Phytochemistry, Vol. 26, No. 6, pp. 1669–1672, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

CYANOGENESIS IN THE GENUS *ZIERIA**

TERENCE M. FLYNN† and IAN A. SOUTHWELL‡

Biological and Chemical Research Institute, PMB 10, Rydalmere, NSW 2116, Australia

(*Revised received* 18 *September* 1986)

Key Word Index—*Zieria*; Rutaceae; cyanogenesis.

Abstract—Examination of leaf material from some 200 specimens representing most of the known *Zieria* taxa showed that cyanogenesis was evident among 69% of the samples.

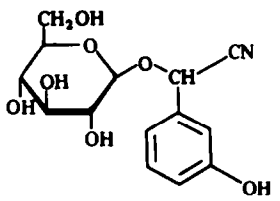
INTRODUCTION

As part of our continuing studies on the phytochemistry of the Australian flora, we are examining the essential oils of the genus *Zieria* (tribe Boronieae), which has recently undergone taxonomic revision [2, 3]. All but one species, *Z. chevalieri* from New Caledonia, are endemic to eastern Australia. The presence of cyanogenic glycosides in certain *Zieria* species [4, 5] including zierin (**1**) in *Zieria laevigata* Sm. [6] and the reported relationship between ingested *Zieria arborescens* ('Stinkwood') and cattle deaths due to respiratory disorders [7] have been well documented [8]. Consequently, part of the leaf material collected for the essential oil studies was used for qualitative screening for cyanide using the picrate colour test [9, 10].

The *Zieria* nomenclature used in this paper is based on the taxa defined by Powell and Armstrong [2] and outlined in Table 2.

RESULTS AND DISCUSSION

Many species and subspecies were highly variable showing both positive and negative results, thus reflecting the known intraspecific variations associated with cyanogenesis [11]. The number and identity of specimens giving positive picrate paper tests are shown in Table 1. Some degree of quantification was obtained by designating 0 to negative results and 3 to strong colorations of intensity equivalent to positive tests from *Eucalyptus cladocalyx* (sugar gum), which contains 0.2–0.3% of hydrocyanic acid [12]. Between these limits were weak (1) and moderate (2) picrate colorations. Species with mean levels ranging from around 2.0 to 3.0 should be viewed as hazardous when ingested, i.e. *Z. arborescens* 'c' form and 'e' form, *Z. compacta*, *Z. laevigata*, *Z. laevigata* var. *fraseri*, *Z. laxiflora* and *Z. smithii* forms 'd' and 'e'. Those with mean levels around 1.0 need to be treated with caution and any species or subspecies giving a positive test must be considered potentially toxic because of geographical, climatic, seasonal, diurnal, age and clonal intraspecific cyanogenic variation [11]. Indeed, this variation was evident with *Z. arborescens* sens. strict., the very subspecies associated with cattle deaths [7]. The only Tasmanian specimen examined gave a strong picrate colour test wheras samples from mainland Australia gave predominantly negative results. This variation is consistent with the essential oil variation of this geographi-



1

* Part 4 in the series "Phytochemistry of the Genus *Zieria*". For Part 3, see ref. [1].

† Present address: Standards Association of Australia, P.O. Box 458, North Sydney, NSW 2060, Australia.

‡ To whom correspondence should be addressed. Present address: Agricultural Research Centre, Wollongbar, NSW 2480, Australia.

Table 1. Occurrence of cyanogenesis in the genus *Zieria*

Taxon	Samples tested	Positive tests	Picrate coloration*	
			Range	Mean
Z. arborescens sens. strict.	13	4	0–2	0.5
Z. arborescens 'b' form	1	1	1	1.0
Z. arborescens 'c' form	13	13	1–3	2.5
Z. arborescens 'd' form	1	1	1	1.0
Z. arborescens 'e' form	5	5	2–3	2.8
Z. arborescens 'f' form	1	1	1	1.0
Z. aspalathoides sens. strict.	7	3	3	0.9
Z. aspalathoides 'b' form	2	0	0	0.0
Z. aspalathoides var. Nov '1'	1	0	0	0.0
Z. aspalathoides var. *obovatum*	1	1	1	1.0
Z. chevalieri	1	1	1	1.0
Z. compacta	4	4	2–3	2.8
Z. cytisoides sens. strict.	10	9	0–3	1.5
Z. cytisoides 'b' form	5	4	0–2	1.0
Z. furfuraceae sens. strict	4	4	1–2	1.3
Z. granulata var. *granulata*	3	3	1–2	1.7
Z. granulata var. *adenodonta*	3	2	0–1	0.6
Z. involucrata	1	0	0	0.0
Z. laevigata sens. strict.	8	7	0–3	2.1
Z. laevigata var. *fraseri*	4	4	1–2	2.0
Z. laxiflora	8	7	0–3	2.3
Z. minutiflora sens. strict.	2	0	0	0.0
Z. minutiflora 'b' form	4	0	0	0.0
Z. murphyi	5	2	0–1	0.4
Z. obcordata	1	0	0	0.0
Z. pilosa	3	0	0	0.0
Z. robusta	3	3	1–3	2.0
Z. smithii sens. strict.	25	14	0–3	0.9
Z. smithii 'b' form	5	5	1–2	1.8
Z. smithii 'c' form	6	4	0–3	1.7
Z. smithii 'd' form	4	4	3	3.0
Z. smithii 'e' form	4	4	2–3	2.8
Z. smithii 'f' form	1	1	1	1.0
Z. sp. aff. *arborescens*	6	4	0–3	1.2
Z. sp. aff. *aspalathoides*	2	0	0	0.0
Z. sp. aff. *involucrata*	2	1	0–2	1.0
Z. sp. aff. *smithii*	3	3	1	1.0
Z. sp. nov. 1	1	1	1	1.0
Z. sp. nov. 11	2	1	0–2	1.0
Z. veronicea	2	0	1–2	1.5

*0 = no colour, 1 = slight, 2 = moderate, 3 = intense.

cally widespread subspecies [1, 13]. Although reduced hydrocyanic acid levels have been reported in aged *Eucalyptus cladocalyx* leaf [12], this screening has shown intense picrate colours in some samples over several years.

EXPERIMENTAL

Plant material. All specimens have been lodged with the N.S.W. Herbarium, Sydney and voucher details are available from J. A. Armstrong, Australian National Botanic Gardens, G.P.O. Box 1777, Canberra, ACT, 2601, Australia. Accession numbers for all the specimens screened are shown in Table 2.

Guignard–Mirande [10] *picrate tests.* Leaf material (< 1 g) was macerated with a stirring rod in the bottom of a micro test tube and treated with a drop of $CHCl_3$. A strip of filter paper moistened consecutively with picric acid and Na_2CO_3 solns was fixed to the mouth of the tube and then stoppered so as to be airtight. The tube was allowed to stand at 20–25° for 24 hr and the resultant colour assessed.

Table 2. *Zieria* nomenclature and accession numbers used in this study

Taxa sampled	Taxa as defined in ref. [2]	Accession Nos
1. *Z. arborescens* sens. strict.	*Z. arborescens* subsp. 'a'	403, 406, 516, 517, 951, 1138, 1378, 1388, 1436, 1437, 1442, 1478, 1479
2. *Z. arborescens* 'b' form (ridged stem form)	*Z. arborescens* subsp. 'b'	398
3. *Z. arborescens* 'c' form (broad leafed form)	*Z. arborescens* subsp. 'c'	118, 862, 1374, 1449a, 1455b, 1458a, 1458b, 1459a, 1464, 1465, 1471, 1473, 1474, 1476, 1477
4. *Z. arborescens* 'd' form (glabrous form)	*Z. arborescens* subsp. 'd'	1211
5. *Z. arborescens* 'e' form (hairy stem form)	*Z. arborescens* subsp. 'e'	455, 465, 1375, 1469, 1470
6. *Z. arborescens* 'f' form (narrow leafed form)	*Z. arborescens* subsp. 'f'	1435
7. *Z. aspalathoides* sens. strict.	*Z. aspalathoides* subsp. 'a'	519, 942, 1305, 1356, 1357, 1424, 1432
8. *Z. aspalathoides* 'b' form (broad leafed form)	*Z. aspalathoides* subsp. 'b'	1447, 1463
9. *Z. aspalathoides* var. *nov.* 1.	*Z.* sp. nov. 'B'	729
10. *Z. aspalathoides* var. *obovatum*	*Z.* sp. nov. 'A'	856
11. *Z. chevalieri*	*Z. chevalieri*	1296
12. *Z. compacta*	*Z. fraseri* subsp. 'b'	207, 829, 943, 943b
13. *Z. cytisoides* sens. strict.	*Z. cytisoides* subsp. 'a'	94, 522, 1111, 1113, 1301, 1431, 1431b1, 1431c3, 1448a, 1448b
14. *Z. cytisoides* 'b' form (coastal form)	*Z. cytisoides* subsp. 'b'	91, 248, 307, 399, 436
15. *Z. furfuracea* sens. strict.	*Z. furfuracea* subsp. 'a'	371, 1454, 1462c, 1462d
16. *Z. granulata* var. *granulata*	*Z. granulata*	402, 1373b, 1468
17. *Z. granulata* var. *adenodonta*	*Z* sp. nov. C	311, 1457b, 1460
18. *Z. involucrata*	*Z. involucrata*	486
19. *Z. laevigata* sens. strict.	*Z. laevigata* subsp. 'a'	390, 525, 1428, 1430, 1440, 1444, 1445, 1484
20. *Z. laevigata* var. *fraseri*	*Z. fraseri* subsp. 'a'	1043, 1201, 1387, 1422
21. *Z. laxiflora*	*Z. laevigata* subsp. 'b'	794, 795, 797, 1404, 1405, 1419a, 1419b, 1455
22. *Z. minutiflora* sens. strict.	*Z. minutiflora* subsp. 'a'	416, 1110
23. *Z. minutiflora* 'b' form (hairy fruited form)	*Z. minutiflora* subsp. 'b'	526, 1410a, 1410b, 1482
24. *Z. murphyi*	*Z. murphyi*	1027, 1288, 1400, 1439, 1443
25. *Z. obcordata*	*Z. obcordata*	1302
26. *Z. pilosa*	*Z. pilosa*	906, 1109, 1427
27. *Z. robusta*	*Z. robusta*	167, 483, 1290
28. *Z. smithii* sens. strict	*Z. smithii* subsp. 'a'	127, 325, 372, 400, 1081, 1084, 1085, 1094, 1128, 1189, 1203, 1385, 1389, 1416, 1420, 1425, 1429, 1446, 1449b, 1459b, 1462b, 1472, 1475, 80-049
29. *Z. smithii* 'b' form (tomentose form)	*Z. smithii* subsp. 'b'	750, 751, 752, 753, 1411
30. *Z. smithii* 'c' form (glabrous form)	*Z. smithii* subsp. 'c'	375, 1021, 1453, 1457a, 80-045, 80-046
31. *Z. smithii* 'd' form (robust mountain form)	*Z.* sp. nov. 'E' subsp. 'a'	1199, 1205, 1332, 1462
32. *Z. smithii* 'e' form (glabrous mountain form)	*Z.* sp. nov. 'E' subsp. 'b'	1406, 1407, 1408, 1409
33. *Z. smithii* 'f' form (prostrate mountain form)	*Z.* sp. nov. 'E' subsp. 'c'	1176
34. *Z.* sp. aff. *arborescens*	*Z.* sp. nov. 'F'	391, 523, 1101, 1280, 1390, 1399
35. *Z.* sp. aff. *aspalathoides*	*Z.* sp. nov. 'H'	1421, 1483
36. *Z.* sp. aff. *involucrata*	*Z.* sp. nov. 'I'	199, 908
37. *Z.* sp. aff. *smithii*	*Z.* sp. nov. 'J'	1450, 1451, 1452
38. *Z.* sp. nov. 1	*Z.* sp. nov. 'K'	1226
39. *Z.* sp. nov. 11	*Z.* sp. nov. 'G'	387, 401
40. *Z. veronicea*	*Z. veronicea*	1433, 1434

Acknowledgement—The authors would like to thank Mr. J. A. Armstrong, Australian National Botanic Gardens, Canberra, for providing most of the plant specimens.

REFERENCES

1. Southwell, I. A. and Armstrong, J. A. (1982) *8th Int. Congr. Ess. Oils, October* 1980, p. 323. Fedarom, Grasse.
2. Powell, J. M. and Armstrong, J. A. (1980) *Telopea* **2**, 85.
3. Armstrong, J. A. (1987) (in preparation).
4. Petrie, J. M. (1912) *Proc. Linn. Soc. N.S.W.* **37**, 222.
5. Smith, F. and White, C. T. (1918) *Proc. R. Soc. Queensl.* **30**, 84.
6. Finnemore, H. and Cooper, J. M. (1936) *J. Proc. R. Soc. N.S.W.* **70**, 175.
7. Stephens, W. H. (1967) *Tasmanian J. Agric.* **38**, 84.
8. McBarron, E. J. (1976) *Medical and Veterinary Aspects of Plant Poisons in New South Wales.* Dept. of Agric., N.S.W., Sydney.
9. Mirande, M. (1909) *C. R. Acad. Sci.* **149**, 140.
10. Eyjolfsson, R. (1970) *Fortschr. Chem. Org. Naturst.* **28**, 74.
11. Jones, D. A. (1972) in *Phytochemical Ecology* (Harborne, J. B., ed.), p. 103. Academic Press, London.
12. Finnemore, H., Reichard, S. K. and Large, D. K. (1935) *J. Proc. R. Soc. N.S.W.* **69**, 209.
13. Southwell, I. A. and Armstrong, J. A. (1987) *Phytochemistry* **26**, 1687.