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INHERITANCE OF TRITERPENE METHYL ETHERS IN *CORTADERIA* (GRAMINEAE)*

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Key Word Index—*Cortaderia*; Gramineae; triterpene methyl ethers; dominant gene control of synthesis; arundoin; cylindrin; α-amyrin methyl ether; β-amyrin methyl ether; parkeol methyl ether.

Abstract—The distribution of triterpene methyl ethers in several generations of interspecific hybrids of *Cortaderia* indicates dominant gene control of their synthesis. The hybrid *C. richardii* × *C. toetoe* is an exception because synthesis of α- and β-amyrin methyl ethers is suppressed in F_1 and F_2, but is restored in the backcross F_1 × *C. toetoe*; this backcross generation was heterozygous for genes for the amyrin methyl ethers, and on selfing segregated in a simple Mendelian ratio.

INTRODUCTION

Triterpene methyl ethers are probably the most significant triterpenoids in the Gramineae [1] even though only a dozen or so have been recognised. If these compounds are to have some taxonomic value at the species level, as they do in part in *Cortaderia* [2–4], and in Japanese species of *Zoysia* [1], it is necessary to have some information on the genetic system controlling their expression. Here we report on the genetic control of triterpene methyl ether synthesis from a study of the distribution of these compounds in epicuticular wax from the species themselves, and from their frequency in various generations of artificially made hybrids. Preliminary results from some F_1 interspecific hybrids were reported earlier [4]. Surface leaf wax only has been examined and no attempt made at isolation from within live tissues.

RESULTS

The distribution of triterpene methyl ethers in species and inter- and intra-specific hybrids is given in Table 1. Arundoin, cylindrin, α- and β-amyrin methyl ethers were reported from *Cortaderia* leaf wax [2–4], and we now report the occurrence of parkeol methyl ether, also found in four species of the grass genus *Chionochloa* [5]. An unidentified triterpene methyl ether, (designated compound R), is present in all New Zealand species of *Cortaderia*; its inheritance can be determined from the frequency of its presence in F_1 and F_2 generations, but we have few data on it other than GLC retention time, TLC and some MS values. Compound R seems to be a tetracyclic triterpene methyl ether because its MS showed no strong characteristic peaks typical of pentacyclic triterpenes [5].

Cortaderia splendens alone is polymorphic for triterpene methyl ethers, having in the one family plants that synthesize (*S*) and plants that do not synthesize (*N*) these compounds [6]. *C. toetoe* which synthesises the same set of triterpenoids as *C. splendens S*, was checked to see if it too was polymorphic. Thirteen plants of an S_1 generation were examined; all contained the same triterpene methyl ethers. This result is interpreted as indicating an absence of polymorphism, but it cannot represent all that might be found in nature.

Leaf wax of F_1 *C. araucana* × *C. selloana* contains both cylindrin and arundoin, and the F_2 segregated 3 arundoin and cylindrin: 1 non-synthesis ($\chi^2 = 0.40$, $n = 30$). There was no evidence for independent assortment. Backcrossing of the F_1 to the recessive parent *C. selloana*, yielded plants that fit the expected ratio 1 synthesis: 1-non-synthesis ($\chi^2 = 0.40$, $n = 10$). A single dominant gene or dominant genes linked in the coupling phase, would satisfy as a genetic analysis for the control of the synthesis of cylindrin and arundoin. Similar explanations would account for F_1 and F_2 results in *C. richardii* × *C. splendens N* for the synthesis of arundoin, parkeol methyl ether and compound R. Segregation in F_2 fits a 3:1 ratio ($\chi^2 = 0.026$, $n = 13$). Again there was no evidence for independent assortment.

The intraspecific F_1 *C. splendens N* × *C. splendens S* yielded the five triterpene methyl ethers found in the synthesising form, again indicating dominance for synthesis. In the sterile F_1 *C. araucana* × *C. toetoe* six triterpene methyl ethers are present; arundoin alone is common to both species. The triterpenoids in this hybrid represent the addition of the two parental complements, and the results once again indicate the action of dominant genes.

F_1 *C. richardii* × *C. toetoe*, and the reciprocal cross, yielded arundoin, parkeol methyl ether and compound R, but lacked α- and β-amyrin methyl ethers. This is the only hybrid where there was evidence in F_1 of the suppression of triterpene methyl ether synthesis. There was no evidence either of triterpene alcohols in the wax [7]. None of 54 plants examined in two F_2 families contained α- and/or β-amyrin methyl ethers; and, as would be expected from F_1 results, the triterpene methyl ethers in F_1 × *C. richardii* were identical with those in F_1. In the backcross F_1 × *C. toetoe*, however, all plants

* Part 3 of a series on *Cortaderia*. For part 2 see Martin-Smith, M., Ahmed, S. and Connor, H. E. (1971) *Phytochemistry* **10**, 2167.

Table 1. Triterpene methyl ethers in species and hybrid generations in *Cortaderia*

	Cylindrin	Arundoin	Parkeol methyl ether	Compound R	α-Amyrin methyl ether	β-Amyrin methyl ether	Ratio in hybrids
Section Cortaderia $2n = 72$ (8x)							
C. araucana Stapf	+	+					
C. selloana (Schult.) Asch. et Graeb.	No triterpene methyl ethers						
Hybrids							
C. araucana ♀ × *C. selloana* ♂							
F_1	+	+					1:0
F_2	+	+					3:
	−	−					1
F_1 ♀ × *C. selloana* ♂	+	+					1:
	−	−					1
F_1 ♀ × *C. araucana* ♂	+	+					1:0
Section Bifida $2n = 90$ (10x)							
C. fulvida (Buch.) Zotov		+	+	+			
C. richardii (Endl.) Zotov		+	+	+			
C. toetoe Zotov		+	+	+	+	+	
C. splendens Connor							
S		+	+	+	+	+	
N	No triterpene methyl ethers						
Hybrids							
C. splendens N ♀ × *C. splendens S* ♂ F_1		+	+	+	+	+	1:0
C. splendens S ♀ × *C. toetoe* ♂ F_1		+	+	+	+	+	1:0
C. richardii ♀ × *C. splendens N* ♂							
F_1		+	+	+			1:0
F_2		+	+	+			3:
		−	−	−			1
C. richardii ♀ × *C. fulvida* ♂ F_1		+	+	+			1:0
C. richardii ♀ × *C. toetoe* ♂ and reciprocal F_1		+	+	+			1:0
F_2		+	+	+			1:0
F_1 ♀ × *C. richardii* ♂		+	+	+			1:0
F_1 ♀ × *C. toetoe* ♂		+	+	+	+	+	1:0
Selfed (F_1 × *C. toetoe*)		+	+	+	+	+	3:
		+	+	+			1
Intersectional hybrid $2n = 81$ (9x)							
C. araucana ♀ × *C. toetoe* ♂ F_1	+	+	+	+	+	+	1:0

contained α- and β-amyrin methyl ethers, arundoin, parkeol methyl ether and compound R—the *C. toetoe* complement. In the progeny from selfing one plant of F_1 × *C. toetoe* there were plants synthesising the five triterpene methyl ethers of *C. toetoe* and others with the three of *C. richardii*; the ratio was 3 *C. toetoe* type: 1 *C. richardii* type ($\chi^2 = 0.20$, $n = 15$). These backcross results would indicate dominant gene control of triterpene methyl ether synthesis, and would, in fact, meet the expectation for an F_1 and an F_2 based on other results reported here. They are, of course, inconsistent with data from true F_1 and F_2 plants.

F_1's from the crosses *C. richardii* × *C. fulvida* and *C. splendens S* × *C. toetoe*, where both parents synthesise identical sets of triterpenoids, yield data of no genetic interest.

DISCUSSION

Dominant gene control of triterpene methyl ether synthesis is in keeping with most conclusions on the inheritance of secondary metabolites. Though the results are presented in terms of triterpene methyl ether inheritance, it is probable—by analogy with methylation in flavonoids—that control of methylation of 3 β-hydroxytriterpenes, each synthesised by an individual gene–cyclase system, is under discussion.

For *Cortaderia* a single dominant gene controlling the methylation of the triterpene alcohols synthesised by a species or hybrid, would fit the results presented, just as would a solution based on single genes, each triterpene methyl ether specific, and linked in the coupling phase. The data do not allow a discrimination between the alternatives.

Evidence opposed to a general application of the proposition of a single dominant methylating gene is found in the results of Ohmoto *et al.* [1] who showed in 14 grasses that triterpene alcohols and triterpene methyl ethers—both of divers structures—may co-occur e.g. β-amyrin and crusgallin in *Zoysia monostachya*; α-amyrin, β-amyrin, and cylindrin in *Festuca arundinacea*; and taraxerol, arundoin, and cylindrin in *Lophatherum gracile*. Unless some special conditions, genetic or biosynthetic, obtain in all those grasses, a single dominant methylating gene would not apply to them. These co-occurrences are very persuasive evidence for a one-gene-one-enzyme specificity for each triterpene methyl ether.

The hybrids *C. richardii* × *C. toetoe* where F_1 and F_2 both failed to synthesise α- and β-amyrin methyl ethers, require further comments. Clearly the *C. richardii* genome differs from that of other species and possesses the dominant capacity for suppressing the synthesis of the amyrin methyl ethers, but in F_1 × *C. toetoe*, suppression having been rendered inoperative, plants synthesise the methyl ethers found in *C. toetoe*. This backcross is heterozygous for genes controlling the synthesis of the amyrin methyl ethers as is evidenced by the 3:1 segregation in the subsequent generation. Our data do not allow a formal genetic solution though possible explanations may lie in preferential pairing in F_1 of chromosomes of the *C. richardii* genome, or gene dosage or genomic balance. The species of *Cortaderia* indigenous to New Zealand, unlike some South American species, are not ideal subjects for genetic experiments [8]. The main problem with the New Zealand species lies in the four year period from seed to first flowering which severely curtails the number of generations that can be produced for study in a reasonable time.

On the current evidence, triterpene methyl ethers are a phenomenon of the Gramineae, exceptions being found in *Neolitsea dealbata* (Lauraceae) [9], and in *Picea* [10] and *Pinus* [11] among the conifers. Sterols and pentacyclic triterpene alcohols seem omnipresent in plants but the methylated forms are primarily a grass characteristic though their role in the biochemical economy of the Gramineae remains unknown.

EXPERIMENTAL

The species examined are parts of the populations used previously [2,4,6].

All F_1 hybrids were produced by hand pollination, F_2 generation by self-pollination of F_1 plants or F_1 interpollination, and backcrosses by hand pollination. All plants were grown in the uniform conditions of the Botany Division experimental gardens at Lincoln, Canterbury. Specimens are deposited at CHR. Leaf waxes were extracted from fresh leaf material and the triterpene methyl ether fraction isolated as previously described [2]. Where necessary they were separated by preparative GLC and identified by comparison with authentic compounds (mp, mmp, IR, GLC R_t's, MS, NMR, TLC) [3–6].

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