Sterol Composition in *Coincya* (Brassicaceae)

Javier Vioque*, Julio Pastor[1] and Eduardo Vioque
Instituto de la Grasa, C.S.I.C., Sevilla, Spain

ABSTRACT: Sterol composition was determined for seed oils and leaf waxes in eleven taxa belonging to the genus *Coincya* (Brassicaceae) on the Iberian Peninsula (Spain and Portugal). Seed sterols ranged from 1.2 to 6.7%. The major components were sitosterol (42.6–54.6%), campesterol (20.4–33.2%), and brassicasterol (10.8–23.5%). In leaf waxes, the major free sterols were sitosterol (40.9–74.2%), campesterol (9.6–17.0%), and cholesterol (4.6–17.0%). In leaf wax esters, the major sterols were sitosterol (22.2–56.5%), cholesterol (7.3–32.8%), and campesterol (5.8–25.6%). An apparent substitution of brassicasterol in free sterols from the seeds by cholesterol in free sterols from the leaves was observed. There was an increase of cholesterol in sterols from leaf wax esters with respect to free sterols from leaves and seeds. In *C. monensis* subsp. *nevadensis,* the composition in sterols from leaf waxes may be an adaptation to low temperatures.
JAOCS 72, 493–495 (1995).

KEY WORDS: Brassicaceae, *Coincya,* sterol composition.

Coincya constitutes a rare wild genus belonging to the Brassicaceae, which is located in Western Europe and North Africa. *Coincya* is included in the tribe Brassiceae, along with other taxa that are well known for their economic and nutritional value, such as *Brassica* and *Sinapis* (1). In spite of the relationship of *Coincya* with these plants, it has not been extensively studied.

The distribution of *Coincya* is centered on the Iberian Peninsula (Spain and Portugal), where it is well represented with eleven of the fourteen taxa currently recognized in the genus (2). We have studied the eleven taxa present on the Peninsula. The majority of the taxa show local distributions that are in accordance with their taxonomic range. The taxa with a more extensive distribution are var. *recurvata* and subsp. *hispida,* which spread mainly along the west and center of the Iberian Peninsula. *Coincya* can be found in very different habitats: alpine, such as subsp. *nevadensis*; arid, such as *C. transtagana;* or in sand dunes, such as var. *johnstonii.*

We studied the free sterol compositions in seed oils and in leaf waxes and the sterol composition in leaf wax esters. These compounds are normally present in all eucariotic organisms. The most abundant sterols in the plant kingdom are sitosterol, campesterol, and stigmasterol. Sterols mainly carry out a structural function as components of cellular membranes, but they may also act as cellular metabolites, hormones, or precursors of hormones (3). Small amounts are also found in leaf waxes.

MATERIALS AND METHODS

Leaves were taken from the medium zone of the basal rosette from several plants in a population. Seed samples were collected at full maturity during late summer. The number of populations studied for each taxon is shown in Tables 1, 2, and 3. These populations were distributed all over the Peninsula, although some taxa have a local distribution. Field collections were made between 1989 and 1991. Voucher specimens of the samples analyzed are deposited in the Herbarium of the Vegetable Biology Department at the University of Seville (Seville, Spain).

Seeds were ground in a mechanical crushing mill after washing. The oil was exhaustively extracted from the resulting flour with *n*-hexane in a Soxhlet-type apparatus for three hours, following International Union of Pure and Applied Chemists recommendations (4). Purification of the free sterols was carried out by thin-layer chromatography (TLC) on silica gel 60-G plates (20 × 20 cm and 0.5 mm thick; Merck, Darmstadt, Germany). The developing solvent was hexane/diethyl ether/acetic acid (70:30:1). The sterols were visualized with iodine vapor and identified with the appropriate standards. After separation, the sterols were washed from the TLC plate, dried, and weighed.

The leaves were soaked for 30 s in chloroform at room temperature for wax extraction. The chloroform extract was filtered and concentrated by rotary evaporation. Separation and purification of free sterols and esters was carried out by TLC on silica gel 60-G plates (20 × 20 cm and 0.5 mm thick). The developing solvent was hexane/diethyl ether/acetic acid (70:30:1). The amount of wax loaded on the plate was always kept below 100 mg. The free sterols 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

*To whom correspondence should be addressed at Instituto de la Grasa (C.S.I.C.), Avda. Padre García Tejero 4, 41012 Sevilla, Spain.

[1]Present address: Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Avda. Reina Mercedes s/n, Apdo 1095, 41080 Sevilla, Spain.

TABLE 1
Seed Free Sterols in *Coincya*[a]

Taxa	Total	Col.	Bra.	Cam.	Stg.	Sit.	Δ^5	Δ^7	n[b]
Coincya transtagana	2.2 ± 0.5	0.5 ± 0.2	12.5 ± 1.2	28.3 ± 3.0	0.8 ± 0.1	54.6 ± 4.8	2.5 ± 0.9	0.7 ± 0.5	3
C. longirostra	1.2 ± 0.7	0.4 ± 0.3	19.0 ± 3.0	33.2 ± 4.1	0.9 ± 0.3	45.2 ± 1.6	1.2 ± 0.3	0.1 ± 0.1	3
C. rupestris									
subsp. *rupestris*	3.2 ± 0.0	1.9 ± 1.0	23.5 ± 0.3	28.7 ± 2.4	2.2 ± 0.7	42.6 ± 3.3	1.0 ± 0.5	0.2 ± 0.1	2
subsp. *leptocarpa*	2.1 ± 1.0	2.9 ± 2.6	18.8 ± 3.0	31.3 ± 2.4	0.8 ± 0.1	42.8 ± 2.1	2.4 ± 1.3	0.9 ± 0.7	3
C. monensis									
subsp. *recurvata*									
var. *recurvata*	3.5 ± 2.7	1.8 ± 1.7	18.0 ± 5.1	31.7 ± 4.6	1.3 ± 1.0	44.6 ± 3.0	2.1 ± 1.6	0.5 ± 0.3	13
var. *johnstonii*	6.7 ± 0.0	10.1 ± 0.0	10.8 ± 0.0	21.6 ± 0.0	3.5 ± 0.0	52.0 ± 0.0	1.2 ± 0.0	0.8 ± 0.0	1
var. *granatensis*	4.5 ± 0.0	10.1 ± 0.0	13.9 ± 0.0	20.4 ± 0.0	3.6 ± 0.0	50.2 ± 0.0	1.1 ± 0.0	0.7 ± 0.0	1
subsp. *hispida*	1.3 ± 1.6	1.4 ± 1.3	18.4 ± 2.6	31.2 ± 2.7	1.0 ± 0.7	45.9 ± 2.9	1.6 ± 1.3	0.5 ± 0.4	17
subsp. *nevadensis*	1.7 ± 0.4	0.8 ± 0.3	23.0 ± 3.0	26.1 ± 2.8	1.9 ± 1.3	43.7 ± 3.4	0.6 ± 0.2	4.0 ± 2.4	3

[a]Results are expressed as average percentages ± SD. Col., cholesterol; Bra., brassicasterol; Cam., campesterol; Stg., stigmasterol; Sit., sitosterol; Δ^5, Δ^5-avenasterol; Δ^7, Δ^7-stigmasterol. [b]Number of populations studied.

(TMAH) (25%) (5): 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. Sterols from esters were purified by TLC as free sterols, as discussed previously.

Sterols were derivatized with a solution of pyridine/hexamethyldisiloxane/trimethylchlorosilane (9:3:1) for gas chromatographic analysis. The analysis of the sterols was carried out with a Hewlett-Packard (HP) GC, model 5890, series II, fitted with a flame-ionization detector and an HP 3390A integrator (Hewlett-Packard, Palo Alto, CA). A capillary column HP-5 (5% Ph Me Silicone, L, 25 m; 0.32 mm i.d.) was used. The injector was maintained at 280°C, the detector was maintained at 300°C, and the column temperature was held at 275°C. Peaks were identified with the appropriate standards and by comparing the chromatograms with those of plants with a well-known sterol composition, such as *Brassica oleracea* or *B. napus*. The taxa are arranged in phylogenetic order in Tables 1, 2 and 3.

RESULTS AND DISCUSSION

The sterol content for seeds in the plants studied averaged around 5%, from 1.2% in *C. longirostra* to 6.7% in var. *johnstonii* (Table 1). These percentages represent total free sterols of the seeds, both internal and external or wax sterols because seeds were ground and the resulting flour was extracted with hexane.

The major sterols are sitosterol (42.6–54.6%), campesterol (20.4–30.2%), and brassicasterol (10.8–23.5%), with lesser amounts (below 10%) of Δ^5-avenasterol, Δ^7-stigmasterol, and cholesterol. These percentages are similar to those observed in other Brassicaceae, such as in *Brassica* (6).

Free sterols constitute one of the minor fractions in waxes of *Coincya*, with percentages between 0 and 5% (7). As was observed in the seeds, the major free sterol is sitosterol (40.9–74.2%), with lesser amounts of campesterol (9.6–17.0%), and cholesterol (4.6–14.0%) (Table 2). In subsp. *nevadensis*, an alpine taxon that suffers the lowest temperatures, the highest abundance of cholesterol (17.0%), campesterol (17.0%), and stigmasterol (13.4%), and the lowest percentage of sitosterol (40.9%) are observed. The low abun-

TABLE 2
Leaf Free Sterols in *Coincya*[a]

Taxa	Col.	Bra.	Cam.	Stg.	Sit.	Δ^5	Δ^7	n[b]
Coincya transtagana	6.6 ± 0.0	0.1 ± 0.0	9.6 ± 0.0	2.9 ± 0.0	74.2 ± 0.0	4.3 ± 0.0	2.3 ± 0.0	1
C. longirostra	7.1 ± 0.4	0.1 ± 0.0	13.2 ± 0.5	4.8 ± 0.4	69.0 ± 2.0	4.7 ± 3.0	1.2 ± 0.5	2
C. rupestris								
subsp. *rupestris*	8.4 ± 0.0	2.9 ± 0.0	12.2 ± 0.0	7.8 ± 0.0	65.9 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1
subsp. *leptocarpa*	13.2 ± 3.7	0.4 ± 0.6	17.0 ± 2.4	6.3 ± 1.9	58.0 ± 4.7	4.5 ± 1.5	0.7 ± 0.7	3
C. monensis								
subsp. *recurvata*								
var. *recurvata*	10.0 ± 3.0	1.1 ± 1.3	14.0 ± 4.3	7.1 ± 4.4	58.6 ± 9.3	6.1 ± 3.3	3.1 ± 2.4	10
var. *johnstonii*	4.6 ± 1.6	0.1 ± 0.1	11.3 ± 2.1	5.0 ± 1.0	73.4 ± 3.7	2.9 ± 1.7	2.9 ± 2.5	2
var. *setigera*	14.0 ± 0.3	6.5 ± 0.5	11.5 ± 2.6	10.0 ± 6.5	50.6 ± 5.4	5.4 ± 1.8	2.0 ± 1.7	3
subsp. *hispida*	5.8 ± 3.6	0.3 ± 0.5	16.9 ± 4.4	4.8 ± 2.2	65.8 ± 7.2	5.9 ± 5.2	1.3 ± 0.8	12
subsp. *puberula*	5.4 ± 1.8	0.2 ± 0.2	15.1 ± 0.5	6.0 ± 2.4	69.0 ± 1.3	3.8 ± 2.3	0.4 ± 0.2	3
subsp. *nevadensis*	17.0 ± 1.6	5.9 ± 0.9	17.0 ± 1.8	13.4 ± 3.9	40.9 ± 5.3	4.8 ± 1.9	1.0 ± 0.4	3

[a]Results are expressed as average percentages ± SD. Abbreviations as in Table 1. [b]Number of populations studied.

TABLE 3
Leaf Ester Sterols in *Coincya*[a]

Taxa	Col.	Bra.	Cam.	Stg.	Sit.	Δ^5	Δ^7	n[b]
Coincya transtagana	17.8 ± 0.0	0.4 ± 0.0	10.4 ± 0.0	1.6 ± 0.0	49.3 ± 0.0	18.7 ± 0.0	1.8 ± 0.0	1
C. longirostra	13.9 ± 1.0	0.4 ± 0.4	15.2 ± 1.0	1.2 ± 0.6	54.6 ± 1.5	14.2 ± 0.6	0.7 ± 0.3	2
C. rupestris								
subsp. *rupestris*	27.5 ± 0.0	0.0 ± 0.0	10.1 ± 0.0	1.3 ± 0.0	51.2 ± 0.0	8.8 ± 0.0	1.1 ± 0.0	1
subsp. *leptocarpa*	32.8 ± 0.0	0.0 ± 0.0	14.6 ± 0.0	1.9 ± 0.0	40.8 ± 0.0	7.8 ± 0.0	2.1 ± 0.0	1
C. monensis								
subsp. *recurvata*								
var. *recurvata*	26.2 ± 8.9	1.8 ± 1.7	11.7 ± 3.0	4.7 ± 3.7	47.0 ± 8.9	7.7 ± 3.9	1.0 ± 0.4	11
var. *johnstonii*	10.7 ± 0.0	0.6 ± 0.0	16.6 ± 0.0	1.3 ± 0.0	54.6 ± 0.0	10.1 ± 0.0	6.1 ± 0.0	1
var. *setigera*	7.3 ± 0.0	8.0 ± 0.0	5.8 ± 0.0	17.5 ± 0.0	43.2 ± 0.0	13.5 ± 0.0	4.7 ± 0.0	1
subsp. *hispida*	20.3 ± 5.9	0.8 ± 0.8	15.8 ± 4.2	3.2 ± 2.2	52.6 ± 7.7	6.2 ± 1.5	1.0 ± 0.6	8
subsp. *puberula*	10.1 ± 0.0	10.0 ± 0.0	25.6 ± 0.0	2.1 ± 0.0	56.5 ± 0.0	3.8 ± 0.0	1.9 ± 0.0	1
subsp. *nevadensis*	28.2 ± 2.0	6.7 ± 1.4	9.6 ± 4.2	27.0 ± 10.5	22.2 ± 5.2	4.9 ± 1.7	1.4 ± 0.9	3

[a]Results are expressed as average percentages ± SD. Abbreviations as in Table 1. [b]Number of populations studied.

dance of sitosterol produces an increase of the remaining compounds, mainly cholesterol. In the free sterol composition of leaf waxes, lower environmental temperatures would produce a decrease in the percentages of sitosterol and stigmasterol, and an increase in the abundance of campesterol and cholesterol, which would be more efficient at these temperatures, as has been suggested by Burden (8). In contrast, in *C. transtagana*, an arid taxon, and var. *johnstonii*, a sand dune taxon, the taxa that suffer the highest hydric stress, higher percentages of sitosterol and lower percentages of campesterol are observed. Finally in var. *setigera*, a taxa with glaucous leaves, high percentages of cholesterol and brassicasterol and a lower percentage of sitosterol are observed.

There are clear differences between the free sterol composition of seeds and leaves. The abundance of brassicasterol decreases from percentages above 10% in seeds to amounts below 1% in leaves. In contrast, cholesterol increases from around 1% in seeds to abundance near 10% in leaves. In general, brassicasterol and campesterol decrease and the rest, mainly cholesterol, increases. So, there is an apparent substitution of brassicasterol by cholesterol in leaves, perhaps because cholesterol, less rigid metabolically, is more useful in structures that are more exposed to the environment, such as leaf waxes.

The major sterol observed in leaf wax esters in *Coincya* is sitosterol, 22.2–56.5% (Table 3). In wax free sterols, subsp. *nevadensis* presents the most divergent composition. In this unique taxon, sitosterol (22.2%) is not the main sterol, and cholesterol, with a percentage of 28.2%, is the most abundant component. Clear differences between the sterol ester composition on the one hand and the free sterol composition from seeds and leaves on the other are observed. In all studied taxa, except in var. *setigera*, the percentages of cholesterol are higher in sterols from esters than in free sterols. So, there is an increase in the abundance of cholesterol with respect to the other two fractions, and less evidence of Δ^5-avenasterol. In contrast, stigmasterol and sitosterol decrease in sterols from esters with respect to free sterols in leaves.

In conclusion, the highest percentages of brassicasterol and campesterol are observed in seed sterols, whereas in wax free sterols, stigmasterol and sitosterol are the most abundant. Finally, sterols from wax steres contain the highest percentages of cholesterol, Δ^5-avenasterol, and Δ^7-stigmasterol.

ACKNOWLEDGMENTS

This work has been supported by CICYT Grants ALI88-0169 and ALI91-0409.

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[Received April 21, 1994; accepted January 26, 1995]