Myrmicarin 430A: a new heptacyclic alkaloid from *Myrmicaria* ants

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A heptacyclic alkaloid, myrmicarin 430A, showing an entirely new carbon skeleton made up of two unbranched C_{15}-chains, is identified from the poison gland secretion of African *Myrmicaria* ants by NMR spectroscopy.

Ants of the myrmicine group produce a large number of alkaloids, including pyrrolidines, piperidines, pyrrolizidines and indolizidines. These compounds exhibit a wide range of biological functions and are of considerable pharmacological interest.[1] Originating from the acetate pool, almost all of the identified ant alkaloids show unbranched carbon chains. We now report the identification of a heptacyclic alkaloid, myrmicarin 430A, from ants of the genus *Myrmicaria*,[2] showing an entirely new skeleton.

Poison gland secretion of the African ant, *Myrmicaria opaciventris*,‡ consists of a mixture of monoterpene hydrocarbons and new alkaloids (Fig. 1). GC–MS analysis revealed the presence of two different groups of alkaloids, consisting of 15 or 30 carbon and 1 or 2 nitrogen atoms, respectively, as shown by high resolution mass spectroscopy. Recently, we identified the main components of the $C_{15}N$ group, myrmicarin 215A, 215B and 217 (Fig. 2).[3]

Whereas these compounds could be isolated by column chromatography under argon,[3] isolation of the main component of the $C_{30}N_2$ set, myrmicarin 430A, failed due to its remarkable sensitivity. Fresh secretion exposed to air at ambient temperature for only 1 h, showed more than 90% decomposition of this alkaloid. Since we could not isolate myrmicarin 430A, we performed NMR spectroscopic analysis of poison gland secretion without any purification. For these experiments, an extract of 30 freshly dissected poison gland reservoirs was submitted to NMR spectroscopy immediately after preparation.

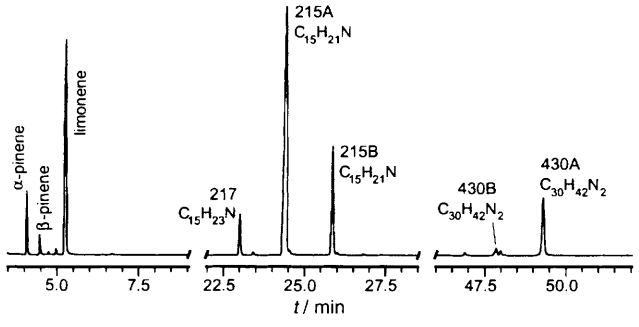

Fig. 1 Gas chromatogram of fresh poison gland secretion from *M. opaciventris* and molecular compositions of the main alkaloids

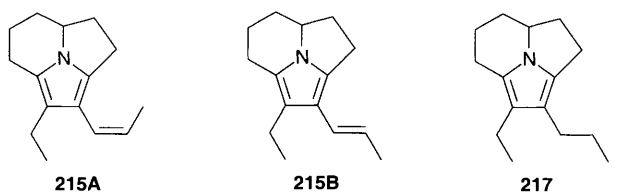

Fig. 2

Although the obtained one-dimensional ^1H and ^{13}C NMR spectra are very crowded especially in the aliphatic regions, careful analysis of phase sensitive (^1H,^1H)-DQ-COSY spectra allowed us to assign all signals of the already identified components myrmicarin 217, 215A and 215B as well as the signals of the monoterpene hydrocarbons. From the remaining crosspeaks three separate additional ^1H spin systems could be constructed (**I**, **II** and **III** in Fig. 3, Table 1). It was shown *via* (^{13}C,^1H)-correlation experiments that these spin systems form the main parts of myrmicarin 430A. In this case, HMQC experiments were insufficient to trace out the proton bearing carbons, since the principally limited resolution of ^{13}C chemical shift in HMQC led to serious crosspeak overlap. Best results were obtained with a sensitivity-enhanced phase sensitive HSQC with gradients,[4] providing sufficient resolution to distinguish between carbons from the $C_{15}N$-alkaloids, myrmicarins 215A/215B/217, and those of myrmicarin 430A.‡ Long-range (^{13}C,^1H) correlation (HMBC) was used to connect the spin systems *via* quaternary carbons (Fig. 3).

Spin system **I** shows chemical shift values for protons and the attached carbons that are very similar to those of myrmicarins 217, 215A and 215B,[3] suggesting a hexahydropyrrolo[2,1,5-*c*,*d*]indolizine subunit as part of the structure of myrmicarin 430A. The long-range correlations of spin system **I** to carbons appearing at δ 111.7, 118.1, 123.6 and 127.6, which are also correlated to an isolated ethyl group, confirm this structural relationship of myrmicarin 430A and myrmicarin 215A/B.

The remaining part of the myrmicarin 430A structure consists of another subunit, bearing spin systems **II** and **III** and the second nitrogen atom. Spin systems **II** and **III** could be connected *via* several long range correlations of the two carbons appearing at δ 145.7 and 154.1 and of a quaternary carbon at δ 62.1, which is also correlated to an isolated ethyl group (Fig. 3). Additionally, a small allylic coupling between the protons 3a-H and 4-H is observed, which corroborates our structural assignment. Relatively high chemical shift values of C-3b and C-8a as well as the characteristically low shift values of the carbons C-4 and C-8 indicate that these carbons form two enamine double bonds. The high sensitivity of myrmicarin

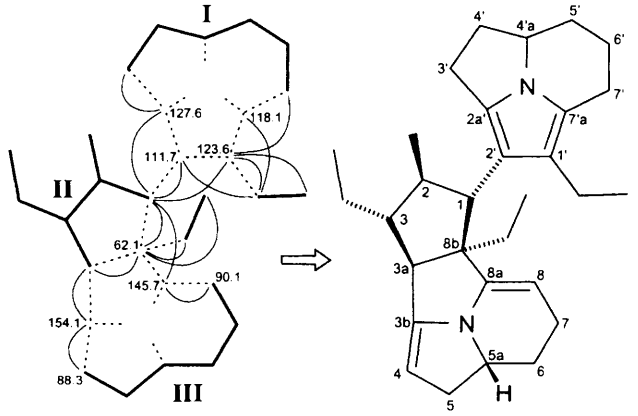

Fig. 3 ^1H Spin systems (**I**, **II** and **III**) and (^{13}C,^1H) long range correlations (HMBC) of quaternary carbon atoms in myrmicarin 430A

Table 1 ^{1}H NMR data (C_6D_6) of myrmicarin 430A; H_c and H_t refer to *cis*- and *trans*-orientation relative to 1-H in the tetracyclus or 4a′-H in the tricyclus

	δ	*J*/Hz
1-H	3.209	$J_{1,2}$ = 12.2
2-H	1.970	$J_{2,2\text{-}CH_3}$ = 6.8; $J_{2,3}$ = 10.5
2-CH_3	0.977	
3-H	1.657	$J_{3,3\text{-}CHaHb}$ = 9.2; $J_{3,3\text{-}CHaHb}$ = 4.0; $J_{3,4}$ = 8.1
3-CH_aH_b	1.352	$J_{3\text{-}CHaHb,3\text{-}CHaHb}$ = 13.1
3-CH_aH_b	1.851	
3-CH_2CH_3	1.156	$J_{3\text{-}CH_2CH_3,3\text{-}CH_2CH_3}$ = 7.4
3a-H	2.536	$J_{3a,4}$ = 0.5
4-H	4.380	$J_{4,5c}$ = 1.6; $J_{4,5t}$ = 2.6
5-H_c	3.098	$J_{5c,5t}$ = 15.0; $J_{5c,5a}$ = 10.8
5-H_t	2.649	$J_{5t,5a}$ = 4.9
5a-H	3.937	$J_{5a,6c}$ = 4.9; $J_{5a,6t}$ = 11.5
6-H_c	1.422	$J_{6c,6t}$ = 12.2; $J_{6c,7c}$ = 3.5; $J_{6c,7t}$ = 3.0
6-H_t	1.300	$J_{6t,7c}$ = 12.0; $J_{6t,7t}$ = 3.0
7-H_c	2.161	$J_{7c,7t}$ = 15.9; $J_{7c,8}$ = 1.7
7-H_t	1.933	$J_{7t,8}$ = 7.0
8-H	4.389	
8b-CH_aH_b	1.469	$J_{8b\text{-}CHaHb,8b\text{-}CHaHb}$ = 13.3
8b-CH_aH_b	1.728	
8b-CH_2CH_3	0.986	$J_{8b\text{-}CH_2CH_3,8b\text{-}CH_2CH_3}$ = 7.3
1′-CH_aH_b	2.680	$J_{1'\text{-}CHaHb,1'CHaHb}$ = 14.6
1′-CH_aH_b	2.930	
1′-CH_2CH_3	1.323	$J_{1'\text{-}CH_2CH_3,1'\text{-}CH_2CH_3}$ = 7.5
3′-H_c	2.694	$J_{3'c,3't}$ = 14.5; $J_{3'c,4'c}$ = 6.4; $J_{3'c,4't}$ = 10.8
3′-H_t	2.558	$J_{3't,4'c}$ = 0.5; $J_{3't,4't}$ = 8.0
4′-H_c	2.067	$J_{4'c,4't}$ = 11.2; $J_{4'c,4a'}$ = 5.3
4′-H_t	1.670	$J_{4't,4a'}$ = 10.2
4a′-H	3.383	$J_{4a',5'c}$ = 3.5; $J_{4a',5't}$ = 11.0
5′-H_c	1.570	$J_{5'c,5't}$ = 12.8; $J_{5'c,6'c}$ = 2.8; $J_{5'c,6't}$ = 4.2
5′-H_t	0.896	$J_{5't,6'c}$ = 13.1; $6_{5't,6't}$ = 2.6
6′-H_c	1.408	$J_{6'c,6't}$ = 13.6; $J_{6'c,7'c}$ = 6.5; $J_{6'c,7't}$ = 11.9
6′-H_t	1.697	$J_{6't,7'c}$ = 1.2; $J_{6't,7't}$ = 6.5
7′-H_c	2.690	$J_{7'c,7't}$ = 15.8
7′-H_t	2.455	

430A can be rationalized as resulting from this double enamine moiety positioned in a considerably strained ring system. Finally, long-range correlations observed for proton 1-H of the tetracyclus allowed us to connect the two subunits *via* carbons C-1 and C-2′ (Fig. 3).

Phase sensitive NOESY spectra revealed several NOE effects, which in addition to (^{1}H,^{1}H)-coupling constants determined the relative configuration of the tetracyclic subunit. Key enhancements are shown in Fig. 4. Owing to overlap with the crosspeaks of the corresponding protons of the pyrrolo[2,1,5-*c*,*d*]indolizine systems in myrmicarins 215A, 215B and 217, the relative configuration at C-4′ in the tricyclus could not be determined.

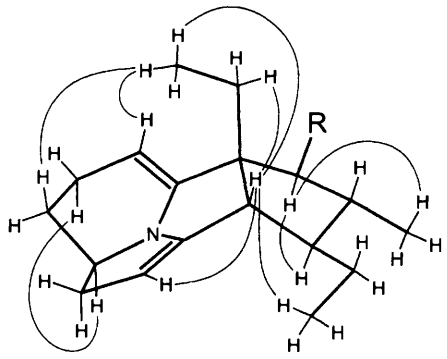

Fig. 4 Key NOE enhancements in the tetracyclic subunit of myrmicarin 430A

In conclusion, myrmicarin 430A shows a new heptacyclic structure, which is among the most complex of the alkaloids identified from insects so far. The carbon skeleton can be seen to consist of two unbranched C_{15}-chains, forming a hexahydropyrrolo[2,1,5-*c*,*d*]indolizine system as in myrmicarin 215 and a new tetracyclic system. It seems to be highly propable, that the 'C_{15}-monomer', myrmicarin 215A, and the 'dimer', myrmicarin 430A, share a common biogenetic precursor.

Footnotes

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‡ Ants were collected at Kitale, Kenya; for details see ref. 3.

§ ^{13}C *NMR data* (C_6D_6) for myrmicarin 430A C-1 (δ 55.33), C-2 (43.38), 2-CH_3 (17.10), C-3 (54.71), 3-CH_2CH_3 (27.53), 3-CH_2CH_3 (12.82), C-3a (48.65), C-3b (154.05), C-4 (88.32), C-5 (41.24), C-5a (55.10), C-6 (28.12), C-7 (22.31), C-8 (90.07), C-8a (145.75), C-8b (62.07), 8b-CH_2CH_3 (26.72), 8b-CH_2CH_3 (17.12), C-1′ (123.57), 1′-CH_2CH_3 (18.79), 1′-CH_2CH_3 (16.58), C-2′ (111.69), C-2a′ (127.63), C-3′ (27.24), C-4′ (37.13), C-4a′ (54.79), C-5′ (29.95), C-6′ (22.90), C-7′ (20.96) and C-7a′ (118.08).

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