Pergamon

PII: S0031-9422(96)00427-X

Phytochemistry, Vol. 43, No. 6, pp. 1201–1203, 1996

0031-9422/96 $15.00 + 0.00

INFRASPECIFIC VARIATION OF INSECTICIDAL SESQUITERPENE DIALDEHYDES IN *PSEUDOWINTERA COLORATA*

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(*Received* 4 *March* 1996)

Key Word Index—*Pseudowintera colorata*; Winteraceae; infraspecific variation; drimanes; sesquiterpene dialdehydes; polygodial; 9-deoxymuzigadial.

Abstract—HPLC and NMR methods are described for determining the levels of the sesquiterpene dialdehydes polygodial and 9-deoxymuzigadial in the foliage of *Pseudowintera colorata*. Analyses of 25 individual plants, from four populations on the South Island of New Zealand, showed two chemotypes: a mixed chemotype with similar levels of polygodial and 9-deoxymuzigadial, and a polygodial chemotype with very low levels of 9-deoxymuzigadial. Only the polygodial chemotype was found in northern and southwestern populations, both chemotypes were found in a central eastern population, and only the mixed chemotype was found in a southeastern population. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

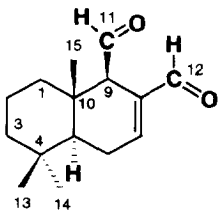
Over 70 sesquiterpenes with the drimane skeleton have been found, in a range of plants, fungi and marine invertebrates [1]. These compounds, particularly the dialdehyde polygodial (**1**), have shown a wide range of biological, especially insecticidal and antifungal, activities [1]. By contrast, only a few rearranged drimanes, in which one methyl group has migrated from C-4 to C-3, have been found [1]. The first rearranged drimane to be reported was a lactone from bark of *Pseudowintera colorata* (Raoul) Dandy (Winteraceae) [2]. *P. colorata*, or horopito, is a shrub endemic to New Zealand, where it is found in most regions, ranging from lowland to montane forests [3]. Our interest in drimanes began when we isolated **1** and 9-deoxymuzigadial **2** as the main insecticidal compounds in *P. colorata* foliage [4]. The rearranged drimane **2** has only been reported from one other source, *Canella winterana* L. (Canellaceae) [5]. Polygodial has previously been isolated, as an antifugal agent, from *P. colorata* by researchers at Canterbury University [6]. These researchers did not report **2**, but they did find the 9α-epimer of polygodial. Therefore, infraspecific variation of sesquiterpene dialdehydes in *P. colorata* seemed possible. We have found infraspecific variation of sesquiterpene hydrocarbons in other New Zealand plants [7]. This was confirmed by analysing individual plants from four widely separated populations of *P. colorata*.

RESULTS

The first requirement to study infraspecific variation was a rapid, sensitive analytical method to separate and quantify the dialdehydes. GC analyses of polygodial have been reported [6, 8], but we found that capillary GC of **1** and **2** gave multiple peaks, with ratios affected by the injector temperature. We assume that this was due to the thermal reactions mentioned by the Canterbury researchers [6]. We were able to obtain baseline separation of **1** and **2** by HPLC on a C_{18} column, with acetonitrile–H_2O (1:1) as the mobile phase. Benzophenone was used as an internal standard for quantitation. Three sequential extractions of the same batch of foliage showed that over 70% of the total dialdehydes were obtained in the first extraction. Replicate analyses of a single batch of horopito foliage showed that the dialdehydes could be quantified by this method with a coefficient of variation of 10% (Table 1).

We also developed a method for rapid quantitative analyses of the sesquiterpene dialdehydes using ^{1}H NMR spectroscopy (see Experimental), which was used to check several horopito samples. The NMR method confirmed HPLC results for both the relative dialdehyde levels in different samples, and for the ratios of **1** to **2**. We did not detect the NMR signals of the 9α-epimer of **1**, found by the Canterbury researchers in *P. colorata* "... from sites throughout the South Island of New Zealand..." [6], in any of our samples.

Sesquiterpene dialdehydes levels in leaves of in-



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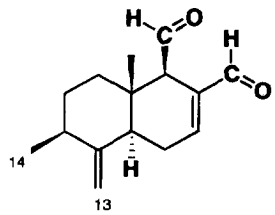

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dividual *P. colorata* plant samples from four different regions on the South Island of New Zealand were analysed by HPLC. The results (Table 1) showed large quantitative variations in the absolute levels of the dialdehydes, which may have been due to sampling leaves of different ages, at different seasons. There were also major qualitative differences in the relative levels of the dialdehydes. All the Dunedin samples (from the southeast of the South Island of New Zealand), and all but one of the Canterbury samples (central east), contained similar levels of both **1** and **2**. By contrast, one Canterbury sample and all the Nelson (north) and Haast (southwest) samples contained higher levels of **1** and almost no detectable **2**. The presence of one **1** individual amongst the mainly mixed individuals in the Canterbury population seems to rule out seasonal and environmental influences on this qualitative variation. Therefore, there seem to be two distinct sesquiterpene dialdehyde chemotypes of *P. colorata*, one with mostly **1** and the other with a mixture of **1** and **2**.

This discovery supports the dictum of Harborne and Turner: "Sampling both within the same population and within different populations is desirable whenever analysing a given plant for its secondary chemistry . . ." [9]. We are not aware of any previous reports of infraspecific variation of drimane sesquiterpenes [1]. It would be interesting to compare the distribution of the two *P. colorata* chemotypes with current theories on New Zealand biogeography [10], but this would require much more extensive sampling.

EXPERIMENTAL

Plant collections. R. Weavers collected *P. colorata* foliage from Sawyers Bay, Dunedin (Dunedin 1: 45° 49′ S, 170° 35′ E) in December 1990 (Plant Extracts Research Unit voucher specimen 901200-01). M. Benn collected from Mt Cargill, Dunedin (Dunedin 2: 45° 49′ S, 170° 34′ E) in January 1992 (920121-01 to -05). W. Harris collected from Pudding Hill Reserve, Mt Hutt, Canterbury (43° 40′ S, 171° 35′ E), in May 1992 (920531-01 to -10). P. Smale collected from Canaan, Nelson (41° 00′ S, 173° 00′ E), in July 1992 (920722-01 to -05). A. Evans collected from Lake Ellery, south of Haast (44° 05′ S, 168° 40′ E), in October 1992 (921029-18 to -22). Plants were air-dried at elevated temps, then stored in sealed plastic bags at room temp. (22°). Samples of leaves and petioles were ground to give fine homogenous powders.

HPLC analyses. A ground plant sample (2.5 g) was homogenized (5 min) with EtOH [25 ml, containing 0.4 mg ml^{-1} benzophenone as int. standard (IS)] with cooling in an ice bath. A subsample of the resulting supernatant (0.5 ml) was filtered through C_{18} (0.5 g) and rinsed with MeCN (2 ml). The cleaned-up extract was filtered (0.45 μm pore size) ready for HPLC analysis on the same day. Cleaned-up extracts (20 μl) were analysed using an RP-18 analyt. column (300 × 3.9 mm, 10 μm), with guard column (4 × 4 mm, 5 μm), at 40°. The mobile phase was MeCN–H_2O (1:1, 1.5 ml min^{-1}), with UV detection at 226 nm. The IS, **2** and **1** peaks had R_ts of *ca* 9.4, 13.1 and 13.6 min, respectively. The actual IS R_t was used to correct the expected R_ts of the **2** and **1** peaks. Injections of solns containing equal concns of IS and purified dialdehydes [4] established response factors (RFs) of 1.85 for **2** and 2.04 for **1**, where: dialdehyde concn = RF × IS concn × (dialdehyde peak area/IS peak area). This relationship, taking into account the ratio of leaf to solvent in the extraction and the IS level, was used to calc. dialdehydes levels in mg g^{-1} dry leaf (Table 1).

NMR analyses. A ground plant sample (0.2 g) was

Table 1. Sesquiterpene dialdehyde levels in *P. colorata* collections

Collection*	Individuals	Polygodial (**1**)†	9-Deoxymuzigadial (**2**)†
Dunedin 1	‡	13 (1)	10 (1)
Dunedin 2	5	10 (7)	7 (3)
Canterbury	9	17 (5)	11 (2)
Canterbury	1	44	0.3
Nelson	5	19 (9)	<0.2
Haast	5	37 (11)	<0.2

*See Experimental for details.

†Mean (standard deviation in parentheses), in mg g^{-1} dry foliage.

‡Composite sample, four replicate analyses.

shaken with $CDCl_3$ (1.0 ml), then allowed to stand at room temp. for *ca* 5 min. The supernatant was then filtered through cotton wool into an NMR tube (5 mm diam.) and DMF (0.3 μl) was added. ^1H NMR spectra (200 MHz) were referenced to $CHCl_3$ at 7.25 ppm. Total dialdehyde levels were estimated from the ratio of the integral of the sesquiterpene aldehyde peaks (9.4–9.6 ppm) with the integral of the DMF aldehyde peak (7.94–8.04 ppm). A spectrum of purified dialdehydes established the approximate relationship: total dialdehyde concn (mg g^{-1} leaf) = 5 × integral ratio. NMR results for total dialdehyde levels showed strong positive correlation with HPLC results, but predicted about 1/5 the absolute level, presumably due to less efficient extraction in the NMR method, which did not employ homogenization. The ratio of **1** to **2** was estimated from the ratio of peak heights of the H-12 aldehyde singlets, at 9.45 ppm for **1** and 9.49 ppm for **2**.

Acknowledgements—We thank the Department of Conservation (Otago and Westland) for permission to collect, the collectors named above, and W. Harris for helpful comments. This research was supported by the New Zealand Foundation for Research, Science and Technology.

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