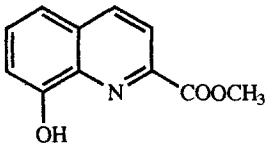


2 R = OH

3 R = Cl



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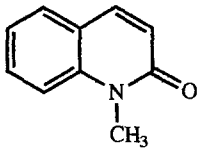

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separation of the two phases, the aqueous layer was washed with CH_2Cl_2 and evaporated under reduced pressure to dryness. The solid residue was chromatographed over a column of Sephadex LH-20 (eluent: MeOH). This led to an authentic sample of Nα-quinaldyl-L-arginine·HCl (38 mg) that was compared to the natural compound **1**: $[\alpha]_D$ 26.8 (c 0.47, MeOH) for the synthetic sample and $[\alpha]_D$ 24.8 (c 0.5, MeOH) for the natural sample; identical Rf (SiO_2; CH_2Cl_2/MeOH/NH_4OH 6:4:0.3) and HPLC retention time (Lichrospher 100 RP-18; CH_3CN/H_2O/TFA 3:7:0.01); identical ^1H NMR and IR spectra were obtained.

Results and discussion

Compound **1** was isolated as an optically active white amorphous solid ($[\alpha]_D$ 24.8 (c 0.5, MeOH)). The ESMS displayed a $(M+H)^+$ ion at m/z 330 Daltons and the ^{13}C NMR spectrum (broad band proton decoupling) indicated the presence of 16 carbon atoms. These data are compatible with the elemental composition $C_{16}H_{20}N_5O_3$.The UV spectrum [λ_{max} in MeOH at 238 (22535), 315 (2066) and 352 nm (1485)] was reminiscent of a heteroaromatic chromophore. The number of nitrogen atoms and a quaternary carbon atom signal at δ 158.8 in the ^{13}C NMR spectrum suggested a guanidinium moiety.

The ^1H and ^{13}C NMR spectra are presented in table 1. Direct C-H connectivities followed from the analysis of the ^1H-detected heteronuclear multiple quantum coherence (HMQC) spectrum. Three independent spin systems could be clearly deduced from the homonuclear correlation spectroscopy (COSY) spectrum: a system of four aromatic protons (H-5 to H-8), a system of two aromatic protons (H-3 + H-4), and the sequence –CH–CH_2–CH_2–CH_2–. In addition, signals at δ 178.1 and 165.8 in the ^{13}C NMR as well as large IR absorption bands at 3364 to 2800 and 1654 cm^{-1} suggested the presence of two carbonyl groups, probably involved in amide and carboxylic acid functions respectively. All these data led us to propose that compound **1** is arginine acylated by quinaldic acid (2). Comparison of the NMR data of **1** with those of these two compounds supported this proposal (table 1). That the quinaldic acid moiety was linked to the α-amino group of arginine followed from the HMBC spectrum. Indeed, the proton at δ 4.51 attributed to HC-10 was found to be correlated to the two carbonyl carbons at δ 178.1 and 165.8.

To confirm this structural hypothesis, an authentic sample of Nα-quinaldyl-L-arginine·HCl was synthesized by coupling quinaldic acid chloride (3) with L-arginine. The chromatographic (Rf, HPLC retention time) and spectroscopic (^1H NMR and IR) properties of the amide so prepared were identical to those of the natural derivative. Since the rotatory power of the two compounds had the same sign, it follows that the configuration of the stereogenic carbon atom of arginine in the natural compound should be *S* (L-series). As far as we know, neither Nα-quinaldyl-L-arginine·HCl (**1**) nor quinaldic acid (**2**) has been isolated from natural sources.

Compound **1** is most probably of endogenous origin since the methanolic extract of the host plant (*Saponaria officinalis*) on which the specimens of *Subcoccinella-24-punctata* were collected was devoid of this alkaloid. Moreover, a few specimens of the same ladybirds collected on alfa-grass were also found to contain Nα-quinaldyl-L-arginine·HCl (detection by HPLC and TLC).

The feeding deterrencies of compound **1**, quinaldic acid and L-arginine·HCl were compared using an assay in which ants (*Myrmica rubra*) were given the choice between either 50 µl of pure sucrose (10^{-1} M) or of the same sucrose solution in which various amounts of the tested substances were dissolved. The testing protocol was as previously described[14]. The frequency of ant visitation to the two solutions was scored and provided the basis for calculation of relative acceptability of the three compounds. Three different concentrations (10^{-2}, 10^{-3} and 10^{-4} M) were tested and each experiment was repeated five times. The percentages of ants feeding on a concentration range of the different compound solutions are reported in table 2. It is clear from the results, that Nα-quinaldyl-L-arginine·HCl proved to be deterrent to ants at much lower concentration than L-arginine·HCl and quinaldic acid. It is highly deterrent at a concentration of 10^{-2} M. The latter concentration is roughly equivalent to the alkaloid concentration in the beetle, suggesting that the compound may act as an effective defense mechanism against ants. Nα-quinaldyl-L-arginine·HCl is more deterrent to ants (concentration

Table 1. ^1H and ^{13}C NMR data of compound **1** [δ (J in Hz)] and comparison with those of quinaldic acid (**2**) and L-arginine·HCl.

#C	Compound **1** [600 MHz]		Quinaldic acid (**2**) [300 MHz][15]		L-Arginine·HCl[300 MHz][16]	
	$^{13}C(CD_3OD)$	$^1H(CD_3OD)$	^{13}C(DMSO)	^1H(DMSO)	$^{13}C(D_2O)$	$^1H(D_2O)$
C-15	178.1	–	–	–	174.1	–
C-9	165.8	–	166.3	–	–	–
C-14	158.6	–	–	–	157.0	–
C-2	150.8	–	148.7	–	–	–
C-8a	148.0	–	146.7	–	–	–
HC-4	138.9	8.42 d(8.5)	137.5	8.60 d	–	–
HC-7	131.5	7.80 dd(8, 8.5)	130.4*	7.92 dd	–	–
HC-8	130.8°	8.13 d(8.5)	129.6*	8.25 d	–	–
C-4a	130.8°	–	128.7	–	–	–
HC-6	129.3	7.66 dd (8, 8)	127.9*	7.80 dd	–	–
HC-5	129.0	7.95 d(8)	128.4*	8.15 d	–	–
HC-3	119.4	8.14 d(8.5)	120.7	8.20 d	–	–
HC-10	55.6	4.51 dd(6, 6)	–	–	54.6	4.13 dd(6, 6)
H_2C-13	42.2	3.24 m	–	–	41.0	3.27 dd
H_2C-11	31.5	1.92/2.02 m	–	–	28.0	2.02 m
H_2C-12	26.1	1.72 m	–	–	24.4	1.88 m

°Signal split into two peaks in the spectrum taken in the mixture $CDCl_3/CD_3OD$.
*Assignments may be interchanged.

Table 2. Percentages of ants (*Myrmica rubra*) feeding on a concentration range of the different compound solutions (100% = total number of ants feeding).

Compounds	10^{-2} M	10^{-3} M	10^{-4} M
Quinaldic acid (**2**)	10*	22*	52
L-arginine·HCl	12*	47	NT
Nα-quinaldyl-L-arginine·HCl (**1**)	2*	8*	24*

The concentrations are in mole/L in sucrose 10^{-1} M. Asterisks indicate significantly lower percentages of ants at the tested solution than at the control solution ($p < 0.05$ in Wilcoxon matched-pairs signed-ranks test). NT = not tested.

for which 50% of the ants are repelled = RD_{50} = 10^{-4} M) than the other Coccinellidae alkaloids for which the deterrenty power to ants has been evaluated: coccinelline ($RD_{50} = 10^{-3}$ M)[2], convergine ($RD_{50} = 7.10^{-4}$ M)[2] and (+)-9-aza-1-methyl-bicyclo-[3.3.1] nonan-3-one ($RD_{50} = 10^{-3}$ M)[3].

Many quinoline alkaloids have been found in plants (e.g. Rutaceae, Compositae) and some microorganisms (e.g. *Pseudomonas* sp.)[17]. They are generally biosynthesized from anthranilic acid[18]. In insects only two quinoline alkaloids have been known until now. As with Nα-quinaldyl-L-arginine·HCl, both were isolated from Coleoptera species. 1-Methyl-2-quinolone (**4**) is one of the major bitter principles of the haemolymph of the Australian Lycidae, *Metriorrhynchus rhipidius*[19], and methyl 8-hydroxyquinoline-2-carboxylate (**5**) is the principal component of the prothoracic defensive gland of the water beetle *Ilybius fenestratus* (Dytiscidae)[20].

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