Pergamon

PII: S0031-9422(97)00505-0

Phytochemistry, Vol. 46, No. 8, pp. 1365-1368, 1997

Printed in Great Britain
0031-9422/97 $17.00+0.00

ESSENTIAL OILS OF *OTACANTHUS*

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(*Received in revised form* 28 *April* 1997)

Key Word Index—*Otacanthus*; Scrophulariaceae; essential oils; chemotaxonomy.

Abstract—Ten taxa of *Otacanthus* were found to contain essential oils ranging from 0.007 to 0.2% of fresh weight, that consisted of monoterpenoids and sesquiterpenoids. Their composition was submitted to principal component analysis and cluster analysis. This provided additional data for the classification of the taxa, especially for the position of intermediate taxa that are difficult to classify on purely morphological grounds. © 1997 Elsevier Science Ltd

INTRODUCTION

In the genus *Otacanthus*, six species have been described so far [1]. They are small shrubs with simple opposite leaves and a terminal spike of showy flowers. Based on a morphological study conducted on 90 herbarium collections, a revision of the genus was carried out. However, some intermediate taxa occur that are presumed to be hybrids, but are difficult to classify on morphological characteristics [2]. On the other hand, it was found by De Pooter *et al.* [3] that the aerial parts of *Otacanthus coeruleus* contain between 0.2 and 0.3% of essential oil on a fr. wt basis. The oil consists of monoterpenes and sesquiterpenes, including α-copaene and the novel sesquiterpene, β-copaen-4α-ol. No other essential oils within *Otacanthus* have been reported previously. Further investigation of the essential oil within this genus was expected to yield interesting results, both for phytochemical reasons and for obtaining additional characters for the taxonomic revision of this genus. In the present work, the following taxa were investigated: *O. coeruleus* Lindley, *O. fluminensis* Kuhlmann, *O. platychilus* (Radlk.) Taub., *O. spec.*, *O. villosus* Philcox, and three intermediate forms that were presumed to be hybrids. For *O. villosus*, three plants from one population were analysed, two of them (no.s 3 and 5) morphologically nearly identical but releasing different odours when crushing the leaves, the third plant (no. 4) exhibiting small morphological differences from the others.

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RESULTS AND DISCUSSION

The investigation taxa of *Otacanthus* all contain essential oil; contents are shown in Table 1 as percent fresh weight. The highest oil content was found in *O. coeruleus* (0.2% fr. wt), and fits with the results of De Pooter *et al.* [3]. In the other taxa, the oil content was below 0.1%, except for *O. villosus*, that contained between 0.1 and 0.2% of essential oils. The lowest yield (0.007% fr. wt) was found in taxon no. 2.

The composition of the essential oils of *Otacanthus* is shown in Table 2. Only the main components, i.e. those that represent at least 5% of the oil in at least one taxon, are shown. All taxa contain a very complex oil, with 32 to 102 compounds in each. All the identified compounds are monoterpenoids or sesquiterpenoids. The six main monoterpenoids are oxygenated compounds: 1,8-cineole, linalool, *trans*-pinocarveol, pinocarvone, myrtenal and myrtenol; most of them belong to the pinane group. Of the 21 main

Table 1. Essential oil content of aerial plant parts of several taxa of *Otacanthus*

No.	Taxon	Content (% fr. wt)
1	coeruleus	0.200
2		0.007
3	villosus	0.175
4	villosus	0.114
5	villosus	0.131
6		0.025
7		0.017
8	spec.	0.070
9	fluminensis	0.030
10	platychilus	0.015

Table 2. Major constituents (%) in volatile oils of several *Otacanthus* taxa

Compound	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
1,8-Cineole	—	1.9	—	—	—	1.1	—	10.0	—	—
Linalool	0.8	0.9	—	0.4	0.3	0.6	10.6	0.4	2.6	0.6
trans-Pinocarveol	13.7	2.1	—	0.1	0.2	0.1	0.4	0.7	—	—
Pinocarvone	6.5	1.4	—	—	—	—	0.3	0.7	—	—
Myrtenal	5.8	1.4	0.7	—	—	—	—	0.8	—	—
Myrtenol	6.0	1.2	—	—	—	—	—	0.2	—	—
R_i 1403	—	5.8	—	—	—	0.2	—	—	0.1	0.6
R_i 1409	—	—	—	—	—	—	—	9.9	—	—
β-Caryophyllene	0.3	9.1	—	—	—	0.9	—	0.8	—	—
α-Humulene	6.7	—	—	—	—	—	0.8	—	1.1	—
R_i 1450	—	—	13.2	—	—	0.5	—	1.5	—	0.4
β-Cadinene	—	0.7	—	—	—	—	8.1	0.8	—	—
R_i 1497	—	—	—	—	1.3	1.6	—	7.1	—	—
Calamenene	—	1.5	—	—	—	—	—	7.4	—	—
R_i 1508	—	—	31.4	—	—	4.8	2.8	—	—	—
δ-Cadinene	0.9	4.6	—	—	—	—	3.3	9.0	—	2.9
R_i 1517	—	—	7.1	—	—	—	—	—	—	—
R_i 1523	—	—	0.8	—	—	—	—	5.1	—	—
Caryophyllene-oxide	0.6	—	—	—	—	—	—	—	5.4	—
β-Copaen-4α-ol	13.0	7.5	—	—	—	—	—	3.3	—	—
R_i 1579	—	—	—	—	—	6.6	—	—	—	—
β-Oplopenon	—	2.9	5.9	—	—	1.6	—	2.8	—	—
R_i 1595	3.6	0.3	17.2	78.0	81.0	0.8	—	3.0	0.3	—
R_i 1600	0.1	2.3	—	—	—	0.7	11.5	—	—	0.2
R_i 1600 bis	—	—	—	—	—	—	5.3	—	—	—
R_i 1662	—	2.7	—	0.3	—	—	—	—	10.9	—
R_i 1662 bis	—	—	—	—	—	—	2.1	5.8	—	—
R_i 1766	—	—	—	—	—	1.8	—	—	35.9	—
R_i 1826	—	—	—	—	0.1	—	—	—	0.7	20.1

Identity of the taxon numbers is as follows: No. 1: *O. coeruleus*; no.s 3, 4 and 5: *O. villosus*; no. 8: *O. spec.*; no. 9: *O. fluminensis*; no. 10: *O. platychilus*; no.s 2, 6 and 7 are intermediate taxa.

sesquiterpenoids, 12 are not identified, six are hydrocarbons and three are oxygenated.

Non-hierarchical cluster analysis for seven clusters within the data of Table 2 resulted in five clusters of one taxon, a cluster containing the intermediate taxa (2, 6 and 7), and a cluster with two numbers of *O. villosus* (no.s 4 and 5). Thus, the plants of *O. villosus* that belong to the same chemotype (no. 4 and no. 5) show morphologically some differences, while the plants that are morphologically nearly identical (no. 3 and no. 5) belong to different chemotypes. The importance of the individual compounds for discrimination between taxa was also given by this method, and the most discriminating compounds appeared to be mostly those that are present in considerable amounts in only one taxon (results not shown). The percentages of 12 of these 'typical' compounds in each taxon are shown in Fig. 1. In *O. coeruleus*, the typical compounds are α-humulene and the pinane monoterpenes, *trans*-pinocarveol, pinocarvone, myrtenal and myrtenol; in *O. villosus*, a compound with R_1 1595 in taxa 4 and 5, and three compounds with R_1 1508, R_1 1450, and R_1 1595 in taxon 3; in *O. spec.*, 1,8-cineole, calamenene and a compound with R_1 1409; in *O. fluminensis*, a compound with R_1 1766; in *O. platychilus*, it is an unknown sesquiterpenoid with R_1 1826. It also appears that the sesquiterpenoid β-copaen-4α-ol, only occurs in *O. coeruleus*, in *O. spec.* and taxon 2.

Hierarchical clustering of the data with Euclidean distance was done with either single linkage or Ward's linkage method (Fig. 2); both methods yielded rather similar clusters. Taxa 4 and 5 of *O. villosus* are most remote from the other taxa, but are very close to each other. Next to be separated are *O. villosus* no. 3 and *O. fluminensis*, while the putative hybrids lie more or less close to *O. platychilus*, *O. coeruleus* and *O. spec.* However, since the distance between the chemotypes of *O. villosus* is larger than between taxa of different species, it appears that the distance between taxa in the dendrogram is not a good measure of their relationship. This is probably the consequence of the unbalanced structure of the data, as described by Clifford and Williams [4], with only one species containing several near-identical taxa.

Principal component analysis yielded 10 factors, the first two factors accounting for 40% of the total variation. The scatter diagrams for each pair of factors yielded similar relative positions of the taxa. The diagram of factor 2 *vs* factor 1 is shown in Fig. 3. Taxa 4 and 5 of *O. villosus* are placed in identical positions, and taxon 3 of the same species lies in their proximity.

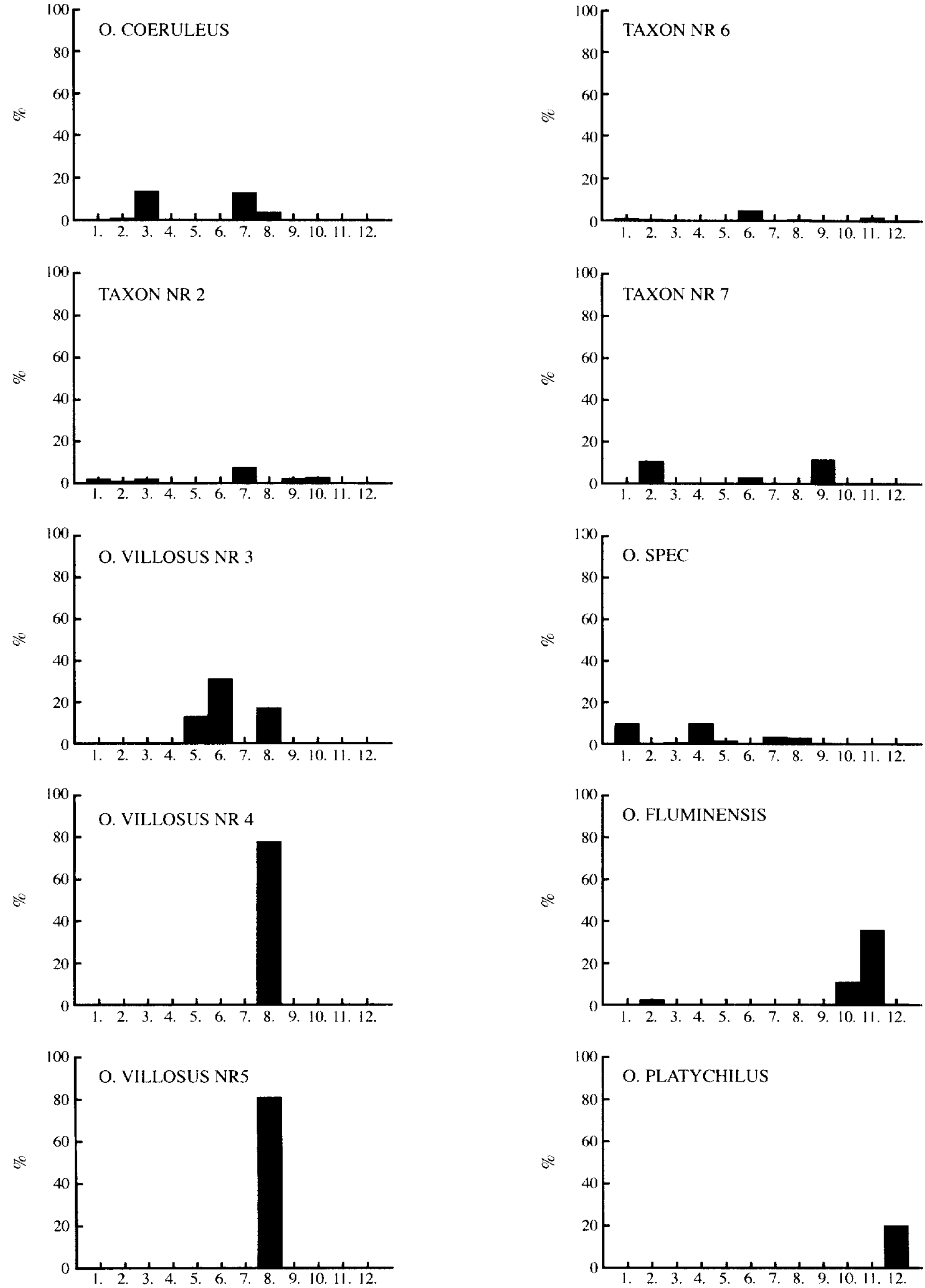

Fig. 1. Percentage of some main components of essential oils of ten *Otacanthus* taxa. Bar 1 represents 1,8-cineole; bar 2 linalool; bar 3 *trans*-pinocarvol; bar 4 R_I 1409; bar 5 R_I 1450; bar 6 R_I 1508; bar 7 β-copaen-4α-ol; bar 8 R_I 1595; bar 9 R_I 1600; bar 10 R_I 1662; bar 11 R_I 1766; bar 12 R_I 1826.

This suggests that the position of the taxa in the scatter diagrams gives a better measure of their relationship than the dendrograms, which can be explained from the fact that they represent two dimensions instead of one. *Otacanthus coeruleus* and taxon 2 are close to each other, with *O. spec.* close by, suggesting that taxon 2 is a hybrid between *O. coeruleus* and *O. spec.* (Fig. 3). Similarly, taxon 6 could be a hybrid between

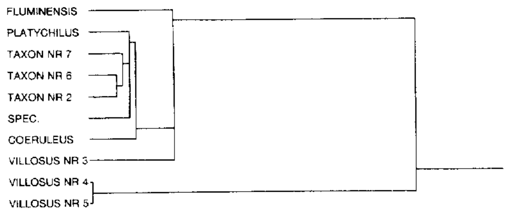

Fig. 2. Dendrogram obtained by application of Ward's linkage method to the Euclidean distances.

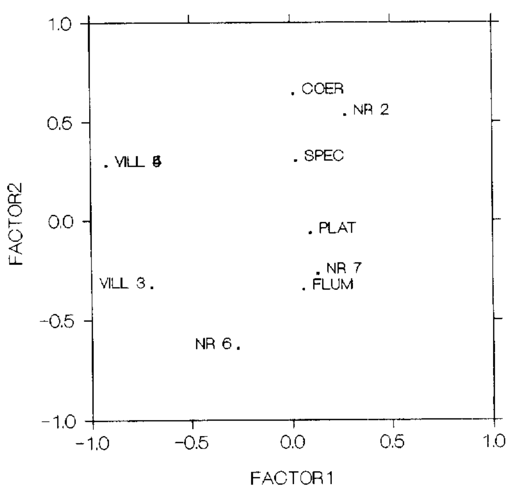

Fig. 3. Scatter diagram of *Otacanthus* taxa.

O. villosus (no. 3) and *O. fluminensis*, as it lies between these two species, and taxon 7 between *O. platychilus* and *O. fluminensis*.

The results of principal component analysis on morphological characters of 85 herbarium specimens (Ronse, in preparation) are completed by the chemical data. The position of taxon 8 in a separate cluster corroborates its placement as a separate species (*O. spec.*), as was done on morphological grounds, although only a few herbarium specimens of the taxon are available. On the basis of morphological characters, the intermediate taxa are placed outside the species clusters, and form themselves two clusters, being most remote from *O. platychilus*. This makes it difficult to classify them, but the chemical data give more indications. Taxon 2 is probably a hybrid between *O. coeruleus* and *O. spec.*; its close relationship to *O. spec.* is confirmed by its morphology and the locality of where it was found. Taxon 6 and taxon 7 are close to *O. coeruleus*, *O. fluminensis* and *O. villosus* according to the morphological results; chemical data confirm a close relationship with the latter two species, while their locality in the close vicinity of populations of *O. villosus* further confirms the close relationship with this species. Within *O. villosus*, two chemotypes were found that do not correspond with the morphological variation within this species. In conclusion, the chemical data give complementary information to the morphological and geographical data, without contradicting them.

EXPERIMENTAL

Plant material. Taxa were numbered and their taxonomic identity, based on morphological study [2], is given between brackets when it is known: no. 1 (*O. coeruleus*): F. Billiet 28-4-1980, between St Philippe and Pointe du Tremblet, La Réunion; voucher F. Billiet and B. Jadin 860, BR and MO. no. 2: H. Boudet Fernandes 9-12-1988, Colatina, Espirito Santo, Brazil; voucher Boudet Fernandes 2681, MBML and BR. no. 3 (*O. villosus*): A. Ronse 17-6-1991, Nova Viçosa, Bahia, Brazil; voucher A. Ronse 5, BR. no. 4 (*O. villosus*): A. Ronse 17-6-1991, Nova Viçosa, Bahia, Brazil; voucher A. Ronse 4, BR. no. 5 (*O. villosus*): A. Ronse 17-6-1991, Nova Viçosa, Bahia, Brazil; voucher A. Ronse 6, BR. no. 6: A. Ronse 22-6-1991, way to Sta Luzia, Canavieiras, Bahia, Brazil. no. 7: A. Ronse 22-6-1991, Canavieiras, Bahia, Brazil; voucher A. Ronse 7, BR. no. 8 (*Otacanthus spec.*): H. Boudet Fernandes 7-9-1989, Itaguaçu, Espirito Santo, Brazil; voucher Boudet Fernandes 2793, MBML and BR. no. 9 (*O. fluminensis*): A. Ronse 15-6-1991, Domingos Martins, Espirito Santo, Brazil; voucher A. Ronse 3, BR. no. 10 (*O. platychilus*): A. Ronse 26-5-1990, C.V.R.D., Linhares, Espirito Santo, Brazil; voucher A. Ronse 1, CVRD.

For each sample, flowering shoots of one to three plants were taken. Fresh plant material was used from plants grown in the greenhouses of the National Botanic Garden of Belgium during 6 months (no.s 4, 5 and 10) or 11 years (no. 1). For the remaining taxa, material was gathered from plants *in situ*, air dried and used for extraction within 2 weeks after collection.

Extraction. Between 20 and 30 g of plant material was rinsed with H_2O and cut, and subjected to extraction for 2 hrs. Extractions were carried out according to the method of ref. [5], adapted to small quantities of plant material by the use of a micro steam distillation-extraction apparatus. GC-MS analysis and identification of the components was as described in ref. [3].

Acknowledgements—We thank the following persons for donating plant material of *Otacanthus*: H. Boudet Fernandes, Museu de Biologia Mello Leitão, Sta Teresa; M. de Souza Menandro, Reserva Florestal da Companhia Vale do Rio Doce, Linhares; R. Kautsky, Domingos Martins, Espirito Santo, Brazil.

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