Phytochemistry, Vol. 25, No. 3, pp. 655–657, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

COUMARINS OF THE RUTOIDEAE: TRIBE DIOSMEAE*

W. E. CAMPBELL, T. MAJAL and P. A. BEAN†

Department of Organic Chemistry, University of Cape Town, Rondebosch 7700, South Africa; † Bolus Herbarium, University of Cape Town, Hiddingh Hall, Orange Street Campus, Gardens, South Africa

(*Revised received* 11 *September* 1985)

Key Word Index—*Agathosma*; *Diosma*; *Empleurum*; Rutaceae; Diosmeae; coumarins; chemotaxonomy.

Abstract—Twenty four species from the genera *Agathosma*, *Diosma* and *Empleurum* (tribe Diosmeae) were investigated for coumarins. Nine simple coumarins were isolated and their chemotaxonomic significance is discussed.

INTRODUCTION

The Diosmeae, one of the five tribes of a subfamily of the Rutaceae, the Rutoideae, is confined mainly to the predominantly winter-rainfall fynbos of the South-West and Southern regions of South Africa. The tribe consists of 10 genera and approximately 260 shrubby and subshrubby species, more than half belonging to the genus *Agathosma* Willd.

Relatively little is known of the chemistry of the Diosmeae. Simple coumarins have been isolated from a few species of the genera *Agathosma*, *Coleonema*, *Diosma* and *Phyllosma* [1–6]. We wish to report here the results of a phytochemical examination of plant material from a further 24 species, collected in connection with the taxonomic revision of certain genera of the tribe.

*Part 1 in the series "Chemosystematic Studies in the Genera of the Diosmeae".

Table 1. Coumarins of the tribe Diosmeae

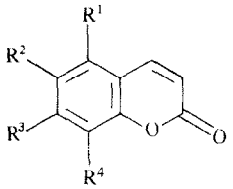
Species	Total coumarin content (% dry wt)	Coumarins identified 1	2	3	4	5	6	7	8	9
Genus *Agathosma* Willd.										
A. abrupta	0.006									+
A. affinis	0.009		+							+
A. capensis	0.005		+							
A. cedrimontana	0.002		+							+
A. ciliaris	0.002		+							
A. collina	0.004		+	+						
A. dielsiana	0.01		+			+				+
A. eriantha	0.006		+							
A. imbricata	0.003		+	+					+	
A. lanceolata	0.004		+							
A. martiana	0.13		+	+		+		+		
A. mucronulata	0.04			+	+		+	+		
A. mundtii	0.003			+	+	+				
A. puberula	0.03					+	+	+		
A. recurvifolia	0.02		+	+		+		+		
A. scaberula	0.007		+							
A. serpyllacea	0.003			+						
A. spinosa	0.002			+				+		
A. thymifolia	0.004			+						
A. unicarpellata	0.002			+						
Genus *Diosma* L.										
D. prama	0.001	+								
D. ranosissima	0.005	+				+				
D. recurva	0.003			+						
Genus *Empleurum* Soland ex Ait										
E. unicapsulare	0.002		+							

RESULTS AND DISCUSSION

The nine coumarins which have been identified are shown in Fig. 1 and the distribution of these compounds in the 24 species from the genera *Agathosma*, *Diosma* and *Empleurum* is given in Table 1.

The total coumarin content of each species varied from 10 μg/g dry wt. (0.001% in *D. prama*) to 1 300 μg/g dry wt (0.13% in *A. martiana*). 7-Oxygenated [7-(3′,3′-dimethylallyloxy)-coumarin (**1**)], 6,7-dioxygenated [6,7-dimethoxycoumarin (**2**) and 6-methoxy-7-(3′,3′-dimethylallyloxy)-coumarin (**3**)], 7,8-dioxygenated [7-(3′,3′-dimethylallyloxy)-8-methoxycoumarin (**4**)], 6,7,8-trioxygenated [6,7,8-trimethoxycoumarin (**5**), 6-methoxy-7-(3′,3′-dimethylallyloxyl-8-hydroxycoumarin (**6**), 6,8-dimethoxy-7-(3′,3′-dimethylallyloxy)-coumarin (**7**) and 6-methoxy-7,8-methylenedioxycoumarin (**8**)] and 5,6,7-trioxygenated coumarins [5,6,7-trimethoxycoumarin (**9**)] were isolated. The results confirm the previous findings [1–6], namely that the Diosmeae would appear to elaborate only the 'simple' coumarins and not furo- and pyrano-coumarins. A further genus, *Empleurum*, has now been added to the four already shown to contain coumarins.

Agathosma martiana, A. mucronulata, A. puberula, A.



	R^1	R^2	R^3	R^4
1	H	H	OPre	H
2	H	OMe	OMe	H
3	H	OMe	OPre	H
4	H	H	OPre	OMe
5	H	OMe	OMe	OMe
6	H	OMe	OPre	OH
7	H	OMe	OPre	OMe
8	H	OMe	O—CH_2—O	
9	OMe	OMe	OMe	H

OPre = $OCH_2CH{=}C(Me)_2$

Fig. 1.

Table 2. Plant materials*

Species	Locality	Date collected	Collector and No.
Agathosma abrupta Pillans	Grootbos, Gansbaai	August 1982	Bean 834
A. affinis Sond.	Kammanassie, Oudtshoorn district	August 1983	Bean 1185
A. capensis Düm.	Kleinjongenskraal, Citrusdal district	August 1982	Bean 792
A. cedrimontana Düm.	Pienaarsvlakte, Cedarberg	September 1983	Bean 1343
A. ciliaris Druce	Mossel river mouth, Stanford district	February 1979	Bean 183
A. collina E. & Z.	Rhenosterkop, Bredasdorp district	November 1980	Bean 491
A. dielsiana Schltr.	Eliaskraal, Bredasdorp district	April 1983	Bean 1126
A. eriantha Stend.	Bredasdorp Limeworks, Quarry Hill	June 1979	Bean 191
A. imbricata Willd.	*ca* 3 km south of the Langebaan Donkergat intersection	August 1979	Bean 246
A. lanceolata Engl.	Paulsberg, Cape Point Nature Reserve	July 1979	Bean 224
A. martiana Sond.	North slopes Outeniqua mountains, Eastern Cape	April 1984	Bean 1438
A. mucronulata Sond.	Koega mountains above Geelhoutboskloof, Eastern Cape	December 1982	Bean 1081
A. mundtii Cham. & Schldl.	North slope and summit, Rooiberg Pass	October 1980	Bean 476
A. puberula Fourcade	South slope, Baviaanskloof mountains, Steytlerville district	September 1980	Bean 452
A. recurvifolia Sond.	Baviaanskloof mountains, Willowmore district	September 1980	Bean 459
A. scaberula Düm.	Potberg coastal plain	June 1979	Bean 204
A. serpyllacea Licht.	Wydgelee, Potberg mountain	April 1981	Bean 577
A. spinosa Sond.	Abour Nook, Avontuur, Oudtshoorn Rd, Uniondale district	September 1980	Bean 469
A. thymifolia Schdl.	*ca* 7 km south of the Langebaan Donkergat intersection	August 1979	Bean 245
A. unicarpellata Pillans	South side Baviaanskloof mountains, Steytlerville district	September 1980	Bean 457
Diosma prama Williams	Witelsbos, Vaalfontein roadside, Zuuranysberg, Eastern Cape	October 1982	Bean 899
D. ranosissima B. & W.	Heerenlogement mountain, Vredendal district	October 1981	Bean 712
D. recurva Clan.	Zoutkloof, Gamka Nature Reserve, Oudtshoorn district	October 1976	Williams 2220
Empleurum unicapsulare (Linn. f.) Skeels	Steenbras water catchment area, Caledon district	October 1982	Williams 3435

*Voucher specimens deposited at the Bolus Herbarium.

recurvifolia and *A. spinosa* are the only taxa investigated so far which contain the 6,7,8-trioxygenated coumarins with an *O*-prenyl group at C-7 (**6** and/or **7**). All five are to be found in the South Eastern Cape and have a close affinity with many similar morphological characters. Three other members of this group, *A. acutissima*, *A. apiculata* and *A. pilifera*, have still to be examined. 7-(3',3'-Dimethylallyloxy)-coumarin (**1**) which was previously found to occur in *Diosma acmaeophylla* [3], has again been obtained from two *Diosma* species (*D. prama* and *D. ranosissima*).

EXPERIMENTAL

Plant material. Aerial parts only were examined and the collection details and herbarium depositions for the 24 species are given in Table 2.

Extraction and isolation of coumarins. Ground dried leaves and stems were extracted with petrol (60–80°). The solvent was removed under red. pres. and the residue treated with NaOH–MeOH. After filtering off the insoluble ppt, the MeOH was removed under red. pres. and the soln extracted with Et_2O. The soln was then acidified, extracted with Et_2O, and the Et_2O dried and removed. Mixtures of coumarins were separated by prep. TLC (silica gel: solvent C_7H_{16}–EtOAc, 7:3). All the coumarins were recrystallized from C_6H_6–hexane, and were identified from their mp, ^1H NMR and MS.

Agathosma abrupta: 15.4 mg of 5,6,7-trimethoxycoumarin **9** from 245 g aerial parts. *A. affinis*: 1.8 mg of 6,7-dimethoxycoumarin (scoparon) **2** and 1.3 mg of **9** from 333 g. *A. capensis*: 13.1 mg of **2** from 275 g. *A. cedrimontana*: 2.0 mg of **2** and 10.8 mg of **9** from 707 g. *A. ciliaris*: 10.1 mg of **2**, from 660 g. *A. collina*: 2.4 mg of **2** and 12.3 mg of 6-methoxy-7-(3',3'-dimethylallyloxy)-coumarin **3** from 354 g. *A. dielsiana*: 8.8 mg of **2**, 4.8 mg of 6,7,8-trimethoxycoumarin **5** and 17.9 mg of **9** from 356 g. *A. eriantha*: 22.0 mg of **2** from 400 g. *A. imbricata*: 3.4 mg of **2**, 4.2 mg of **3**, and 3.3 mg of 6-methoxy-7,8-methylenedioxycoumarin (dracunculin) **8** from 353 g. *A. lanceolata*: 12.4 mg of **2** from 350 g. *A. martiana*: 4.4 mg of **2**, 853.8 mg of **3**, 4.5 mg of **5**, and 3.3 mg of 6,8-dimethoxy-7-(3',3'-dimethylalloxy)-coumarin (puberulin) **7** from 660 g. *A. mucronulata*: 6.2 mg of **3**, 2.3 mg of 7-(3',3'-dimethylallyloxy)-8-methoxycoumarin **4**, 4.4 mg of 6-methoxy-7-(3',3'-dimethylallyloxy)-8-hydroxycoumarin (capensin) **6** and 218.1 mg of **7** from 654 g. *A. mundtii*: 3.4 mg of **3**, 2.8 mg of **4** and 3.2 mg of **5** from 353 g. *A. puberula*: 5.4 mg of **5**, 6.3 mg of **6** and 113 mg of **7** from 470 g. *A. recurvifolia*: 7.9 mg of **2**, 62.8 mg of **3**, 3.2 mg of **5** and 2.8 mg of **7** from 355 g. *A. scaberula*: 4.6 mg of **2** from 650 g. *A. serpyllacea*: 2.9 mg of **3** from 103 g. *A. spinosa*: 2.9 mg of **3** and 3.0 mg of **7** from 252 g. *A. thymifolia*: 25.6 mg of **3** from 661 g. *A. unicarpellata*. 6.7 mg of **3** from 310 g.

Diosma prama: 3.7 mg of 7-(3'-3'-dimethylallyloxy-coumarin **1** from 648 g. *D. ranosissima*: 5.0 mg of **1** and 12.1 mg of **4** from 340 g. *D. recurva*: 8.8 mg of **3** from 265 g.

Empleurum unicapsulare: 6.4 mg of **2** from 311 g.

Acknowledgements—Financial assistance from the Council for Scientific and Industrial Research and from the University of Cape Town is gratefully acknowledged.

REFERENCES

1. Finkelstein, N. and Rivett, D. E. A. (1976) *Phytochemistry* **15**, 1080.
2. Campbell, W. E. and Cragg, G. M. L. (1979) *Phytochemistry* **18**, 688.
3. Campbell, W. E., Provan, G. J. and Waterman, P. G. (1982) *Phytochemistry* **21**, 1457.
4. Gray, A. I. (1981) *Phytochemistry* **20**, 1711.
5. Khalid, S. A. and Waterman, P. G. (1983) *Phytochemistry* **22**, 9.
6. Gray, A. T., Meegan, C. J. and O'Callaghan, N. B. (1983) *Phytochemistry* **22**, 9.