Leaf Wax Alcohols in *Coincya* (Brassicaceae)

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Free and ester alcohol compositions have been determined for leaf waxes in ten taxa belonging to the genus *Coincya* (Brassicaceae) on the Iberian Peninsula (Spain and Portugal). Size of leaf wax alcohols in the genus *Coincya* varies between 20 and 31 carbon atoms. This series is dominated by alcohols with an even number of carbon atoms. The most abundant alcohols are C_{24}, C_{26} and C_{28} among even and C_{25}, C_{27} and C_{29} among odd alcohols, both in free alcohols and in wax esters.

KEY WORDS: Brassicaceae, *Coincya*, leaf wax alcohols.

Coincya constitutes a rare wild genus belonging to the Brassicaceae, and its geographical range is the Mediterranean Region and Central Europe. *Coincya* is included in the tribe *Brassiceae*, along with other taxa that are well known for their economic and nutritional value, such as *Brassica* or *Sinapis* (1). *Coincya* presents a distribution centered in the Iberian Peninsula (Spain and Portugal), where it is well represented with eleven of the fourteen taxa recognized currently in the genus (2). We have studied ten of the eleven taxa present on the Peninsula.

The alcohols studied constitute a class of compounds that form a homologous series with saturated, linear, aliphatic chains and a terminal hydroxile group. All of the alcohols studied here are solid and almost odorless at room temperature.

These compounds are normally present in all leaf waxes and generally range from C_{20} to C_{36} with mainly even chainlengths (3). Because of the relationship of *Coincya* with well-known commercial plants, such as *Brassica* or *Sinapis*, it is of interest to study their chemical composition. Besides, the relationship of alcohols from waxes with the environment, and the possible correlations that could be established between chemical composition and changes in environmental parameters, such as temperature, also make their study interesting.

MATERIALS AND METHODS

Leaf waxes were collected from the wild in Spain and in Portugal between 1989 and 1991. Waxes were extracted from leaves taken from the medium zone of the basal rosette from several plants in a population. The number of populations studied for every taxon is shown in Tables 1 and 2. These populations were distributed from all over the Peninsula, although some taxa have a local (Seville, Spain) distribution. Voucher specimens of the samples analyzed are deposited in the Herbarium of the Vegetable Biology Department from the University of Seville, Seville, Spain.

The leaves were soaked for 30 s in chloroform at room temperature. The chloroform extract was filtered and concentrated by rotary evaporation. Separation and purification of free alcohols and esters was carried out by thin-layer chromatography (TLC), on silica gel 60-G plates (20

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TABLE 1

Free Fatty Alcohols in *Coincya*[a]

Taxa	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	n[b]
Coincya transtagana	3.5 ± 2.5	0.9 ± 0.3	3.8 ± 2.2	0.5 ± 0.2	4.6 ± 0.1	1.4 ± 0.2	53.5 ± 7.1	5.0 ± 0.7	22.5 ± 3.8	1.6 ± 0.3	2.7 ± 0.1	0.3 ± 0.3	2
C. longirostra	7.8 ± 4.6	1.0 ± 0.8	6.5 ± 3.7	0.8 ± 0.6	14.2 ± 3.9	4.1 ± 1.4	36.9 ± 9.2	7.8 ± 5.3	10.4 ± 3.0	7.6 ± 5.7	2.1 ± 1.1	1.0 ± 0.9	4
C. rupestris													
subsp. *rupestris*	2.4 ± 0.0	1.7 ± 0.0	3.0 ± 0.0	0.6 ± 0.0	12.6 ± 0.0	2.6 ± 0.0	38.1 ± 0.0	2.1 ± 0.0	15.2 ± 0.0	13.6 ± 0.0	3.5 ± 0.0	4.6 ± 0.0	1
subsp. *leptocarpa*	3.5 ± 1.0	1.4 ± 1.2	3.4 ± 0.9	0.8 ± 0.4	10.2 ± 2.7	2.7 ± 0.3	31.0 ± 7.7	11.0 ± 4.2	11.2 ± 1.6	15.3 ± 5.2	4.2 ± 1.6	5.3 ± 2.6	3
C. monensis													
subsp. *recurvata*													
var. *recurvata*	3.9 ± 3.3	1.1 ± 0.8	5.4 ± 2.1	1.0 ± 0.7	11.6 ± 8.6	4.8 ± 2.1	42.2 ± 7.5	10.3 ± 7.2	10.2 ± 3.0	5.7 ± 2.9	2.6 ± 3.0	1.2 ± 1.3	10
var. *johnstonii*	2.2 ± 2.1	5.0 ± 0.7	1.4 ± 0.7	1.3 ± 0.3	2.3 ± 0.1	3.2 ± 0.2	22.6 ± 1.3	14.6 ± 1.1	12.5 ± 1.0	24.7 ± 4.4	7.5 ± 2.4	3.0 ± 0.5	2
var. *setigera*	2.7 ± 0.5	0.6 ± 0.2	2.9 ± 0.3	0.6 ± 0.1	4.8 ± 1.4	2.1 ± 0.6	27.0 ± 5.4	6.2 ± 1.4	11.0 ± 1.5	19.3 ± 3.7	7.1 ± 1.3	16.4 ± 6.4	6
subsp. *hispida*	5.7 ± 6.0	0.8 ± 0.9	4.0 ± 1.4	2.1 ± 1.3	8.0 ± 3.1	5.7 ± 2.1	36.4 ± 7.6	17.2 ± 6.8	8.4 ± 1.8	7.8 ± 3.1	2.6 ± 1.7	1.7 ± 0.9	12
subsp. *puberula*	2.8 ± 1.0	1.3 ± 0.5	2.1 ± 0.4	0.9 ± 0.3	9.4 ± 0.3	6.0 ± 1.8	40.9 ± 1.6	15.2 ± 4.7	11.1 ± 1.8	7.8 ± 0.2	1.7 ± 0.1	1.3 ± 0.7	2
subsp. *nevadensis*	2.1 ± 0.8	1.8 ± 0.6	5.2 ± 2.2	6.6 ± 1.9	4.5 ± 1.5	17.9 ± 2.2	20.4 ± 4.4	27.0 ± 6.6	5.0 ± 0.9	7.7 ± 1.9	1.2 ± 0.4	0.6 ± 0.3	4

[a]Results are expressed as average percentages ± SD.
[b]Number of populations studied.

TABLE 2

Esters Fatty Alcohols in *Coincya*[a]

Taxa	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	n[b]
Coincya transtagana	7.7 ± 4.9	0.5 ± 0.4	5.4 ± 1.4	0.6 ± 0.3	8.7 ± 3.1	1.9 ± 0.5	54.6 ± 6.9	2.1 ± 0.3	15.0 ± 3.7	1.2 ± 0.2	2.5 ± 0.1	0.2 ± 0.2	2
C. longirostra	3.9 ± 0.3	0.5 ± 0.2	4.8 ± 0.3	0.5 ± 0.1	26.8 ± 1.6	4.0 ± 1.4	45.3 ± 3.0	1.8 ± 0.7	7.9 ± 0.5	1.7 ± 0.4	2.7 ± 0.5	0.0 ± 0.0	3
C. rupestris													
subsp. *rupestris*	3.4 ± 0.0	0.2 ± 0.0	5.4 ± 0.0	0.4 ± 0.0	27.0 ± 0.0	2.4 ± 0.0	44.2 ± 0.0	2.0 ± 0.0	10.1 ± 0.0	3.2 ± 0.0	1.7 ± 0.0	0.0 ± 0.0	1
subsp. *leptocarpa*	3.8 ± 0.5	0.3 ± 0.1	6.9 ± 1.5	0.5 ± 0.2	25.2 ± 1.8	1.7 ± 0.3	46.2 ± 0.4	1.6 ± 0.2	9.8 ± 0.1	2.1 ± 0.6	2.2 ± 0.2	0.0 ± 0.0	2
C. monensis													
subsp. *recurvata*													
var. *recurvata*	4.1 ± 2.5	0.5 ± 0.4	7.9 ± 1.9	0.6 ± 0.3	14.2 ± 4.2	3.2 ± 1.3	53.4 ± 5.0	2.6 ± 0.8	9.4 ± 1.7	1.9 ± 0.9	2.4 ± 2.3	0.0 ± 0.0	11
var. *johnstonii*	2.5 ± 0.6	0.4 ± 0.3	6.8 ± 0.1	0.6 ± 0.2	9.4 ± 1.1	2.1 ± 0.3	55.1 ± 2.0	2.8 ± 0.2	16.1 ± 0.9	1.5 ± 0.1	2.9 ± 0.2	0.0 ± 0.0	2
var. *setigera*	5.9 ± 1.2	0.5 ± 0.2	5.9 ± 1.2	1.0 ± 0.4	12.9 ± 2.5	1.9 ± 0.4	51.6 ± 1.8	2.8 ± 1.1	9.4 ± 0.6	6.9 ± 1.1	1.3 ± 0.6	0.0 ± 0.0	6
subsp. *hiispida*	3.6 ± 2.3	0.5 ± 0.3	10.7 ± 2.0	0.8 ± 0.4	20.8 ± 5.5	2.7 ± 0.7	47.4 ± 5.6	2.0 ± 0.9	7.9 ± 2.3	1.4 ± 1.1	2.1 ± 1.7	0.2 ± 0.3	12
subsp. *puberula*	3.6 ± 2.3	0.7 ± 0.5	6.4 ± 3.7	1.1 ± 0.3	19.6 ± 8.4	3.4 ± 0.8	48.6 ± 1.6	2.6 ± 1.2	9.4 ± 3.8	1.1 ± 0.6	3.6 ± 3.9	0.0 ± 0.0	3
subsp. *nevadensis*	7.4 ± 3.0	0.8 ± 0.5	35.3 ± 6.2	1.6 ± 0.2	13.2 ± 2.4	2.2 ± 0.4	33.2 ± 7.4	1.2 ± 0.3	4.3 ± 1.3	0.4 ± 0.3	0.7 ± 0.5	0.0 ± 0.0	4

[a]Results are expressed as average percentages ± SD.
[b]Number of populations studied.

× 20 cm and 0.5 mm thick; Merck, Darmstadt, Germany). The amount of wax loaded on the plate was always kept below 100 mg. The developing solvent was hexane/diethyl ether/acetic acid (70:30:1). The free alcohols and esters were visualized with iodine vapor and identified with the appropriate standards. After separation, the two fractions were washed from the TLC plate and dried. The transesterification of the esters was carried out with tetramethyl ammonium hydroxide (TMAH) (25%): About 10 mg of esters were placed into a screw-cap vial and dissolved in 300 μL diethyl ether. Then 20 μL of the 25% TMAH was added to the vial and shaken for 2 min. Deionized water (200–500 μL) was added to the vial and shaken to separate the top clear ether layer. The ether layer contained the methyl esters, alcohols and sterols from esters (4). Alcohols from esters were purified by TLC as free alcohols, as discussed previously.

The gas-chromatographic analysis of the alcohols was carried out with a Hewlett-Packard (HP) GC, model 5890, series II, fitted with a flame-ionization detector and an HP 3390 A integrator (Hewlett-Packard, Palo Alto, CA). A capillary column HP-5 (5% Ph Me silicone, L, 25 m; i.d., 0.32 mm) was used. The injector and detector temperatures were maintained at 275°C. The column temperature was held at 260°C. The components of a population of *C. monensis* subsp. *recurvata* var. *recurvata* were identified by gas chromatography/mass spectrometry as their trimethylsilyl derivatives. All mass spectra showed the fragmentation $M^+ - CH_3$ as base peak. So, for example, the mass spectra of the C_{24} trimethylsilyl derivative alcohol showed the following fragmentation: 411 (100); 398 (5.0); 300 (6.5); 239 (7.7); 179 (10.0); and 75 (88.5).

The components of the other taxa were identified by comparison with the retention times of those of the population of *C. monensis* subsp. *recurvata* var. *recurvata*. The taxa are arranged in phylogenetic order in Tables 1 and 2.

RESULTS AND DISCUSSION

In a previous paper (5), we described the quantitative contents of the main components of leaf waxes in *Coincya*. Among these are free alcohols and ester alcohols.

The major free alcohols observed in leaf waxes in *Coincya* vary from 20 to 31 carbon atoms (Table 1). This range does not differ much from that observed in other plants, such as *B. oleracea* (6,7) or *Tilia tomentosa* (8). Although even and odd alcohols are present, in general, the first show higher abundances; thus, the even/odd ratio may vary between 90.6:9.7 in *C. transtagana* and 38.4:61.6 in subsp. *nevadensis*. The most abundant free alcohols are C_{24}, C_{27}, C_{28} and C_{29}.

In taxa that present glaucous leaves, as in var. *setigera*, higher contents of longer alcohols are observed, such as C_{29}, C_{30} or C_{31}. In contrast, in subsp. *nevadensis*, an alpine taxon that suffers the lowest temperatures, higher abundances of the odd alcohols C_{23}, C_{25} and C_{27} are observed.

The major alcohols observed in leaf wax esters in *Coincya* vary from 22 to 31 carbon atoms, such as in the free alcohols (Table 2). The most abundant ones are C_{24} and C_{26}, as has been observed in other plants, such as *Chionochloa* (9) or *Zea mays* (10). As in free alcohols, the compounds with an even number of carbon atoms are more abundant, although in leaf wax esters the differences between even and odd alcohols are bigger than in free alcohols. Thus, the even/odd ratio may vary between 94.1:6.2 in subsp. *nevadensis* and 87.0:13.1 in var. *setigera*. The abundance of odd alcohols almost never reaches 5%. In general, in comparison with free alcohols, the abundance of odd alcohols and larger even alcohols is reduced in wax esters.

As has been observed previously in other fractions of leaf waxes, such as hydrocarbons (11), taxa that have glaucous leaves show the highest contents of C_{29}; thus, var. *setigera*, the most glaucous taxon, present the highest average abundance of C_{29} with 6.9%. In contrast, in subsp. *nevadensis*, higher values of shorter alcohols, such as C_{22}, are observed with an average abundance of 35.3%.

In conclusion, in alcohols from leaf wax esters of *Coincya*, a predominance of even molecules is observed more markedly than in free alcohols. Glaucous character is not as well correlated with the content of C_{29} such as in free alcohols, mainly because of the higher predominance of even alcohols in this group. On the other hand, the higher abundances of odd alcohols observed in subsp. *nevadensis* in free alcohols are not found in alcohols from wax esters; instead, an increase of shorter molecules, such as C_{22}, is observed.

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REFERENCES

1. Padley, F.B., F.D. Gunstone and J.L. Harwood, in *The Lipid Handbook* (edited by F.D. Gunstone, J.L. Harwood and F.B. Padley) Chapman and Hall, 1986, p. 49.
2. Leadlay, E.A., and V.H. Heywood, *Bot. Jour. Linn. Soc. 102*:313 (1990).
3. Hamilton, S., and R.S. Hamilton, in *Topics in Lipid Chemistry Vol. 3*, edited by F.D. Gunstone, Elek Science, London, 1972, p. 199.
4. Metcalfe, L.D., and C.N. Wang, *J. Chromatogr. Sci. 19*:530 (1981).
5. Vioque, J., J. Pastor and E. Vioque, *Bot. Jour. Linn. Soc.*, 1994, in press.
6. Sutter, E., *Canad. J. Bot. 62*:74 (1984).
7. Macey, M.J.K., and H.N. Barber, *Phytochemistry 9*:13 (1970).
8. Gulz, P.-G., E. Muller and R.B.N. Prasad, *Ibid. 30*:769 (1991).
9. Cowlishaw, H.G., R. Bickestaffe and H. Young, *Ibid. 22*:119 (1983).
10. Avato, P., G. Bianchi and N. Pogna, *Ibid. 29*:1571 (1990).
11. Tulloch, A.P., in *Chemistry and Biochemistry of Natural Waxes*, edited by P.E. Kolattukudy, Elsevier, Amsterdam, 1976, p. 236.

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