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# Alkaloids of *Nicotiana africana*

Species of the genus *Nicotiana* are widely distributed in America, the South Pacific and Australia[1,2] but only one occurs naturally on the continent of Africa. The aptly named *N. africana* was discovered on several isolated mountains in central Namibia in 1965 and was first described in 1975.[3] It is considered to be an endemic relict of considerable age — the phytogeographical and phylogenetic history of the species, as well as its correct taxonomic position within the genus, has remained a puzzle.

In contrast with the commercial and pharmaceutical importance of tobacco alkaloids, their potential value in chemosystematics has remained virtually unexplored. Alkaloids are generally conservative taxonomic characters and are often more useful at the generic than specific level.[4–6] Available information on *Nicotiana*,[7] however, indicates substantial quantitative differences (and even qualitative differences) between species (see Table 2). Since alkaloids may ultimately provide useful clues about the evolution of the genus *Nicotiana*, we have investigated the major leaf alkaloids of *N. africana*. This appears to be the first study of alkaloids in the African species. Because of the characteristic electron impact mass spectral fragmentation patterns of bipiperidyl alkaloids[8–12] and the powerful analytical technique of capillary gas chromatography combined with mass spectrometry, sample limitations were not too restrictive and we could positively identify most of the alkaloids of this interesting species.

## Materials and methods

A single plant of authentic *N. africana* (9 g dry weight) was available for extraction. The material was collected on the Brandberg in central Namibia. Air-dried leaves (1.00 g per sample) were homogenized in 15 ml 0.05 M $H_2SO_4$ and left standing at room temperature for 30 min. After filtration, the homogenate was applied to glass columns (270 × 27 mm) with coarse celite (24 g), made basic with ammonia (4 ml) and eluted with 100 ml $CH_2Cl_2$. After evaporation of the solvent, the alkaloidal material was dissolved in 50 µl MeOH, of which 1-µl quantities were used for gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS).

GC/MS analyses were performed on a Varian 3700 mass spectrometer, operating at 70 eV. Retention times were established with two different GC systems as shown below. GC spectra were obtained with a DB-1 fused silica capillary column [30 m × 0.25 mm internal diameter; $N_2$ as carrier gas at 4 ml $min^{-1}$; column temperature 150° to 320° at 6° $min^{-1}$, 15 min isotherm (system 1) or 150° to 280° at 4° $min^{-1}$, 15 min isotherm (system 2); injector 230°C; PND detection 300°C (system 1) or FID detection 275°C (system 2); split ratio 30:1; injection volume 1 µl]. In addition to authentic samples of nicotine, nornicotine and anabasine, several extracts from roots, stems, leaves and seeds of *N. tabacum* (obtained from commercial cultivations) and *N. glauca* (a troublesome invader in most parts of southern Africa) were used for comparative GC and GC/MS studies. The quantity of *N. africana* material was insufficient to allow isolation of the minor alkaloids, but a pure sample (3 mg) of anatabine (the only major alkaloid) was obtained by preparative thin-layer chromatography on Merck 60 silica gel plates (0.25 mm layer thickness) with $CHCl_3$:cyclohexane:$Et_2NH$ (4:5:1) as eluent. Determination of the optical rotation of this sample showed that it was the (±) form.

## Results and discussion

Analysis of three different leaf extracts by capillary GC and GC/MS showed the presence of one major and seven minor alkaloids (Table 1). Nicotine (**1**), nornicotine (**2**) and anabasine (**4**) were identified by direct comparison with authentic samples (MS spectra and GC retention times in two different systems) and published mass spectral data.[8] Anatabine (**3**) [(±) form] and *N'*-methylanabasine (**5**) were identified by comparison of their mass spectra with literature data.[8–11,13] On the basis of characteristic EIMS fragmentation patterns,[8–12] we could identify two new bipiperidyl alkaloids, *N'*-ethylanabasine (**6**) and *N'*-acetylanabasine (**7**). These two compounds occur in trace amounts (a total of less than 0.0019 mg of each was present in our sample), so that it was not yet possible to

Table 1. Distribution and yields of alkaloids in leaf samples of *Nicotiana africana*. The distribution of alkaloids, given as a percentage of total yield, was determined by gas chromatography using peak area.

| Detectable alkaloids (GC, GC/MS) | Retention time (min) (system 1)* | sample 1 young leaves | sample 2 mature leaves | sample 3 senescent leaves |
|---|---|---|---|---|
| nicotine | 6.65 | trace | trace | trace |
| nornicotine | 6.94 | trace | 1.6 | 1.7 |
| anatabine [(±) form)] | 7.60 | 88.6 | 84.3 | 76.4 |
| anabasine | 8.51 | trace | trace | trace |
| 3-(*N'*-formyl-2-piperidinyl)-*N*-methyl-1,4,5,6-tetrahydropyridine | 8.83 | 10.1 | 13.1 | 19.2 |
| *N'*-acetylanabasine | 10.41 | trace | trace | trace |
| *N'*-methylanabasine | 12.41 | 1.3 | 1.0 | 2.7 |
| *N'*-ethylanabasine | 13.18 | trace | trace | trace |
| total yield (mg/g dry weight) | | 0.97 | 1.12 | 1.16 |

*GC parameters are given under Materials and methods.

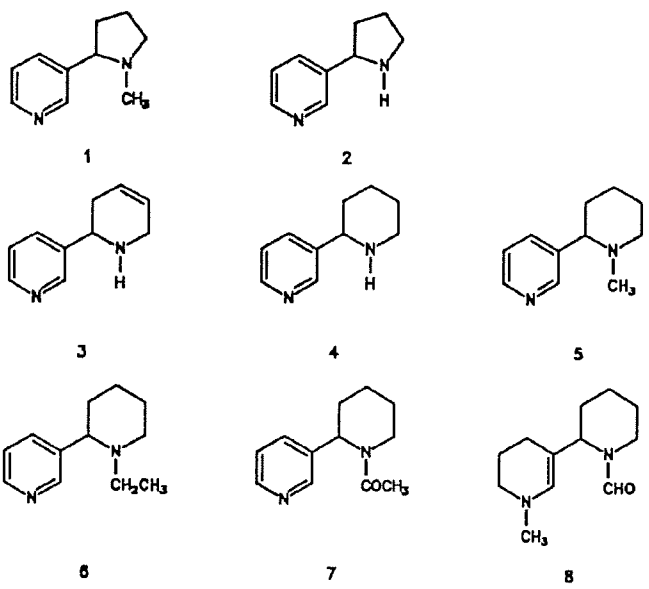


Fig. 1. Structures of alkaloids.

Table 2. Major alkaloids of species of *Nicotiana*.[7]

| | |
|---|---|
| *N. affinis* | nicotine 1′-*N*-oxide |
| *N. africana* | anatabine [(±) form] |
| *N. glauca* | anabasine [(±) form, = neonicotine] |
| *N. glutinosa* | anatabine [(*S*) form] |
| *N. plumbaginifolia* | solaplumbine |
| *N. sylvestris* | nicotine 1′-*N*-oxide |
| *N. tabacum* | nicotine [(*S*) form] |

determine the absolute configuration at the single chiral centre. Instead of simply ignoring these trace compounds, we decided to report their presence so that other workers may look out for them in extracts of other species in order to confirm the proposed structures and determine absolute configurations. The product identified as *N*′-ethylanabasine showed fragmentation of the molecular ion (*m/z* 190), corresponding to the loss of a methyl group followed by the loss of a methylene group. The consequent fragmentation peaks were practically identical to those observed in the mass spectrum of the parent molecule anabasine. Comparison of the mass spectra of **6** and *N*′-methylanabasine, which are virtually identical in fragmentation, confirmed our assignment of the structure of **6**. A strong fragmentation peak with *m/z* 161 from the molecular ion (*m/z* 204) of **7** suggested the presence of an acetyl functional group in the molecule. The absence of other fragment ions between the molecular ion and the base peak (*m/z* 161) excluded other general functional groups as the substituent in this molecule. An unknown compound with a molecular ion of *m/z* 208 was tentatively identified as 3-(*N*′-formyl-2-piperidinyl)-*N*-methyl-1,4,5,6-tetrahydropyridine (**8**). Compound **8**, with a molecular ion of *m/z* 208, was reminiscent of the spectra of bipiperidyl compounds with carbonyl-type substituents on the nitrogen atoms. The first significant fragmentation was the loss of a methyl group (*m/z* 193), followed by a weak ion with *m/z* 190. The latter was the result of the loss of water from the molecular ion. This behaviour is typical of molecules containing formyl groups.[10] From the $M^+–CH_3$ ion fragment, the loss of water produced an *m/z* 175 ion. Further fragmentation ions were in accordance with a bipiperidyl skeleton. Owing to insufficient material, we were as yet unable to record nuclear magnetic resonance data of this compound in order to confirm the proposed structure.

Nicotine (**1**). GC/MS *m/z* (rel. int.): 162 [$M^+$] (21), 161 (16), 133 (29), 119 (9), 84 (100).

Nornicotine (**2**). GC/MS *m/z* (rel. int.): 148 [$M^+$] (50), 147 (54), 120 (30), 119 (100), 70 (49).

Anatabine (**3**) [(±) form]. GC/MS *m/z* (rel. int.): 160 [$M^+$] (100), 145 (14), 131 (23), 118 (18), 107 (36), 106 (50), 105 (61), 82 (31), 80 (30), 54 (74). Accurate mass: Calc. for $C_{10}H_{12}N_2$ 160.10016, found 160.28160.

Anabasine (**4**). GC/MS *m/z* (rel. int.): 162 [$M^+$] (46), 133 (48), 119 (37), 106 (43), 105 (51), 92 (19), 84 (100), 80 (25).

*N*′-methylanabasine (**5**). GC/MS *m/z* (rel. int.): 176 [$M^+$) (94), 161 [$M^+–CH_3$] (11), 159 (10), 148 (28), 147 (100), 135 (13), 130 (13), 120 (48), 119 (87), 111 (26), 105 (47), 98 (48), 92 (50), 85 (17), 79 (31), 70 (83), 65 (40), 57 (26), 51 (43), 43 (75), 41 (85).

*N*′-ethylanabasine (**6**). GC/MS *m/z* (rel. int.): 190 [$M^+$] (27), 175 [$M^+–CH_3$] (19), 161 [$M^+–C_2H_5$) (7), 147 [$M^+–C_2H_5N$] (62), 133 [$M^+–C_2H_5NCH_2$] (6), 131 (8), 120 (55), 119 (33), 105 (20), 93 (17), 92 (18), 78 (17), 70 (96), 57 (20), 55 (19), 51 (22), 43 (100), 41 (46).

*N*′-acetylanabasine (**7**). GC/MS *m/z* (rel. int.): 204 [$M^+$] (3), 161 [$M^+–CH_3CO$) (100), 147 [$M^+–CH_3CON$] (19), 132 (28), 130 (23), 117 (33), 109 (7), 105 (12), 92 (18), 83 (12), 77 (16), 71 (42), 69 (30), 65 (14), 57 (22), 55 (24), 53 (15), 43 (77), 41 (48).

3-(*N*′-formyl-2-piperidinyl)-*N*-methyl-1,4,5,6-tetrahydropyridine (**8**, tentative structure). GC/MS *m/z* (rel. int.): 208 [$M^+$) (27), 193 [$M^+–CH_3$] (19), 190 [$M^+–H_2O$] (3), 175 [$M^+–CH_3–H_2O$)] (31), 149 (13), 147 (12), 139 (5), 137 (4), 133 (7), 121 (39), 119 (14), 109 (12), 105 (18), 95 (12), 93 (14), 91 (14), 81 (12), 79 (16), 77 (13), 69 (100), 57 (11), 55 (28), 53 (13), 43 (33), 41 (77).

Large quantities of racemic anatabine (**3**), in combination with several minor alkaloids, seem to be characteristic of the African species but it should be noted that the (*S*) form of anatabine is the major alkaloid of *N. glutinosa*[7,13] and the second most common alkaloid in *N. tabacum*.[7] Racemic anatabine also occurs in tobacco.[7] The comparison of major alkaloids in Table 2 indicates that *N. africana* is more closely related to *N. glutinosa* than to any other species for which data are available. Future studies will show if *N. africana* is indeed best accommodated in the subgenus *Petunioides*, as proposed earlier,[3] or if it is not perhaps better placed in the Australian endemic section *Suaveolentes*. A rigorous comparison of a wider range of species may lead to a better understanding of infrageneric affinities.

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