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COMPOSITION OF THE LEAF SURFACE GUM OF SOME *NICOTIANA* SPECIES AND *NICOTIANA TABACUM* CULTIVARS

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Abstract—The compositions of the leaf surface gums of various *Nicotiana* species grown under similar field conditions have been analysed and compared with those of some *Nicotiana tabacum* cultivars Diterpenes could only be detected in the extracts of two of the 16 *Nicotiana* species, labdanoids have been detected for the first time in *N tomentosa* The relative quantitative distributions of *n*- and methyl-branched alkanes in the leaf surface gums proved to be specific for different species

INTRODUCTION

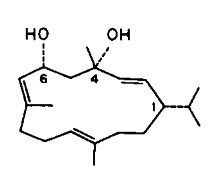
The diterpenes of the leaf surface gum of *Nicotiana tabacum* are considered to be aroma precursors [1–3] from which compounds with aroma properties are formed during curing and fermentation The diterpenes are synthesized in the trichomes and secreted on the leaf surface [4] A growth inhibiting activity has, *inter alia*, been attributed to these compounds [5, 6] *N tabacum* is assumed to be a hybrid of *N sylvestris* (male) and *N tomentosiformis* (female) [7] *N sylvestris* produces diterpenoids of the cembranoid type, whereas *N tomentosiformis* contains labdanoids Accordingly, the leaf surface gum of *N tabacum* either contains only cembranoids (Burley and Virginia) or additional labdanoids (Oriental) [1]

In the past, little information has been published about the composition of the leaf surface gum in *Nicotiana* species It is, however, known that *N glutinosa* and *N otophora* contain diterpenes of the labdanoid type [8], whereas 45 *Nicotiana* species not named in detail are said to contain no cembranoids [9] In order to obtain further information on this point, the leaf surface gums of 16 *Nicotiana* species and seven *N tabacum* cultivars have been analysed

RESULTS AND DISCUSSION

The methylene chloride extracts of the leaf surface gums were analysed by GC and GC/MS without further purification or fractionation to avoid artefacts or loss of material All the wax extracts investigated here contained a mixture of normal-, 2- and 3-methylalkanes with strong variation in total content and composition for the different species Since the total content of the leaf surface gum depends on leaf position, climate, soil, etc [10, 11], only the quantitative composition of the hydrocarbons, in relation to each other, was compared to distinguish between the species and also between the cultivars For a given species, this ratio is independent of leaf position and growing site, as could be confirmed for some species in the two consecutive years 1978 and 1979 The results for the individual *Nicotiana* species and *tabacum* cultivars are summarized in Table 1, for greater clarity the percentages for the alkanes are summarized as main constituents (H, > 10 %), minor compounds (N, 2–10 %) and trace compounds (S, ≤ 2 %) There is clearly a species-specific pattern for all the *Nicotiana* species Accordingly, it should be possible to establish a chemosystematic subdivision of *Nicotiana* species on the basis of the distribution of alkanes present in the leaf surface gum

A similar effect is indicated for the individual *tabacum* cultivars although this is less marked and will require further confirmation with other varieties All the *N tabacum* varieties under investigation contained diterpenoids of the cembranoid type, the main products in each case being α- and β-2,7,11-cembratriene-4,6-diol (**1**, **2**) There are very marked variations in the contents of both these compounds (Table 1), their concentration being below the normal detection limit in glandless tobacco Ti 1112, so that they could only be detected by mass fragmentography This extremely low concentration is in agreement with the results of Severson *et al* [12] Since the concentration of the diterpenes not only depends on the leaf position [10, 11] but is also affected by genetic and environmental factors [11], the percentages specified in Table 1 must be regarded as no more than



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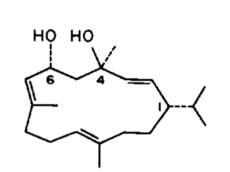

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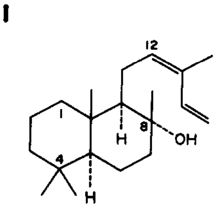

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Table 1 Composition of the leaf surface gum of different *N tabacum* varieties and *Nicotiana* species

Plant material	Alkanes*																Terpenes
	n-C_{27}	n-C_{28}	i-C_{29}	n-C_{29}	i-C_{30}	a-C_{30}	n-C_{30}	i-C_{31}	a-C_{31}	n-C_{31}	i-C_{32}	a-C_{32}	n-C_{32}	i-C_{33}	a-C_{33}	n-C_{33}	
N tabacum																	
Ti 1112	N	S	N	N	N	N	S	H	S	H	S	N	N	N	S	H	0 5% Cembratrienediol
Virgin Bright	N	S	S	N	S	H	S	N	S	H	S	H	N	S	S	N	5% Cembratrienediol
NC 2326	N	S	N	N	S	H	N	H	S	H	S	H	N	N	S	N	7% Cembratrienediol
Vinica	N	S	N	N	S	H	S	N	S	H	S	H	S	N	S	N	8% Cembratrienediol
Burley B5	N	S	N	N	S	N	S	N	S	H	S	N	S	S	S	N	8% Cembratrienediol
Amarelinho	N	S	S	N	S	N	S	N	S	N	S	N	S	N	S	N	25% Cembratrienediol, 5% Labdanoids
Kurztag	S	S	S	S	S	N	S	N	S	N	S	S	S	S	S	S	75% Cembratrienediol
N accuminata	N	S	N	H	S	H	S	S	S	N	S	S	S	S	S	S	0
N africana	S	S	N	H	S	H	S	N	S	H	S	N	S	S	S	N	0
N alata	N	S	N	N	S	N	S	H	S	H	S	H	N	N	S	H	0
N debneyi	S	S	S	N	S	N	N	H	S	H	S	H	N	N	S	H	Triterpenes
N glauca	S	S	S	S	S	S	S	S	S	H	S	S	S	S	S	N	0
N knightiana	S	S	H	N	S	H	N	H	S	H	S	N	S	N	S	H	0
N langsdorfii	S	S	N	N	S	N	S	H	S	H	S	N	N	H	S	H	0
N longiflora	S	S	N	H	S	N	N	H	S	H	S	N	N	S	S	H	0
N megalosiphon	S	S	N	H	S	H	N	H	N	H	S	H	N	N	S	H	0
N paniculata	S	S	H	N	S	H	N	H	S	H	S	N	S	N	S	N	Triterpenes
N plumbaginifolia	N	S	N	H	S	N	N	N	S	H	S	S	N	S	S	N	0
N repanda	S	S	H	H	S	H	N	N	S	H	S	S	S	S	S	N	0
N rustica	N	S	H	H	S	H	N	N	S	H	S	S	S	S	S	N	0
N sanderae	N	S	N	N	S	N	S	H	N	N	S	H	N	N	N	H	0
N sylvestris	N	S	N	N	S	N	S	N	S	H	S	N	S	N	S	H	25% Cembratrienediol
N tomentosa	N	N	N	N	N	H	S	N	S	H	S	N	S	S	S	N	Sesquiterpenes, 5% Labdanoids

*i = *Iso*, a = *anteiso*, S = trace (≤ 2%), N = minor compound (2–10%), H = main compound (> 10%)

rough approximations The particularly high content of *ca* 75% for α- and β-cembratrienediol (**1**, **2**) in the leaf surface gum of Kurztag (short day) tobacco should be stressed Kurztag tobacco is a cultivar requiring short days for the formation of flowers, so that it does not succeed in flowering at all, or only very late in the year, in our latitude

In addition to α- and β-2,7,11-cembratriene-4,6-diol (**1**, **2**) it was possible to detect 2,7,11-cembratriene-4-ol, α- and β-8,11-oxido-2,6,12(20)-cembratriene-4-ol and 2,6,11-cembratriene-4,8-diol in all varieties except Ti 1112, each of these compounds being present at concentrations less than 5% of that of the 2,7,11-cembratriene-4,6-diols The native Brazilian Amarelinho was the only cultivar in which labdanoids, especially (12*Z*)-abienol (**3**), could be found In this case the ratio of cembranoids to labdanoids was *ca* 5 1 This is in agreement with the results of Reid [13]

Diterpenes could only be detected in the leaf surface gums of two of the 16 *Nicotiana* species (Table 1) *N sylvestris* contains compounds of the cembranoid type, here the qualitative and quantitative composition of the diterpenes resembles that of the *N tabacum* species under investigation However, the overall concentration of the diterpenes seems to be distinctly higher in relation to that of the *N tabacum* cultivars of the same growing region (Burley B 5, NC 2326, Vinica, Virgin Bright) The leaf surface gum of *N tomentosa* contains a series of terpenes of the labdanoid type, the following having been tentatively identified by mass spectrometry 8,12-epoxylabd-14-ene-13-ols, 12,15-epoxy-12,14-labdadiene-8-ol, and 15,16-epoxy-8-hydroxy-13(16),14-labdadiene-12-one [14, 15] These compounds can be formed by photooxidation of (12*Z*)-abienol (**3**) [14] which could also be identified In addition to the compounds listed above, it was possible to detect some sesquiterpene alcohols and aldehydes The mass spectra for two compounds suggest the presence of dihydrofarnesols [16], whereas others probably relate to cyclic aldehydes of mass 220

Triterpenes, such as cycloartenol, campesterol, sitosterol and stigmasterol were detected by TLC in the extracts from *N paniculata* and *N debneyi* These compounds are generally found in the cytoplasm of other species, including *N tabacum*, and Reid [personal communication] has already detected these triterpenes in the leaf surface gum of *N debneyi*

The results indicate, that the occurrence of diterpenoids, especially cembranoids, is restricted in the genus *Nicotiana* to only a few species

EXPERIMENTAL

Plant material Apart from *N tabacum* Ti 1112, a glandless variety from North Carolina, and the native *N tabacum* Amarelinho from Brazil, all the samples were taken from the 1978 and 1979 crops in the experimental field at Schleswig-Holstein Leaves from all stalk positions *ca* 8 weeks after planting in the field were taken for analysis

Extraction and analysis of leaf surface gum 10–15 leaves were dipped twice into 1 l of CH_2Cl_2 for *ca* 20 sec with gentle agitation The combined solns were dried, filtered and concd *in vacuo* at 35° to a defined vol GC was carried out with a Carlo Erba Science Fractovap 2901 (FID) with a 25 m glass capillary column (WCOT) coated with WG 11 The oven temp was programmed from 60 to 180° at 3°/min and from 180 to 210° at 5°/min The integration was made with a Spectra Physics labor data system SP 4000 MS (EI) were taken with a Micromass 70/70 from Vacuum Generators at 70 eV, source temp 190°, coupled with a Carlo Erba Science Fractovap 2101

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