Phenolic lactones as chemotaxonomic indicators in the genera *Leucadendron* and *Leucospermum* (Proteaceae)

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Analysis by paper chromatography has shown that 58 out of 59 *Leucadendron* spp., so far studied, contain the phenolic lactone leucodrin as a leaf metabolite; similarly, out of 30 *Leucospermum* spp. studied, about half contain conocarpin, a diastereoisomer of leucodrin, while a further number contain leucodrin itself. These results have a bearing on the taxonomy of these two genera of the family Proteaceae and indicate the possibility of chemotaxonomic assistance in the grouping of the species of these genera.

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Introduction

Leucodrin is a phenolic spiro-bislactone (1) whose structure was established chemically (Perold & Pachler 1964; 1966) and by X-ray diffraction analysis (Diamond & Rogers 1964). It is widely spread in the genus *Leucadendron* and is in many instances accompanied by its hydroxylated analogue, leudrin (2) (Highet et al. 1976). Conocarpin is a diastereoisomer (3) of leucodrin and its structure has also been demonstrated chemically (Kruger & Perold 1970), while its stereochemistry was confirmed by X-ray diffraction analysis of a derivative of it (Boeyens et al. 1982). Conocarpin is generally accompanied, in methanol extracts of plant material, by its ring-opened methylation product, reflexin (4) (Perold et al. 1972).

Leucodrin was previously isolated in pure form from *Leucadendron procercum* (= concinnum), *L. salignum* (= adscendens) and *L. microcephalum* (= stokoei) (Rapson 1938), and subsequently from *L. argenteum* (Glennie & Perold 1980) and *L. tinctum* (unpublished work from this laboratory). Conocarpin was isolated in pure form from *Leucospermum conocarpodendron* (Kruger & Perold 1970), while both conocarpin and reflexin were isolated from *Leucospermum reflexum* (Perold et al. 1972). Both genera
belong to the family Proteaceae which is the only family in which these two phenolic dilactones have so far been reported as generically typical metabolites.

A paper-chromatographic analysis was subsequently developed to allow screening of a large number of spp. for these leaf constituents; the optimal conditions follow hereunder. Thin layer chromatography on silica gel was more rapid, but afforded less information, as leucodrin and conocarpin showed the same R_f value in various solvent mixtures.

Materials and Methods

Authentic samples of leaves of 59 Leucadendron spp. and of 30 Leucospermum spp. were obtained from the National Botanic Gardens, Kirstenbosch, either as fresh material or as herbarium specimens (Compton Herbarium, Kirstenbosch Botanic Garden).

An air-dried sample (200 mg) was continuously extracted (Soxhlet) with methanol for 16 h, the extract evaporated to dryness and the residue shaken at room temperature with five times (volume in cm^3) its mass (in g) of ethanol-water (1+1 v/v). This solution was filtered (cotton wool) to afford a clear filtrate for analysis.

A Whatman No. 2 filter paper sheet (46 x 16 cm) was drawn through a solution of glycerol (10%) in methanol and allowed to dry in air for 30 min. Spots of 1, 2 and 5 mm^3 of the extract solution were applied at 2 cm spacing together with a reference spot of leucodrin (20 μg) on a base line 6 cm

Table 1 Paper chromatograms of leaf extracts of Leucadendron

| Taxa                                      | hR_t values |
|-------------------------------------------|-------------|
|                                           | >100 100 90 80 70 60 50 40 30 20 10 |
| **Section: Leucadendron**                |             |
| Subsection: Villosa                       |             |
| L. brunioides Meisn.                      | O R O      |
| L. coriaceous Phillips & Hutch.           | OR R M     |
| L. dubium (Buck ex Meisn.) Phillips & Hutch. | R R RB M  |
| L. galpinii Phillips & Hutch.             | R M        |
| L. levisanus (L.) Berg.                   | OR R R O   |
| L. linifolium (Jacq.) R.Br.               | OR R       |
| L. stellare (Sims) Sweet                  | OR R       |
| Subsection: Membranaceae                  |             |
| L. arcuatum (Lam.) Williams               | R M        |
| L. pubescens R. Br.                       | R M Y      |
| L. remotum Williams                       | R M        |
| Subsection: Nervosa                       |             |
| L. nervosum Phillips & Hutch.             | R B YB     |
| Subsection: Leucadendron                  |             |
| L. album (Thub.) Fourcade                 | R M R YB   |
| L. argenteum (L.) R.Br.                   | R M P Y    |
| L. dregei E. Mey. ex Meisn.               | R O P Y    |
| L. rubrum Burm. F.                        | R R B      |
| Subsection: Cuneata                       |             |
| L. laxum Williams                         | R R M      |
| L. verticillatum (Thub.) Meisn.           | R R M      |
| Subsection: Nucifera                      |             |
| L. barkerae Williams                      | R M Y      |
| L. burchellii Williams                    | R M Y      |
| L. cadens Williams                        | R M Y      |
| L. daphnoides (Thub.) Meisn.              | R R R B    |
| L. gaberrium (Schltr.) Compton.           | R OR       |
| L. gyldenense Williams                    | R Y Y      |
| L. loranthisfolium (Salisb. ex Knight) Williams | R B     |
| L. roodii Phillips                        | R M        |
| L. sessile R. Br.                         | R R B B    |
| L. shielae Williams                       | R R B      |
| L. tinctum Williams                       | R M        |
| L. tradouwense Williams                   | R R        |
| Subsection: Venticosa                     |             |
| L. chamelae (Lam.) Williams               | R YB YB    |
**Table 1 (continued)**

| Taxa | h\(R_L\) values |
|------|------------------|
|      | >100 | 100 | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 |
| **Section: Alatosperma** | | | | | | | | | | | |
| **Subsection: Trigona** | | | | | | | | | | | |
| *L. conicum* (Lam.) Williams | R | M |
| *L. floridanum* R. Br. | R | R | M |
| *L. rourkei* Williams | R | R | B | Y |
| *L. salicifolium* (Salisb.) Williams | R | R | M |
| *L. uliginosum* R. Br. | R | R | M |
| s.s. *glabratum* Williams | | | | | | | | | | | |
| *L. uliginosum* R. Br. | R | R | M |
| s.s. *uliginosum* | | | | | | | | | | | |
| **Subsection: Brunneobracteata** | | | | | | | | | | | |
| *L. microcephalum* (Gandoger) Gandoger & Schinz | R | R | M | P |
| **Subsection: Alata** | | | | | | | | | | | |
| *L. cryptopterophorum* Guthrie | R | R | B | P |
| *L. diemontianum* Williams | RB | R |
| *L. discolor* Phillips & Hutch. | R |
| *L. eucalyptifolium* Buek ex Mein. | R | Y | R |
| *L. flexuosum* Williams | R | R | B | R |
| *L. foedum* Williams | R | R | R | O |
| *L. gandogeri* Schinz ex Gandoger | R | R | M | B |
| *L. lanigerum* var. Buek ex Mein. | R | R | R | Y |
| *laevigatum* Williams | | | | | | | | | | | |
| *L. modestum* Williams | R | B | B |
| *L. procerum* (Salisb. ex Knight) Williams | R | R | M |
| *L. salignum* Berg. | R | | | |
| *L. spissifolium* (Salisb. ex Knight) Williams | R | R | B | R |
| *L. spissifolium* | R | R | M |
| s.s. *fragrans* Williams | | | | | | | | | | | |
| *L. spissifolium* | R | R | M |
| s.s. *natalense* (Thode & Gilg) Williams | | | | | | | | | | | |
| *L. spissifolium* | R | R | M |
| s.s. *philippisi* (Hutch.) Williams | R | R | M |
| *L. strobilinum* (L.) Druce | | | | | | | | | | | |
| *L. xanthococous* (O. Kuntze) K. Schum. | | | | | | | | | | | |
| *L. xanthococous* hybrid | R | R | R | R | R | O |
| ex *coniferum*? | | | | | | | | | | | |
| **Subsection: Compressa** | | | | | | | | | | | |
| *L. muirii* Phillips | R | M |
| *L. nobile* Williams | R | M |
| *L. platyspermum* R. Br. | R | R | Y | B | R |
| *L. tereifolium* (Andr.) Williams | R | R | R | R | M |

h\(R_L\) = 100 × \(R_F\), i.e. 100 × the ratio of the distance travelled by a spot to that travelled by the reference spot for leucodrin on the same chromatogram. Colours of spots are entered as: B = brown; M = mauve; O = orange; P = plum; R = red; Y = yellow.

from the narrow edge of the paper. The prepared sheet was equilibrated for 10 h in a tank over water saturated with n-butanol and toluene, and then eluted by descending chromatography with n-butanol-toluene (1:1 v/v, saturated with water) for 6 h when the solvent front was at some 30 cm beyond the base line. The sheet was air-dried and sprayed with Pauly's reagent, i.e. diazotized sulphanilic acid (50 mg) in 10% sodium carbonate solution in water (10 cm²). The same spots could be revealed with Fast Blue B but the colours were then practically all of the same blue tint and hence less informative.

The colour of each spot was assessed after the sprayed chromatogram had dried in air for 30 min. The h\(R_L\) (= 100 × \(R_F\)) values for individual spots were calculated relative to the distance travelled by the standard spot of pure leucodrin. The h\(R_L\) values were rounded off in decades (Tables 1 & 2) and were found to be replicable within a few per cent. With this system the leucodrin (reference) spot appeared at \(R_F\) 0.65.

**Discussion**

Of the 59 *Leucadendron* spp. analysed (Table 1) all but one show red spots at h\(R_L\) 100 for leucodrin and 29 of them show spots at h\(R_L\) 50–60 for leudrin. The single exception is
Leucadendron platyspermum which shows spots only above hR_L 100 and below hR_L 80. Spots above hR_L 100 occur at various positions with various colours for various spp. and are frequently not well defined. Spots below hR_L 100 are also often streaky, except for well-defined plum-coloured spots at hR_L 10 in the subsection A lata, identified as corresponding to arbutin (hydroquinone β-D-glucoside) by running the pure compound as a reference.

Of the 30 Leucospermum spp. studied (Table 2), 14 spp. show the co-occurrence of conocarpin and (as expected) reflexin as red spots at hR_L 90 and 80 respectively. In the case of 12 spp. (in Groups 3, 7, 8, and 9) chromatograms show the pattern, found for Leucadendron spp., of red and mauve spots at hR_L 100 and 60 for leucodrin and leudrin respectively. Four out of the six representatives of Group 4 of the genus Leucospermum show still different behaviour in that only brown and yellow, but no red spots are shown, i.e. neither leucodrin nor conocarpin is present in these instances. Of the remaining two representatives of this Group, Leucospermum cordatum shows the presence of conocarpin and

Table 2 Paper chromatograms of leaf extracts of Leucospermum in groups

| Group                  | hR_L values |
|------------------------|-------------|
|                        | >100 | 100 | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 |
| 1 Crassicaudex Rourke  |       |     |    |    |    |    |    |    |    |    |    |
| L. cuneiforme (Burm. f.) Rourke |       |     |    |    |    |    |    |    |    |    |    |
| L. innovans Rourke     |       |     |    |    |    |    |    |    |    |    |    |
| 2 Conocarpodendron Buek ex Endl |       |     |    |    |    |    |    |    |    |    |    |
| L. conocarpodendron (L.) Buek | R   | R   | M  | R  |    |    |    |    |    |    |    |
| L. glabrum Phillips    | R    | R   | M  | R  |    |    |    |    |    |    |    |
| L. pluridens Rourke    | R    | R   | M  | R  |    |    |    |    |    |    |    |
| 3 Tumiditubus Rourke   |       |     |    |    |    |    |    |    |    |    |    |
| L. erubescens Rourke   | R    | R   | M  | R  |    |    |    |    |    |    |    |
| L. fulgens Rourke      | R    | R   | M  | R  |    |    |    |    |    |    |    |
| L. muiri Phillips      | R    | R   | M  | R  |    |    |    |    |    |    |    |
| L. praecox Rourke      | R    | R   | M  | R  |    |    |    |    |    |    |    |
| L. spathulatum R. Br.  | R    |     |   M |    |    |    |    |    |    |    |    |
| L. atriculatum Rourke  | R    |     |   M |    |    |    |    |    |    |    |    |
| 4 Brevifilamentum Rourke |       |     |    |    |    |    |    |    |    |    |    |
| L. cordatum Phillips   | R    | R   |     |    |    |    |    |    |    |    |    |
| L. cordifolium (Salis. ex Knight) Fourcade | B   | YB  |    |    |    |    |    |    |    |    |    |
| L. lineare R. Br.      | Y    | YB  |    |    |    |    |    |    |    |    |    |
| L. patersonii Phillips | Y+Y  | Y   | Y  |    |    |    |    |    |    |    |    |
| L. totum (L.) R. Br.   | Y+Y  | Y   | Y  |    |    |    |    |    |    |    |    |
| L. vestitum (Lam.) Rourke | R+Y | YB  | YB |    |    |    |    |    |    |    |    |
| 5 Cardinistylus Rourke |       |     |    |    |    |    |    |    |    |    |    |
| L. catherinae Compton  | R    | R   | R  |    |    |    |    |    |    |    |    |
| L. formosum (Andr.) Sweet | R   | R   | R  |    |    |    |    |    |    |    |    |
| L. reflexum Buek ex Mein. | R   | R   | R  |    |    |    |    |    |    |    |    |
| 6 Leucospermum         |       |     |    |    |    |    |    |    |    |    |    |
| L. tomentosum (Thunb.) R. Br. | R   | R   | R  | YB | R  | M  | R  | R  |    |    |    |
| 7 Diastelliodaea Phillips |     |     |    |    |    |    |    |    |    |    |    |
| L. bolusii Gandoger    | R    |     | M  |    |    |    |    |    |    |    |    |
| L. caligerum (Salisb. ex Knight) Rourke | R   |     | M  |    |    |    |    |    |    |    |    |
| L. heterophyllum (Thunb.) Rourke | R   |     | M  |    |    |    |    |    |    |    |    |
| L. royenifolium (Salisb. ex Knight) Stapf | R   | R   | R  |    |    |    |    |    |    |    |    |
| L. truncatulum (Salisb. ex Knight) Rourke | R   | R   | R  |    |    |    |    |    |    |    |    |
| L. wittebergense Compton | Y   | R   | R  |    |    |    |    |    |    |    |    |
| 8 Xericola Rourke      |       |     |    |    |    |    |    |    |    |    |    |
| L. alpinum (Salisb. ex Knight) Rourke | R   |     | M  |    |    |    |    |    |    |    |    |
| 9 Crinitae Phillips    |       |     |    |    |    |    |    |    |    |    |    |
| L. mundii Mein.        | R    |     | M  |    |    |    |    |    |    |    |    |
| L. oleifolium (Berg.) R. Br. | R   |     | M  |    |    |    |    |    |    |    |    |

hR_L = 100 × R_L, i.e. 100 × the ratio of the distance travelled by a spot to that travelled by the reference spot for leucodrin on the same chromatogram. Colours of spots are entered as: B = brown; M = mauve; O = orange; P = plum; R = red; Y = yellow.
reflexin, while *Leucospermum patersonii* shows the presence of leucodrin and leudrin.

There are 81 known (Williams 1972) spp. of the genus *Leucadendron* and in 58 out of 59 spp. examined so far, leucodrin (and frequently leudrin) appears as a leaf constituent. There are 47 established (Rourke 1972) members of the genus *Leucospermum*; conocarpin and reflexin appear as leaf constituents of about half the 30 spp. studied, while leucodrin and leudrin occur in a somewhat smaller number of these spp. The atypical behaviour of four out of the six representatives of Group 4 of the genus *Leucospermum*, in that neither of the two series of phenolic lactones occurs, is still being studied.

These findings have a direct bearing on the proposed mechanism of the biogenesis of these phenolic lactones (Diamand & Rogers 1964; Glennie & Perold 1980), viz. that the two diastereoisomeric series of compounds arise via enzyme-mediated coupling between p-coumaric acid and L-galactono-γ-lactone (or their biochemical equivalents) in either of two mirror-image relationships in different subdivisions of these two genera. Where the stereo-specificity of enzyme systems is genetically controlled, these findings may have a bearing on the possibility of an evolutionary relationship between the genera *Leucadendron* and *Leucospermum* (Rourke 1975, pers. comm.).

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