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THE ALKALOID CONTENTS OF SIXTY *NICOTIANA* SPECIES

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Key Word Index—*Nicotiana*; Solanaceae; leaves; roots; alkaloids; nicotine; nornicotine; anabasine, anatabine; distribution; chemotaxonomy.

Abstract—The alkaloid content (nicotine, nornicotine, anabasine, and anatabine) in leaves and roots of 60 *Nicotiana* species were analyzed by GC. All species contained alkaloids, the amounts varying with the species. There was no clearcut correlation between alkaloid amounts and subgeneric or sectional classification. The alkaloid content in the floral parts and immature and mature fruits of *Nicotiana tabacum* were also analyzed.

INTRODUCTION

Although alkaloids have been studied in 52 of 66 *Nicotiana* species, the data available are qualitative or at best semiquantitative [1–5]. In 1959, Jeffery analyzed alkaloids in 25 *Nicotiana* species by paper chromatography and determined their contents by a colorimetric method [6]. Since then, no quantitative analysis of alkaloids has been conducted except for total alkaloid or nicotine content, which was determined by steam distillation and spectrophotometry [7, 8].

Here, we report quantitative data based on capillary GC on the four alkaloids (nicotine, nornicotine, anabasine and anatabine) in leaves and roots of 60 species of *Nicotiana*. Alkaloids of floral parts and fruits of *Nicotiana tabacum* were analyzed also.

RESULTS AND DISCUSSION

The nicotine, nornicotine, anabasine and anatabine content in leaves and in roots of 60 out of 66 *Nicotiana* species are listed in Table 1. In addition to these four alkaloids, unidentified basic components were observed in both leaves and roots. Generally roots contain more such unknown compounds than leaves; identification and quantitation were not attempted in this study.

Total alkaloid content of each species (the sums of the contents of four alkaloids) varied from 20 μg/g dry wt. (0.002%; *N. alata*) to 29 600 μg/g dry wt. (2.96%; *N. sylvestris*) in leaves, and from 272 μg/dry wt. (0.027%; *N. langsdorfii*) to 24 817 μg/g dry. wt. (2.48%; *N. velutina*) in roots. With a few exceptions (Table 1) nicotine, nornicotine, anabasine and anatabine were detected in both leaves and roots of all species. The amounts of these four alkaloids as μg/g dry wt varied from species to species; nicotine, from 12 (*N. kawakamii*) to 23 688 (*N. sylvestris*) in leaves, from 178 (*N. langsdorfii*) to 13 115 (*N. velutina*) in roots; nornicotine from trace amounts (*N. alata*) to 8445 (*N. glutinosa*) in leaves, from trace amounts (*N. forgetiana*) to 5526 (*N. trigonophylla*) in roots; anabasine, from trace amounts (13 species) to 7546 (*N. glauca*) in leaves, from trace amounts (10 species) to 8655 (*N. cordifolia*) in roots; anatabine, from trace amounts (3 species) to 1229 (*N. fragrance*), in leaves from 34 (*N. velutina* and *N. hesperis*) to 2480 (*N. spegazzinii*) in roots.

Nicotine was the dominant alkaloid in leaves of 33 species, nornicotine of 24 species, anabasine of 2 species (*N. glauca* and *N. debneyi*) and anatabine of *N. otophora* only, whereas in roots nicotine predominated in 51 species, nornicotine in 2 species (*N. alata* and *N. africana*), anabasine in 7 species (*N. glauca, N. solanifolia, N. benavidesii, N. cordifolia, N. debneyi, N. maritima* and *N. hesperis*), and anatabine in no species. The concentrations of anatabine and anabasine were higher in roots than in leaves in most species except for anabasine in *N. glauca, N. tabacum, N. repanda* and *N. hesperis*, and anatabine in *N. alata, N. corymbosa* and *N. hesperis*. No such tendency was noted with regard to nicotine and nornicotine.

Alkaloid content in the generative organs of *N. tabacum* are shown in Tables 2 and 3. Both calyces and upper leaf contained considerable amounts of nicotine, stigma and style, ovaries, and stamina a lesser amount and immature and mature seeds trace amounts only. Other alkaloids did not occur in detectable amounts (except in calyces) in our experiments [9, 10].

Our data show that all *Nicotiana* species contain alkaloids: The amount and ratio of total and individual alkaloids present in a plant depend on the species. No clearcut correlation between alkaloid pattern and classification of the genus *Nicotiana* seems to exist. There are some discrepancies between our data and those reported previously [1–5], but these may be due to inaccurate methods previously used for analyses of these alkaloids, or to variations in the timing of harvest [1].

EXPERIMENTAL

Plants. The 60 species of *Nicotiana* used for this study were identified morphologically [11–13] in the Iwata tobacco experimental station of the Japan Tobacco & Salt Public Corporation. The seeds of the species collected there by self-pollination were used. All plants were grown in a greenhouse. All leaves except withered ones from five plants of each species were collected when several flowers had opened. Roots of less than 1 mm diameter were also collected As some species (see Table 1) did not bloom, the leaves and the roots were harvested 5 months after germination. The harvested tissues were lyophilized, powdered

Table 1. Alkaloid contents of *Nicotiana* species

Subgenus, section and species	Leaves					Roots				
	Total content (μg/g dry wt)	Nicotine	Nornicotine	Anabasine	Anatabine	Total content (μg/g dry wt)	Nicotine	Nornicotine	Anabasine	Anatabine
		(% of total)					(% of total)			
RUSTICA										
Paniculatae										
N. glauca Graham	8872	12.5	1.5	85.1	0.9	5246	35.5	2.8	51.3	10.4
N. paniculata L.	8592	83.3	15.6	0.2	0.9	8566	49.0	46.4	1.5	3.1
N. knightiana Goodspeed	9319	90.7	8.3	0.2	0.8	7358	63.7	32.2	1.6	2.5
N. solanifolia Walpers	848	3.2	81.4	15.4	tr.	9326	27.7	10.0	60.3	2.0
N. benavidesii Goodspeed*	2166	82.7	1.3	14.8	1.2	14666	44.9	0.8	48.1	6.2
N. cordifolia Philippi	789	58.4	6.1	29.0	6.5	13435	26.4	2.5	64.4	6.7
N. raimondii Macbride*	7001	82.2	8.1	8.9	0.8	15786	66.7	2.5	28.1	2.7
Thyrosiflorae										
N. thyrosiflora Bitter ex Goodspeed										
Rusticae										
N. rustica L.	7752	96.4	0.9	1.1	1.6	8439	81.6	1.7	6.6	10.1
TABACUM										
Tomentosae										
N. tomentosa Ruiz & Pavon*	361	17.2	66.5	tr.	16.3	2920	38.8	36.8	4.7	19.7
N. tomentosiformis Goodspeed*	2536	19.2	68.4	1.0	11.4	1560	61.2	16.4	2.8	19.6
N. otophora Grisebach*	377	6.9	32.9	tr.	60.2	7924	61.3	27.0	0.6	11.1
N. setchellii Goodspeed*	2285	7.4	83.0	7.5	2.1	6494	51.5	26.7	16.5	5.3
N. glutinosa L.	9309	6.0	90.7	0.3	3.0	14670	83.0	3.2	3.0	10.8
N. kawakamii Ohashi*	223	5.4	85.2	tr.	9.4	2390	70.8	17.6	tr.	11.6
Genuinae										
N. tabacum L.	11462	94.8	3.0	0.3	1.9	2176	81.3	6.0	1.7	11.0
PETUNIOIDES										
Undulatae										
N. undulata Ruiz & Pavon	3359	95.3	2.9	1.3	0.5	5596	67.7	14.0	13.5	4.8
N. arentsii Goodspeed	6487	91.3	2.4	4.8	1.5	3500	58.7	9.0	25.6	6.7
N. wigandioides Koch & Fintelmann*	5388	91.8	0.3	4.7	3.2	7424	73.1	1.0	19.2	6.7
Trigonophyllae										
N. trigonophylla Donal	1106	1.8	94.9	tr.	3.3	14458	55.8	38.2	0.4	5.6
Alatae										
N. sylvestris Spegazzini & Comes*	29601	80.0	19.1	0.2	0.7	7864	89.9	6.3	1.0	2.8
N. langsdorffii Weinmann	2580	96.0	0.6	tr.	3.4	272	65.4	8.8	tr.	25.7
N. alata Link & Otto	26	100.0	tr.	—	—	1998	37.7	46.4	tr.	15.8
N. forgetiana Hort. ex Hemsley	43	100.0	—	—	—	1752	92.0	tr.	tr.	8.0
N. bonariensis Lehmann	73	100.0	tr.	—	—	2088	53.6	37.3	4.2	4.9
N. longiflora Cavanilles*	42	100.0	tr.	—	—	6460	75.3	17.8	0.3	6.6
N. plumbaginifolia Viviani	423	12.5	84.6	tr.	2.9	5232	83.6	13.1	tr.	3.2

Repandae										
N. repanda Willdenow ex Lehmann	3265	1.2	76.7	11.9	10.2	9140	54.1	35.2	3.0	7.7
N. stocktonii Brandegee*	11099	71.7	25.7	0.2	2.9	12168	89.5	2.4	0.6	7.5
N. nesophila Johnston	10641	46.1	46.3	0.4	7.2	14426	80.6	8.3	1.0	10.1
Noctiflorae										
N. noctiflora Hooker	3172	21.2	76.0	2.3	0.5	6868	54.3	6.6	32.1	7.0
N. petunioides (Grisebach) Millán										
N. acaulis Spegazzini										
N. ameghinoi Spegazzini										
Acuminatae										
N. acuminata (Graham) Hooker	768	83.6	12.8	tr.	3.6	2404	62.2	32.1	tr.	5.7
N. pauciflora Remy	712	82.3	15.7	tr.	2.0	5232	57.2	38.9	tr.	3.9
N. attenuata Trorry ex Watson	22269	98.4	0.8	tr.	0.8	2484	89.8	5.2	tr.	5.0
N. longibracteata Philippi										
N. miersii Remy	4408	17.4	82.2	tr.	0.4	5434	94.6	3.7	0.4	1.3
N. corymbosa Remy	8519	86.1	11.9	tr.	2.0	4170	77.9	19.3	tr.	2.8
N. linearis Philippi										
N. spegazzinii Millán	1678	8.2	68.0	tr.	23.8	12584	62.5	16.9	0.8	19.8
Bigelovinae										
N. bigelovii (Torrey) Watson	7801	96.8	2.3	tr.	0.9	2196	91.7	4.9	tr.	3.4
N. clevelandii Gray	8945	98.5	0.3	tr.	1.2	5218	92.6	1.4	0.5	5.5
Nudicaules										
N. nudicaulis Watson	1814	3.8	66.2	13.6	16.4	11014	70.7	11.9	8.7	8.7
Suaveolentes										
N. benthamiana Domin	3602	80.8	0.6	16.1	2.5	3826	68.4	1.4	26.3	3.9
N. umbratica Burbidge	43	51.2	48.8	tr.	tr.	9932	43.4	23.5	27.8	5.3
N. cavicola Burbidge	285	16.8	76.9	6.3	tr.	3798	58.0	9.1	25.4	7.5
N. debneyi Domin	2457	31.1	15.8	46.0	7.1	3038	34.7	1.4	53.2	10.7
N. gossei Domin	12169	98.8	0.3	0.2	0.7	7222	73.8	0.9	17.9	7.5
N. amplexicaulis Burbidge	4959	98.5	0.3	tr.	1.2	7648	71.8	3.1	11.9	13.2
N. maritima Wheeler	608	7.2	70.4	15.8	6.6	14030	20.8	30.0	44.5	4.6
N. velutina Wheeler	5276	2.8	88.4	8.1	0.7	24817	52.9	13.4	32.3	1.4
N. hesperis Burbidge	4108	52.1	0.4	44.3	3.2	1930	22.1	1.2	74.9	1.8
N. occidentalis Wheeler	519	9.4	75.1	15.4	tr.	6490	41.5	14.6	39.5	4.4
N. simulans Burbidge	258	14.0	67.0	19.0	tr.	6624	45.1	6.4	44.3	4.2
N. megalosiphon Heurck & Muell	319	18.5	42.0	39.5	tr.	5566	50.5	1.8	43.7	4.0
N. rotundifolia Lindley	4949	96.6	0.8	2.1	0.5	4912	53.9	1 1	40.7	4.3
N. excelsior J. M. Black	18902	95.9	1.1	0.9	2.1	4772	66.5	1.4	20.0	12.1
N. suaveolens Lehmann	4954	85.0	13.6	0.9	0.5	6658	51.7	9.9	29.0	9.4
N. ingulba J. M. Black	669	7.5	44.8	42.2	5.5	4804	46.3	4.5	42.3	6.9
N. exigua Wheeler*	7692	70.0	26.0	1.5	2.5	12040	60.1	1.8	21.2	16.9
N. goodspeedii Wheeler	730	5.5	68.4	21.1	5.0	4284	67.8	1.3	26.6	4.3
N. rosulata (S. Moore) Domin	992	24.9	70.8	3.0	1.3	3376	85.6	1.7	10.5	2.2
N. fragrans Hooker	14985	91.0	0.4	0.4	8.2	13344	81.4	0.8	1.1	16.7
N. africana Merxmüller & Buttler*	6776	4.7	92.4	0.3	2.6	7698	45.0	45.1	1.0	8.9

*Species did not bloom. —, Not detected.

Table 2. The alkaloid content in the floral organs of *Nicotiana tabacum* (μg/g dry wt)

Organ	Nicotine	Nornicotine	Anabasine	Anatabine
Stigma + style	14	—	—	—
Stamen	14	—	—	—
Ovary	80	—	—	—
Ovary base*	156	—	—	—
Petal	86	—	—	—
Calyx	1435	—	—	—
Flower axis	254	—	—	—
Upper leaf†	1895	—	—	—

—, Not detected.

*An ovary was disected into upper and lower parts, where most ovules attached to the former and the latter were colored reddish brown, the lower part is tentatively designated as the ovary base.

†The leaf just below the floral branches.

Table 3. The alkaloid content in immature and mature capsules of *Nicotiana tabacum* (μg/g dry wt)

Plant part	Nicotine	Nornicotine	Anabasine	Anatabine	Total
Immature					
Wall of ovary	15	—	—	—	15
Seed	tr.	—	—	—	tr.
Placenta	16	—	—	—	16
Calyx	6078	24	15	—	6117
Flower axis	109	—	—	—	109
Mature					
Wall of ovary	43	—	—	—	43
Seed	10	—	—	—	10
Placenta	36	—	—	—	36
Calyx	2607	—	—	—	2607
Flower axis	317	—	—	—	317

and kept in a desiccator until use. Samples were stirred with 2 ml 1 N HCl (containing *ca* 200 mg of isoquinoline as an internal standard) in a glass homogenizer, 2 ml CH_2Cl_2 added, thoroughly mixed and centrifuged (4500 rpm, 15 min). The acid aqueous layer separated was adjusted to pH 11 with 6 N NaOH, 2 ml CH_2Cl_2 added, thoroughly shaken and centrifuged (4500 rpm, 30 min). This procedure was performed three times for each sample. The CH_2Cl_2 layer was separated and dried under N_2. The residue was dissolved in 100 μl MeOH A 1 μl aliquot of the sample was then subjected to GC.

Preparation of the standard mixture solution. Picrates of isoquinoline, nicotine, nornicotine, anabasine and anatabine (10 mmol each) were dissolved in alkaline solution and extracted with CH_2Cl_2. The extract was dried under N_2 The residue was dissolved in 1 ml MeOH. A 0.2 μl aliquot was used for calibration.

Gas chromatography. GC was accomplished with a Shimadzu GC-4CM with an integrator (Hewlett Packard 3390A) using a 50 m × 0.32 mm i.d. flexible fused silica capillary column coated with methylsilicone gum, a He carrier gas flow rate of 50 ml/min, split ratio of 1:40. Column temperature was raised from 140° to 240° at 2°/min. Retention times of isoquinoline, nicotine, nornicotine, anabasine and anatabine were 3.8, 4.6, 5.3, 6.3 and 6.7 min, respectively

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