

Analysis of the essential oil from leaves of *Croton zambesicus* Muell. Arg. growing in Benin

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ABSTRACT: The leaves of *Croton zambesicus* are widely used in African folk medicine as a water decoction to treat hypertension, microbial infections and fever associated with malaria. Cytotoxic trachylobane and pimarane diterpenes have been isolated from a dichloromethane extract of the leaves. In order to check whether these compounds are present in the essential oil, we have analysed its composition by GC-FID and GC-MS. The major constituents of the oil are caryophyllene oxide (19.5%), β -caryophyllene (10.8%), α -copaene (6.3%), linalool (6.1%) and β -pinene (5.2%). Only the less polar diterpenes are present in the oil: *ent*-trachyloban-3-one (3.0%), *ent*-trachyloban-3 β -ol (0.5%), isopamara-7, 15-dien-3 β -ol (1.0%), *ent*-trachylobane (0.7%), sandaracopimaradiene (0.4%) and kaurene (2.1%). Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: *Croton zambesicus*; Euphorbiaceae; essential oil composition; diterpenes; trachylobane; isopimarane

Introduction

Croton zambesicus Muell. Arg. (family Euphorbiaceae) (syn. *C. amabilis* Muell. Arg., syn. *C. gratissimus* Burch.) is a shrub or a small tree reaching 10 m in height. It is a Guineo-Congolese species widespread in Tropical Africa. The leaf decoction is used in Benin as an antihypertensive, an antimicrobial (urinary infections) and to treat fever associated with malaria.^{1,2} In Zambia and South Africa, the leaves, characterized by a pleasant aromatic odour, are used as a home deodorant.³

Recently we have isolated isopimarane and trachylobane diterpenes from a dichloromethane extract of the leaves.^{4,5} These compounds showed cytotoxic activity on several cell lines.^{4,5} Previous studies on the composition of the essential oil from the leaves of *C. zambesicus* described linalool (9.9%) and β -caryophyllene (9.9%) as the major constituents in a sample from Tchad.⁶ In a sample from Cameroon, the major constituents were limonene (19.2%), β -pinene (18.9%), β -caryophyllene (15.8%) and α -pinene (8.7%).⁷ In order to complete the analysis of the essential oil obtained from different samples of this plant and check whether diterpenes may be present in the essential oil, GC-FID and GC-MS analysis have been performed on a sample of plant growing in Benin. This is the first time that diterpenes are quantified and identified in the essential oil.

Experimental

Plant Material

Leaves of *C. zambesicus* were collected on 6 February 2003 in Agon in the Atlantic Department in Benin (20 km to the north of Cotonou). They were dried the same day in the dark at 25 °C. After drying, the plant material was powdered and stored at room temperature in a dry, dark place till analysis. The plant was identified by a Beninese botanist, Professor V. Adjakidje (Université d'Abomey-Calavi). A sample has been deposited in the herbarium of the National Botanical Garden of Belgium in Meise (BR S.P. 848.108).

Oil Isolation

Leaves (120 g) were hydrodistilled for 4 h in a Clevenger-type apparatus, as described in the *European Pharmacopoeia*.⁸ The distillation was made in triplicate in order to determine the yield of the essential oil. Essential oil was stored at –20 °C until analysis. Oil yield (% v/w) obtained from the leaves was 0.45%. The essential oil was diluted 10 times in *n*-hexane prior to GC-FID and GC-MS analysis.

GC-FID

GC analysis were performed on a Thermo Finnigan Focus GC under the following conditions: injector

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of domestication over a long period.⁴ The use of essential oils as a taxonomic descriptor coupled with genetic fingerprinting^{14,15} would provide in basil a potent combination for taxonomic classifications of this genus.

The groups of *Ocimum* species based on morphological characteristics do not correspond to the groups based on volatile oils constituents. Each group of volatile oil profiles can occur in different species, for example, the citral/spathulenol type occurs in both *O. × citriodorum* and *O. basilicum*; linalool-rich types are frequent in *O. basilicum* varieties, *O. minimum*, *O. kilimandscharicum* and *O. × citriodorum*. The presence of methyl-cinnamate-rich types in both *O. americanum* and *O. basilicum* clearly shows that species and essential oil profiles do not necessarily correspond. Basil cultivars are the result of many years of breeding and selection. As morphological traits and chemical characteristic segregation are not necessarily linked, plants can obtain morphological characteristics from one parent or chemical characteristics from the other. Therefore, several variations can be found at infraspecific level.

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References

- Simon JE, Quinn J, Murray RG. *Advances in New Crops Research*, Janick J, Simon JE (eds). Timber Press, Portland, Oregon, 1990: 484–489.
- Albuquerque UP, Andrade LHC. *Anales Jard. Bot. Madrid*, 1998; **6**(1): 43–64.
- Gupta R. *G-15 Gene Banks Med. Arom. Plants Newsl.*; 1994; **5**/6: 1–3.
- Vieira RF, Simon JE. *Econ. Bot.*, 2000; **54**(2): 207–216.
- Simon JE, Chadwick AF, Craker LE. *Herbs: an Indexed Bibliography 1971–1980; the Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plant of the Temperate Zone*. Archon Books, Hamden, 1984; 7–9.
- de Baggio T, Belsinger S. *Basil, an Herb Lover's Guide*. Interweave Press, Colorado, 1996.
- Darrah H. *The Cultivated Basils*. Buckeye, Missouri, 1980.
- Darrah H. *Econ. Bot.*, 1984; **28**: 63–67.
- Marotti M, Piccaglia R, Giovanelli E. *J. Agric. Food Chem.*, 1996; **44**: 3926–3929.
- Harley MM, Paton A, Harley RM, Cade PG. *Grana*, 1992; **31**: 161–176.
- Paton A. *Kew Bull.*, 1992; **47**(3): 403–435.
- Tucker AO. *Herbs, Spices and Medicinal Plants*, Vol I, Craker LE, Simon JE (eds). Haworth Press, New Jersey, 1986; 33–80.
- Paton A, Putievsky E. *Kew Bull.*, 1996; **51**(3): 1–16.
- Vieira RF, Grayer R, Paton A, Simon JE. *Biochem. Syst. Ecol.*, 2001; **29**: 287–304.
- Vieira RF, Goldsbrough P, Simon JE. *J. Am. Soc. Hort. Sci.*, 2003; **128**(1): 94–99.
- Maffei M. *Biochem. Syst. Ecol.*, 1990; **18**(7/8): 493–502.
- Maffei M, Paracino V, Sacco T. *Acta Hort.*, 1993; **330**: 159–169.
- Canigual S, Vila R, Vicario G, Tomas X, Adzet T. *Biochem. Syst. Ecol.*, 1994; **22**(3): 307–315.
- Keskitalo M, Linden A, Valkonen JPT. *Theor. Appl. Genet.*, 1998; **96**(8): 1141–1150.
- Adams RP, Demeke T, Abulfatih HA. *Theor. Appl. Genet.*, 1993; **87**: 22–26.
- Charles DJ, Simon JE. *J. Am. Soc. Hort. Sci.*, 1990; **115**(3): 458–462.
- Morales MR, Simon JE. *Hortscience*, 1997; **32**(1): 148–149.
- Grayer RJ, Kite GC, Goldstone FJ, Bryan SE, Paton A, Putievsky E. *Phytochemistry*, 1996; **43**(5): 1033–1039.
- Sarin, YK, Agarwal SG, Thappa RK, Singh K, Kapahi BK. *J. Essent. Oil Res.*, 1992; **4**: 515–519.
- Demissew, S. *J. Essential Oil Res.*, 1993; **5**(5): 465–479.
- Lawrence BM. *Advances in Labiatae Science*, Harley RM, Reynolds T (eds). The Royal Botanical Garden, London, 1992; 399–436.
- Hegnauer R. *Chemotaxonomie der Pflanzen*, vol IV. Birkhauser, Basel, 1966; 313–314.
- Gottlieb OR. *Micromolecular Evolution, Systematics, and Ecology: an Essay into a Novel Botanical Discipline*. Springer, Berlin, 1982.
- Egerton-Warburton LM, Ghisalberti EL, Considine JA. *Biochem. Syst. Ecol.*, 1998; **26**: 873–888.
- Cool LG, Hu Z, Zavarin E. *Biochem. Syst. Ecol.*, 1998; **26**: 899–913.
- Timmermann BN, McLaughlin SP, Hoffmann J. *Biochem. Syst. Ecol.*, 1987; **4**: 401–410.

temperature, 220 °C; detector temperature, 240 °C; column, DB-5 capillary column (30 m × 0.25 mm i.d., coating thickness 0.25 µm; J&W Scientific, Agilent Technologies); oven temperature programme, from 60 °C to 240 °C at 3 °C/min; carrier gas, helium at 1 ml/min; injection, 1 µl (10% hexane solution); split ratio, 1/30. The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization, all relative response factors being taken as 1.

GC-MS instrument

GC-EIMS analyses were performed using a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm i.d., coating thickness 0.25 µm; J&W Scientific, Agilent Technologies) and a Varian Saturn 2000 ion trap mass detector. Injector and transfer line temperatures, 220 °C and 240 °C, respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas, helium at 1 ml/min; injection, 1 µl 10% hexane solution; split ratio, 1/30.

Identification of the compounds

Identification of the constituents was based on comparison of their retention times with those of authentic samples, comparing their linear retention indices (LRI) relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 95 and Adams) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data.^{9–13} Trachylobane and pimarane were identified by comparing their LRI and their mass spectra with pure compounds isolated from the plant. Standard solutions of *ent*-trachyloban-3-one, *ent*-trachyloban-3β-ol and isopimarane-7, 15-dien-3α-ol were prepared at 0.1 mg/ml in dichloromethane. The percentages of the components were calculated from the GC peak areas, using the normalization method.

Results and Discussion

Essential oil yield was determined as 0.45% (v/w) and as reported in Table 1, this essential oil is characterized by a high proportion of sesquiterpenes (66.3%), while the monoterpenes are present in smaller amount (21.2%) and the diterpenes are present in a relatively high proportion (12.5%). Some of these diterpenes have been identified as trachylobane, isopimarane and kaurene derivatives. Among the diterpenes isolated from the dichloromethane extract of the leaves, only the less polar compounds are present in the oil. The major diterpene is *ent*-trachyloban-3-one (3.0%). It is also the major diterpene present in the

Table 1. Percentage composition of the essential oil from the leaves of *Croton zambesicus*

Constituents	LRI*	GC/FID
α-Thujene	933	0.4
α-Pinene	942	1.3
Camphene	959	0.5
Sabinene	979	1.0
β-Pinene	984	5.2
p-Cymene	1030	0.7
Limonene	1035	0.4
1,8-Cineole	1039	1.7
γ-Terpinene	1064	0.2
cis-Sabinene hydrate	1075	0.3
Linalool	1098	6.1
trans-Pinocarveol	1147	0.8
Camphor	1154	2.3
Pinocavone	1162	0.6
Borneol	1165	0.5
4-Terpineol	1177	0.4
Myrtenol	1193	1.2
Cyclosativene	1368	0.6
α-Copaene	1376	6.3
β-Bourbonene	1384	3.1
Cyperene	1402	0.9
β-Cedrene	1418	0.3
β-Caryophyllene	1418	10.8
α-Humulene	1454	1.6
allo-Aromadendrene	1461	1.6
γ-Murolene	1477	1.0
Germacrene D	1480	4.8
α-Murolene	1499	0.5
Cuparene	1505	0.3
trans-γ-Cadinene	1513	1.0
δ-Cadinene	1524	1.1
Caryophyllene oxide	1581	19.5
Guaiol	1595	0.7
Humulene oxide II	1606	2.3
ent-Trachylobane	1989	0.7
Sandaracopimaradiene	1996	0.4
Kaurene	2034	2.1
ent-Trachyloban-3-one	2226	3.0
ent-Trachyloban-3β-ol	2242	0.5
Isopimarane-7,15-dien-3β-ol	2244	1.0
Total identified (%)		87.7
Yield (% v/w)		0.45

* Linear retention index on DB-5 column.

dichloromethane extract of the leaves (data not shown). Unfortunately, because of lack of reference compounds, we could not identify all the diterpenes in the oil. No previous reports have described the presence of trachylobane, isopimarane and kaurene diterpenes in the essential oil of this plant.

The major compounds of the essential oil are caryophyllene oxide (19.5%), β-caryophyllene (10.8%), α-copaene (6.3%), linalool (6.1%) and β-pinene (5.2%). In comparison to previous studies on the essential oil of leaves, this sample from Benin contains much more caryophyllene oxide (4.6% in a sample from Tchad, 0.3% in a sample from Cameroon)^{6,7} and almost the same amount of β-caryophyllene as in the sample from Tchad (9.9%) but less than in the sample from Cameroon

of domestication over a long period.⁴ The use of essential oils as a taxonomic descriptor coupled with genetic fingerprinting^{14,15} would provide in basil a potent combination for taxonomic classifications of this genus.

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- Simon JE, Quinn J, Murray RG. *Advances in New Crops Research*, Janick J, Simon JE (eds). Timber Press, Portland, Oregon, 1990: 484–489.
- Albuquerque UP, Andrade LHC. *Anales Jard. Bot. Madrid*, 1998; **6**(1): 43–64.
- Gupta R. *G-15 Gene Banks Med. Arom. Plants Newsl.*; 1994; **5**/6: 1–3.
- Vieira RF, Simon JE. *Econ. Bot.*, 2000; **54**(2): 207–216.
- Simon JE, Chadwick AF, Craker LE. *Herbs: an Indexed Bibliography 1971–1980; the Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plant of the Temperate Zone*. Archon Books, Hamden, 1984; 7–9.
- de Baggio T, Belsinger S. *Basil, an Herb Lover's Guide*. Interweave Press, Colorado, 1996.
- Darrah H. *The Cultivated Basils*. Buckeye, Missouri, 1980.
- Darrah H. *Econ. Bot.*, 1984; **28**: 63–67.
- Marotti M, Piccaglia R, Giovanelli E. *J. Agric. Food Chem.*, 1996; **44**: 3926–3929.
- Harley MM, Paton A, Harley RM, Cade PG. *Grana*, 1992; **31**: 161–176.
- Paton A. *Kew Bull.*, 1992; **47**(3): 403–435.
- Tucker AO. *Herbs, Spices and Medicinal Plants.*, Vol I, Craker LE, Simon JE (eds). Haworth Press, New Jersey, 1986; 33–80.
- Paton A, Putievsky E. *Kew Bull.*, 1996; **51**(3): 1–16.
- Vieira RF, Grayer R, Paton A, Simon JE. *Biochem. Syst. Ecol.*, 2001; **29**: 287–304.
- Vieira RF, Goldsbrough P, Simon JE. *J. Am. Soc. Hort. Sci.*, 2003; **128**(1): 94–99.
- Maffei M. *Biochem. Syst. Ecol.*, 1990; **18**(7/8): 493–502.
- Maffei M, Paracino V, Sacco T. *Acta Hort.*, 1993; **330**: 159–169.
- Canigueral S, Vila R, Vicario G, Tomas X, Adzet T. *Biochem. Syst. Ecol.*, 1994; **22**(3): 307–315.
- Keskitalo M, Linden A, Valkonen JPT. *Theor. Appl. Genet.*, 1998; **96**(8): 1141–1150.
- Adams RP, Demeke T, Abulfatih HA. *Theor. Appl. Genet.*, 1993; **87**: 22–26.
- Charles DJ, Simon JE. *J. Am. Soc. Hort. Sci.*, 1990; **115**(3): 458–462.
- Morales MR, Simon JE. *Hortscience*, 1997; **32**(1): 148–149.
- Grayer RJ, Kite GC, Goldstone FJ, Bryan SE, Paton A, Putievsky E. *Phytochemistry*, 1996; **43**(5): 1033–1039.
- Sarin, YK, Agarwal SG, Thappa RK, Singh K, Kapahi BK. *J. Essent. Oil Res.*, 1992; **4**: 515–519.
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- Lawrence BM. *Advances in Labiate Science*, Harley RM, Reynolds T (eds). The Royal Botanical Garden, London, 1992; 399–436.
- Hegnauer R. *Chemotaxonomie der Pflanzen*, vol IV. Birkhauser, Basel, 1966; 313–314.
- Gottlieb OR. *Micromolecular Evolution, Systematics, and Ecology: an Essay into a Novel Botanical Discipline*. Springer, Berlin, 1982.
- Egerton-Warburton LM, Ghisalberti EL, Considine JA. *Biochem. Syst. Ecol.*, 1998; **26**: 873–888.
- Cool LG, Hu Z, Zavarin E. *Biochem. Syst. Ecol.*, 1998; **26**: 899–913.
- Timmermann BN, McLaughlin SP, Hoffmann J. *Biochem. Syst. Ecol.*, 1987; **4**: 401–410.