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The Major Phenolic Compounds in the Leaves of *Cyclopia* Species (Honeybush Tea)

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Key Word Index—Fabaceae; Podalyrieae; *Cyclopia*; flavanones; xanthones; chemotaxonomy.

Abstract—The phenolic compounds of the leaves of *Cyclopia* species (tribe Podalyrieae) are of both chemotaxonomic and commercial interest, as the leaves are used to brew a herbal drink known as honeybush tea. Despite the commercial importance of *Cyclopia*, virtually nothing was known about the chemistry of the leaves prior to the present work. Methanolic extracts from leaves of 22 species were screened for the presence and distribution of phenolic compounds. Three major constituents of the leaves were identified as mangiferin (a xanthone) and glycosides of the flavanones hesperitin and isosakuranetin. The combination of these three compounds is a unique character for *Cyclopia*, as none of them are present in any of the other genera of the tribes Podalyrieae and Liparieae. Various combinations of the three compounds occur in the different infrageneric groups, but the species are remarkably similar. These results are thus of chemotaxonomic significance at the generic rather than infrageneric level. Copyright © 1996 Elsevier Science Ltd

Introduction

Cyclopia Vent. (tribe Podalyrieae) is a genus of ± 24 species of woody legumes endemic to the fynbos region of South Africa. The leaves of several species are used to brew a traditional herbal tea. This product, known as honeybush tea, is made from the leaves of mainly two species, *Cyclopia intermedia* E. Mey. and *C. subternata* Vogel, both of which are being developed as commercial crop plants. The only known reports of chemical analyses of *Cyclopia* leaves date back to 1870 and 1881 (Watt and Breyer-Brandwijk, 1962) where the presence of unknown and unidentified substances were mentioned. As part of a chemotaxonomic survey of phenolic compounds in the tribes Podalyrieae and Liparieae, the major constituents in the leaves of *Cyclopia* species were investigated.

Materials and Methods

Plant material. Plant material was collected by A. L. Schutte and B.-E. van Wyk and herbarium voucher specimens are housed in the Rand Afrikaans University Herbarium (JRAU) unless otherwise stated. Voucher numbers and locality details are given in Table 1. The genus *Cyclopia* is currently under revision by one of the authors (A. L. Schutte) and the nomenclature used in Table 1 is preliminary only, as some new taxa are yet to be formally described.

Procedures. Dry leaf material was powdered and extracted at room temperature with reagent grade methanol for 4 h. The extract was filtered through celite and passed through a C_{18} Chromapack cartridge to remove substances of high retention time.

Samples were then taken to dryness on a rotary evaporator, redissolved in methanol, mixed with distilled water (1 : 1) and subjected to HPLC analysis. The HPLC system comprised a Phenomenex IB-Sil column (C_{18} reverse phase, 5 μm particle size, 250 mm × 4.6 mm internal diameter, flow rate 1 ml min^{-1}, 20 μl

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sample loop) and a 30–100% linear gradient of methanol in 1% acetic acid–water over 20 min. Detection was by diode array detector, using two channels (A: 280 ± 40 nm; B: 330 ± 70 nm).

One-dimensional paper chromatography was employed to isolate mangiferin from air-dried leaves of *C. pubescens* Eckl. & Zeyh. (*Schutte 685*) according to the method of Mabry *et al.* (1970) (R_f=0.35 in TBA; R_f=0.15 in 15% acetic acid) (yield: 48 mg g^{-1} dry wt.). The xanthone was identified with ^{1}H NMR and ^{13}C NMR and the spectra agreed closely with literature values.

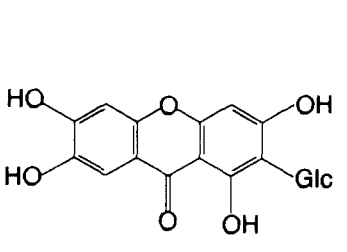
Preparative HPLC was used to isolate the *O*-glycoside of hesperitin from air-dried leaves of *C. meyeriana* Walp. (*Van Wyk 2779*). The HPLC system consisted of a Beckman Ultrasphere column (C_{18} reverse phase, 5 μm particle size, 250 mm × 10 mm internal diameter, flow rate 4.5 ml min^{-1}, 1 ml sample loop) and a 35–65% linear gradient over 14 min, followed by a gradient of 65–100% methanol in 1% acetic acid–water for 2 min. The compound was obtained from a fraction with R_t 6.8 min (yield: 1 mg g^{-1} dry wt.). The identity of the aglycone was established by ^{1}H NMR and EI–MS, but the exact position and identity of the sugar moiety is being further investigated.

Preparative TLC was used to isolate the *O*-glycoside of isosakuranetin from air-dried leaves of *C. dregeana* Kies (*Schutte 715*). Silica gel plates (2 mm layer thickness) (Merck) were developed in eluant system F1 of Rockenbach (1991) and the compound (R_f=0.50) eluted with methanol (yield: 47.3 mg g^{-1} dry wt.). The eluant was taken to dryness and hydrolysed in 4 N HCl for 30 min at 95°C. After filtration, the sample was purified, using a C_{18} Chromapack cartridge. The aglycone was identified by ^{1}H NMR and EI–MS. Future work will focus on identification of the *O*-glycoside.

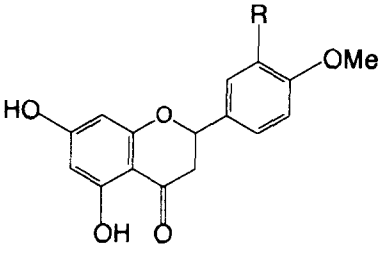
Results and Discussion

The results of a survey of 52 samples from 22 *Cyclopia* species are summarised in Table 1. Only three major phenolic compounds were present in the leaves: a xanthone *C*-glycoside, mangiferin, and *O*-glycosides of hesperitin and isosakuranetin, two flavanones.

Mangiferin is widely distributed in plants and has been reported from six families of ferns (Murakami and Tanaka, 1988) and several flowering plants, e.g. *Mangifera indica* L. (Anacardiaceae) (Buckingham, 1994). Hesperitin has been reported from the Rutaceae, Asteraceae, Lamiaceae (Bohm, 1988) and from the Polemoniaceae, Rosaceae and Araceae (Buckingham, 1994). Isosakuranetin is known from the Asteraceae, Rosaceae, Lamiaceae (Bohm, 1988) and Betulaceae, Gentianaceae and Rutaceae (Buckingham, 1994). Mangiferin has been reported from two species of the legume genus *Hedysarum* L. (Buckingham, 1994), but the distribution of flavanones are poorly known in the Fabaceae. In an extensive chemotaxonomic survey (De Nysschen, in prep.), mangiferin, hesperitin and isosakuranetin were found to be absent from all the genera of the Podalyrieae and Liparieae as circumscribed by Van Wyk and Schutte (1995), so that they are useful chemical markers (autapomorphies) for the genus *Cyclopia.* The chemical uniqueness of *Cyclopia* agrees with its morphological isolation within the tribe Podalyrieae. It is, for example, the only genus with trifoliolate leaves and paired bracts; all the other genera have either pinnate or simple leaves and single bracts. It is interesting to note that alkaloids appear to be



mangiferin



isosakuranetin: R = H
hesperitin: R = OH

SCHEME 1

TABLE 1. DISTRIBUTION OF MAJOR PHENOLIC COMPOUNDS IN LEAVES OF *CYCLOPIA*. Major compounds: 1 = mangiferin, 2 = hesperitin and 3 = isosakuranetin. +: Compound constitutes less than 10% of total absorbance; + +: between 10 and 50%; + + +: above 50%. Retention time is given below each compound

			Major compounds		
			1	2	3
Species	Voucher number	Locality	9.6	12.2	14.7
Cyclopia sp. nov.	*Vlok & Schutte 129*	Kamanassie	+ + +	+ +	
C. aurescens Kies	*Schutte & Van Wyk 771b*	Seweweekspoort Peak	+ +	+	
	Schutte 775	Klein Swartberg	+ + +	+	
	Schutte 824	Swartberg	+ + +	+ +	
	Vlok & Schutte 176	Klein Swartberg	+ +	+	+
C. bolusii Hofmeyr & E. Phillips	*Schutte & Vlok 749*	Swartberg Pass	+ +	+ +	
	Vlok 1243 (SAAS)	Swartberg	+ + +		
	Vlok 2539	Swartberg	+ + +		
C. bowieana Harv.	*Schutte 526*	Ruitersberg	+ +		
C. burtonii Hofmeyr & E. Phillips	*Schutte 641*	Swartberg	+ + +	+ +	
	Schutte 643	Swartberg	+ +	+ +	
	Vlok & Schutte 189	Swartberg	+ + +	+	
C. buxifolia (Burm. f.) Kies	*Schutte 544*	Jonkershoek		+ +	+ +
	Schutte 602	Jonkershoek		+	+ +
	CM Van Wyk 1992 (STE)	Wintershoek		+ +	+ + +
	Richfield s.n. (NBG)	Wellington		+ +	+ + +
	Vlok & Schutte 227	Langeberg	tr	+ + +	+ +
C. dregeana Kies	*Schutte 715a–c*	Du Toitskloof		+ +	+ + +
	Malan 12 (NBG)	Bainskloof	+	+ +	+ + +
C. falcata (Harv.) Kies	*Schutte 598*	Wintershoek	+ +	+ +	+
	Schutte 612	Franschoek Pass	+ + +	+	
C. galioides (P. J. Bergius) DC.	*Schutte 550*	Cape Point	+ +		
	Schutte 788	Cape Point	+ +	+	
	Schutte 789	Cape Point	+ +	+	
C. genistoides (L.) R. Br.	*Schutte 614*	Constantiaberg	+ +	+	
	Schutte 615	Constantiaberg	+ + +	+	
	Schutte 621	Pringle Bay	+ +	+	+
C. genistoides (L.) R. Br. var. *ovalifolia* Kies	*Esterhuysen 6469* (BOL)	Kamanassie	+ + +		
	Esterhuysen 33447 (BOL)	Goudini Sneeukop		+ +	+ +
	Vlok 2685	George Peak	+ +		
	Vlok & Schutte 250	Hottentots Holland		+ +	
C. intermedia E. Mey.	*Schutte 310*	Misgund East	+ +	+	+ +
	Schutte 645	Swartberg	+ + +		
	Schutte 658	Faniesberg	+ + +		
	Schutte 724b–c	Teeberg	+ +		
C. latifolia DC.	*Compton 6588* (NBG)	Constantiaberg		+ +	+ +
	Esterhuysen 28650a (S)	Constantiaberg		+ +	+ +
C. longifolia Vogel	*Gray* s.n.	Van Staadens River	+ + +		
C. maculata (Andrews) Kies	*Schutte 609*	Jonkershoek	+ + +	+	
	Schutte 636	Garcia State Forest	+ +	+ +	
C. meyeriana Walp.	*Kotze 296* (NBG)	Bainskloof		+	+ +
C. montana Hofmeyr & E. Phillips var. *glabra* Hofmeyr & E. Phillips	*Schutte 557*	Matroosberg	+ + +	+	
C. plicata Kies	*Schutte 670b*	Hoopsberg	+ + +		
C. pubescens Eckl. & Zeyh.	*Schutte 685*	Port Elizabeth	+ + +		
	Schutte 686	Port Elizabeth	+ + +		

TABLE 1—CONTINUED

Species	Voucher number	Locality	Major compounds 1 9.6	2 12.2	3 14.7
C. sessiliflora Eckl. & Zeyh.	*Spreeth s.n.* (STE)	Swellendam State Forest	+ + +		
	Lamb 15 (STE)	Garcia State Forest	+ + +		
C. subternata Vogel	*Schutte* 638	Outeniqua Pass	+ + +	+ +	
	Schutte 639	Outeniqua Pass	+ +		
	Schutte 681	Plettenberg bay	+ +		
C. squamosa A. L. Schutte	*Esterhuysen 35695* (BOL)	Wemmershoek Peak		tr	

absent from *Cyclopia* (Van Wyk and Schutte, 1995), while all the other genera within the two tribes accumulate large amounts of quinolizidine and piperidyl alkaloids.

Mangiferin and the two flavanones occur sporadically in virtually all of the species and their presence or absence do not conform with present ideas about infrageneric relationships (Schutte, in prep.). In *Cyclopia* it is therefore obvious that phenolics are of limited taxonomic value at the species level but of considerable interest at the generic level. It would be interesting to study the role of xanthones and flavanones in the quality and taste of the commercial product.

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References

Bohm, B. A. (1988) The minor flavonoids. In *The Flavonoids* (Harborne, J. B., ed.), pp. 329–388. Chapman and Hall, London.

Buckingham, J., ed. (1994) *Dictionary of Natural Products* **2**, 1699–1700; **4**, 3765–3766; **5**, 5611. Chapman and Hall, Cambridge.

Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer-Verlag, New York.

Murakami, T. and Tanaka, N. (1988) Occurrence, structure and taxonomic implications of fern constituents. *Fortschrifte der chemie organischer Naturstoffe* **54**, 1.

Rockenbach, J. (1991) Phytochemische untersuchungen an Rubiaceae und Oliniaceae unter besonderer berücksichtigung des merkmals der cyanogenese. Ph.D. thesis, University of Münster.

Van Wyk, B.-E. and Schutte, A. L. (1995) Phylogenetic relationships in the tribes Podalyrieae, Liparieae and Crotalarieae. In *Advances in Legume Systematics* 7 (Doyle, J. J. and Crisp, M., eds), pp. 283–308. Royal Botanic Gardens, Kew.

Watt, J. M. and Breyer-Brandwijk, M. G. (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa.* Second Edition. E. & S. Livingstone, Edinburgh.