Cuticular Wax Profiles of Leaves of Some Traditionally Used African Bignoniaceae

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The cuticular waxes, obtained by chloroform extraction from the leaves of four African Bignoniaceae, *Newbouldia laevis*, *Markhamia acuminata*, *Spathodea campanulata* and *Kigelia africana* were analysed by GC-MS. The principal constituents were represented by a homologous series of *n*-alkanes (C_{23}–C_{33}), *n*-alcohols (C_{18}–C_{30}) and related carboxylic acids (C_{16}–C_{36}). For *N. laevis* and *M. acuminata*, ursolic and oleanolic acid were the most abundant wax components (52 and 60%, respectively), followed by the C_{29}, the C_{31} and the C_{33} *n*-alkanes. The predominant components of *S. campanulata* were *n*-alcohols (35%), with octacosanol and triacontanol as the most abundant ones, while *K. africana* is distinguished from these three members by the conspicuous absence of triterpenoic acids and the predominance of *n*-alkanes (70%) with hentriacontane and tritriacontane as the main representatives. Other notable constituents were sterols, albeit present in trace amounts. The wax profiles are discussed in terms of taxonomic characters.

Key words: Bignoniaceae, Cuticular Wax, Chemotaxonomic Characters

Introduction

Members of the Bignoniaceae are common in the tropical areas of the New World, while only a few occur in the rain forests and savannah zones of Africa. Although many species are cropped (Janzen, 1975; Shepherd *et al*., 2000; Nyberg *et al.,* 2002) and employed in traditional medical systems for the treatment of various diseases (Burkill, 1985), little attention has been paid to the chemical constituents of plants of the Bignoniaceae. The systematic application of morphological characters of, *e.g.*, fruits and seeds to the establishment of subfamilial boundaries is limited and has produced seven tribes, with at least for the Tecomeae tribe considerable heterogeneity (Gentry, 1980; Armstrong, 1985). The present study was therefore initiated to re-examine the taxonomic grouping of the medicinally used plants *Newbouldia laevis* Seem., *Markhamia acuminata* (Klotzsch) K. Schum., and *Spathodea campanulata* Beauv. (tribe Tecomeae) and *Kigelia africana* (Lam.) Benth. (tribe Coleeae) on the basis of their leaf wax compositions as chemical characters. The role of surface waxes in plant defence is well established (Schoonhoven et al., 1998), with long chain hydrocarbons and derivatives thereof as principle constituents (Baker, 1982). Terpenoids and flavonoids also occur, the latter being reported for cuticular waxes of some species and used as taxonomic features (Stermitz *et al*., 1992; Wollenweber *et al*., 1996; Blatt *et al*., 1998; Alcerito *et al*., 2002).

This paper deals with compositional studies of the cuticular waxes of *N. laevis*., *M. acuminata*., *S. campanulata* and *K. africana* and their possible taxonomic significance.

Materials and Methods

Plant material

Adult leaves of *N. laevis* were provided by the Royal Botanical Garden of Belgium, adult leaves of *M. acuminata*, *S. campanulata* and *K. africana* were obtained from the Botanical Garden of Berlin (Germany). All species were grown in a tropical greenhouse under similar conditions. Voucher specimens are deposited at the Institut für Pharmazie, Pharmazeutische Biologie, Freie Universität Berlin, Berlin, Germany.

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Extraction

Four to six leaves of each plant were extracted by immersion in 50 ml chloroform for 30 s. The wax extracts were filtered over defatted cotton and the solvent subsequently evaporated under reduced pressure. After gravimetric determination, the extracts were dissolved in chloroform (2 mg/ml) with tetracosane as internal standard (100 μg/ml) and analysed by GC-MS and GC-FID. Measurements of the surface area were performed by tracing the photocopied image of each leaf using standard calibration paper.

Qualitative and quantitative analysis of the wax fractions

Aliquots (100 μl) of the wax fractions were dried and then silylated with 20 μl BSTFA (*N,N*-bistrimethyl-silyltrifluoroacetamide; Macherey-Nagel) in the presence of 10 μl pyridine at 70 °C for 30 min. Qualitative analyses were carried out by GC-MS using a Hewlett-Packard HP 5890 II instrument, coupled with a quadrupole mass selective detector (HP MSD 5971). A DB-1 fused-silica capillary column (30 m x 0.32 mm i.d., film thickness 0.1 μm; Fisons) was used with a temperature programme of 50–200 °C (40 °C min^{-1}) and 200–300 °C (3 °C min^{-1}), 300 °C (20 min); pressure programme: 10 kPa (40 min), 10–100 kPa (10 kPa min^{-1}), 100 kPa (30 min); helium was the carrier gas at a flow rate of 2 ml/min. For MS analysis, the ionisation energy was 70 eV. The compounds were identified on the basis of their retention times and mass-spectral fragmentation patterns compared with those of reference compounds stored on our spectrometer database.

Quantification of identified constituents was performed by injecting 1 μl of the samples (on-column injector; FID; hydrogen as carrier gas). The following temperature and pressure programmes were used: 50 °C (2 min), 50–200 °C (40 °C min^{-1}), 200–320 °C (3 °C min^{-1}), 320 °C (20 min); 40 kPa (40 min), 40–150 kPa (10 kPa min^{-1}), 150 kPa (30 min).

Results and Discussion

The selection of plants was prompted by the synonym citation of some of the specimens, *e.g. Markhamia acuminata* syn. *Spathodea acuminata* and *Newbouldia laevis* syn. *Spathodea laevis* (Missouri Botanical Garden), giving rise to some confusion in their botanical classification. However, *S. campanulata, M. acuminata* and *N. laevis* are morphologically well characterized and can be distinguished by their spathaceous calyx splitted on adaxial (*S. campanulata, M. acuminata*) or abaxial side (*N. laevis*), respectively. Furthermore, the latter two members can be differentiated by their ovules and fruits (Hutchinson and Dalziel, 1963; Steentoft, 1988). These aspects have obviously been ignored in the past.

The average total wax load for the leaves of *N. laevis*, *M. acuminata*, *S. campanulata* and *K. africana* were 18.5, 14.7, 1.6 and 0.6 $\mu g/cm^2$, respectively. From the chloroform extracts about 62% (*N. laevis*), 67% (*M. acuminata*), 52% (*S. campanulata*) and 79% (*K. africana*) of their constituents were unambiguously identified by GC-MS analyses and the results are presented in Table I. Unfortunately, separate GC-MS analyses of the wax extracts on a Restek Rtx®-5Sil column using a Finnigan MD 800 instrument and comparison of the mass spectra with those of Wiley and NIST Library by means of AMDIS failed to identify further constituents. In each case, the lipophilic fractions consisted of a complex mixture with hydrocarbons and oxygenated triterpenoids as the dominating constituents. Several homologous series were identified, including *n*-alkanes (C_{23}–C_{33}), *n*-alcohols (C_{18}–C_{30}) and related carboxylic acids (C_{16}–C_{36}), which are typical constituents of cuticular waxes (Baker, 1982). Conspicuously, large gaps were observed in these series (Table I), a finding that can not be satisfactorily explained. A range of sterols was also detected, although in trace amounts (< 0.01%). To our knowledge, the occurrence of stigmasterol (*M. acuminata, S. campanulata*) and campesterol (*S. campanulata*) was not previously described for leaf extracts of the indicated members.

Compositional studies of the wax extracts of *N. laevis* and *M. acuminata* revealed the presence of considerable amounts of oxygenated triterpenoids, accounting for 52% and 60% of the identified constituents, respectively, followed by long-chain *n*-alkanes (C_{26}–C_{33}) with ca. 9% and 7%, respectively (Table I). The triterpenoid components in the waxes of *N. laevis* and *M. acuminata* were oleanolic acid (14.5% and 23%, respectively) and ursolic acid (around 37%), with the latter as the major component in each case. It should also be noted that hederagenin occurred in *M. acuminata* as a trace component, but was apparently absent or only present below detection limits

Table I. Cuticular wax profiles and distribution of hydrocarbons, alcohols, fatty acids, triterpenoids and sterols for *N. laevis*, *M. acuminata*, *S. campanulata* and *K. africana*.

Species/ Substance class	*N. laevis*		*M. acuminata*		*S. campanulata*		*K. africana*	
	a	b	a	b	a	b	a	b
n-Alkanes								
Tricosane C_{23}	–	–	–	–	–	–	*	0.5
Hexacosane C_{26}	0.1 ± 0.006	0.4	–	–	–	–	–	–
Heptacosane C_{27}	0.1 ± 0.006	0.2	0.1 ± 0.002	0.1	◆	0.7	*	1.7
Octacosane C_{28}	0.1 ± 0.004	0.4	–	–	–	–	–	–
Nonacosane C_{29}	0.5 ± 0.11	2.9	0.1 ± 0.021	0.6	◆	1.0	0.1 ± 0.012	7.0
Triacontane C_{30}	0.1 ± 0.013	0.6	–	–	–	–	–	–
Hentriacontane C_{31}	0.5 ± 0.07	3.0	0.4 ± 0.037	2.9	◆	1.7	0.3 ± 0.04	42.5
Dotriacontane C_{32}	0.1 ± 0.017	0.4	–	–	–	–	–	–
Tritriacontane C_{33}	0.2 ± 0.026	1.4	0.5 ± 0.015	3.2	◆	2.3	0.1 ± 0.02	18.2
Σ	1.7	9.3	1.1	6.8	0.1	5.7	0.5	69.9
n-Alcohols								
Octadecanol C_{18}			–	–	–	–	*	0.6
Eicosanol C_{20}			–	–	*	0.6	*	1.2
Hexacosaol C_{26}			–	–	0.1 ± 0.006	2.0	–	–
Heptacosanol C_{27}			–	–	*	0.5	–	–
Octacosanol C_{28}			*	0.1	0.2 ± 0.064	14.8	–	–
Triacontanol C_{30}			–	–	0.3 ± 0.129	17.1	–	–
Σ	–	–	*	0.1	0.6	35.0	*	1.8
Fatty acids								
Hexadecanoic C_{16}	–	–	◆	0.2	◆	0.9	◆	1.2
Octadecanoic C_{18}	–	–	◆	0.1	◆	0.5	◆	1.5
Hexacosanoic C_{26}	◆	0.2	◆	0.1	–	–	–	–
Dotriacontanoic C_{32}	◆	0.3	–	–	–	–	–	–
Hexatriacontanoic C_{36}	–	–	–	–	◆	0.9	–	–
Σ	◆	0.5	◆	0.4	◆	2.3	◆	2.7
Esters								
C_{44}	–	–	–	–	–	–	◆	4.9
Triterpenoids								
Ursolic acid	7.0 ± 1.02	37.9	5.2 ± 0.320	36.6	◆	1.8	–	–
Oleanolic acid	2.7 ± 0.50	14.5	3.3 ± 0.165	23.1	0.1 ± 0.043	6.7	–	–
Hederagenin	–	–	*	*				
Lupeol					*	*		
α-Amyrin					*	*		
Σ	9.7	52.4	8.5	59.7	0.1	8.5	–	–
Sterols								
β-Sitosterol			*	*	*	*	*	*
Stigmasterol			*	*	*	*		
Campesterol					*	*	*	*
Cholesterol							*	*

[a] Abundance on leaf surface [$\mu g/cm^2$].
[b] Percent of total wax.
* Trace amounts < 0.01%.
◆ Trace amounts < 0.1 $\mu g/cm^2$.
Each species was tested with $n = 4–6$ replicates and results are expressed as mean values ± SD.

(< 1 ng/μl) in the remaining three species investigated. With regard to the identified *n*-alkanes, the C_{29}, the C_{31} and the C_{33} were the most abundant compounds, consistent with the composition of most waxes (Gülz, 1994).

The total amount of chloroform soluble surface wax constituents of the leaves of *S. campanulata* was markedly lower than those of *N. laevis* and *M. acuminata* (vide supra), placed within the same tribe Tecomeae. Also worthy of mention was the

finding that the cuticular wax chemical profile of *S. campanulata* differed significantly from those of the latter tribal-associated members by the considerably reduced levels of triterpenoic acids (8.5%) and the increased level of primary alcohols (35%), with octacosanol and triacontanol as the predominant ones. Other striking features appeared to be the significantly higher percentage of fatty acids and the diversity of sterols in *S. campanulata*, suggesting less taxonomic affinity.

The picture that emerged from examining the wax profile of *K. africana* disclosed differences, as compared to overall amounts and distribution within the terpenoids, the hydrocarbons and their oxygenated analogues. Conspicuously, triterpenoic acids were absent, while the diversity of sterols was somewhat reminiscent of that of *S. campanulata*. Here, the *n*-alkanes constituted the most abundant group (70%), with the C_{31} (42.5%) and C_{33} (18%) as the main components.

With regard to the identified wax profile, shared key characters support the location of *N. laevis*, *M. acuminata*, *S. campanulata* in a common tribe, Tecomeae, as originally delineated by morphological features (Gentry, 1980). Although *S. campanulata* appears chemically distinct, it would be imprudent to exclude this member from this tribe on limited chemical grounds without various additional non-chemical and chemical criteria. The remarkable absence of any triterpenoids and the conspicuous abundance of *n*-alkanes in the cuticular wax of *K. africana* support its treatment as a separate member as reflected by the placement in the Coleeae (Gentry, 1980). Although the results are consistent with the present taxonomic grouping, analyses of fresh plant material collected from several individuals of each species at different developmental stages and from plants of natural population will be needed to ascertain the stability and the individual variability of the chemical characters. Since only single specimens maintained in greenhouse conditions were available, appropriate studies were excluded accordingly. In an alternative approach, gene analysis is currently in progress for extracting further taxonomic information from the sequence variation of ndhF and rbcL genes.

Finally, with regard to their traditional uses, the presence of triterpenoic acids in considerable amounts is significant in that these compounds are well known to possess antimicrobial (Collins and Charles, 1987) and potent antitumoural activity (Liu, 1995). Accordingly, the use of leaves of *N. laevis*, *M. acuminata* and *S. campanulata* in traditional medicine for the treatment of skin cancer may, at least in part, be rationalised in terms of the presence of these triterpenoic acids with established antitumoural activities, while the utilization of *K. africana* should be based on the presence of naphthoquinones. However, the complexity of extracts necessitates that traditional uses have to be evaluated on a rational basis in each case.

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Alcerito T., Barbo F. E., Negri G., Santos D. Y. A. C., Meda C. I., Young M. C. M., Chávez D., and Blatt C. T. T. (2002), Foliar epicuticular wax of *Arrabidea brachypoda*: flavonoids and antifungal activity. Biochem. Syst. Ecol. **30**, 677–683.

Armstrong J. E. (1985), The delimitation of Bignoniaceae and Scrophulariaceae based on floral anatomy and the placement of problem genera. Am. J. Bot. **72**, 755–766.

Baker E. A. (1982), Chemistry and morphology of plant epicuticular waxes. In: The Plant Cuticle (Cutler D. F., Alvin K. L., and Price C. E., eds.). Academic Press, London, pp. 139–165.

Blatt C. T. T., Santos M. S., and Salatino A. (1998), Flavonoids of Bignoniaceae from the "cerrado" and their possible taxonomic significance. Plant Syst. Evol. **210**, 289–292.

Burkill H. M. (1985), The Useful Plants of West Tropical Africa, Vol. 1. Royal Botanic Gardens, Kew, London.

Collins M. A. and Charles H. P. (1987), Antimicrobial activity of carnosol and ursolic acid: two anti-oxidant constituents of *Rosmarinus officinalis* L. Food Microbiol. **4**, 311–315.

Gentry A. H. (1980), Bignoniaceae, Part I. Flora Neotropica Monogr. 25. Botanical Garden, New York, pp. 1–130.

Gülz P. G. (1994), Epicuticular leaf waxes in the evolution of the plant kingdom. J. Plant Physiol. **143**, 453–464.

Hutchinson J. and Dalziel J. M. (1963), Flora of West Tropical Africa, Vol. II. Royal Botanic Gardens, Kew, London.

Janzen D. H. (1975), Ecology of Plants in the Tropics. Edward Arnold, London.

Liu J. (1995), Pharmacology of oleanolic acid and ursolic acid. J. Ethnopharmacol. **49**, 57–68.

Missouri Botanical Garden (2003), www.mobot.org/MOBOT/research link w^3-TROPICOS.

Nyberg G., Ekblad A., Buresh R., and Hoegberg P. (2002), Short-term patterns of carbon and nitrogen mineralization in a fallow field amended with green manures from agroforestry trees. Biol. Fertil. Soils **36**, 18–25.

Schoonhoven L. M., Jeremy T., and van Loon J. J. A. (1998), Insect-Plant Biology: from Physiology to Evolution. Chapman & Hall, London, pp. 31–82.

Shepherd G., Buresh R. J., and Gregory P. J. (2000), Land use affects the distribution of soil inorganic nitrogen in smallholder production systems in Kenya. Biol. Fertil. Soils **31**, 348–355.

Steentoft M. (1988), Flowering Plants in West Africa. Cambridge University Press, Cambridge.

Stermitz F. R., Arslanian R. L., and Castro O. (1992), Flavonoids from the leaf surface of *Godmania aesculifolia* (Bignoniaceae). Biochem. Syst. Ecol. **20**, 481.

Wollenweber E., Dörr M., and Gomez L. P. D. (1996), Exudate flavonoids in *Godmania aesculifolia* (Bignoniaceae). Biochem. Syst. Ecol. **24**, 481–482.