Glucosinolates in the Subantarctic Crucifer Kerguelen Cabbage (*Pringlea antiscorbutica*)

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Glucosinolates in the subantarctic Kerguelen cabbage (*Pringlea antiscorbutica*) were determined by HPLC. Glucoerucin (**6**) was present only in the seeds, whereas sinigrin (**2**), gluconapin (**3**), *n*-butyl glucosinolate (**4**), glucoraphanin (**1**), and glucotropaeolin (**5**) were present in both the seeds and leaves. High concentrations of glucosinolates, precursors of bioactive isothiocyanates, were found in the leaves of Kerguelen cabbage. In particular, the lack of unhealthy β-hydroxylated aliphatic side-chain glucosinolates is supportive of this vegetable being a possible dietary source with a high nutritional value.

Pringlea antiscorbutica R. Br. (Brassicaceae) is an endemic perennial plant from the Kerguelen phytogeographical province in the southern Indian Ocean (68−70° E, 48−50° S).[1] This so-called "Kerguelen cabbage" is a fleshy herb with a rosette of a few green leaves surrounding a heart of numerous white leaves, a thick prostrate stem, and a taproot. Inflorescences produce many seeds in silicles.[1−3] The leaves and stem, consumed by sailors since James Cook's expedition in 1776, proved antiscorbutic, and the plant was therefore considered as "an inestimable blessing to ships touching at this far-distant isle".[2] Its strong cresslike taste was not disliked by the Germans, English, or Americans, whereas the French used to cook the Kerguelen cabbage twice before they would eat it.[4] One of the most successful plants under the harsh subantarctic climate at Kerguelen, *P. antiscorbutica* is tolerant to chilling, frequent freeze−thawing, and some salt exposure.[5] The species displays several physiological and biochemical features possibly related to this tolerance to stresses. Proline is present at high levels in leaves and accumulates in response to a saline treatment, while glutamine, γ-aminobutyric acid, and alanine have been detected by ^{13}C NMR spectroscopy.[5−7] Glucose is particularly abundant in the leaves, with some sucrose and fructose.[5,6] Polyamines are abundant in *P. antiscorbutica*, with high constitutive levels of agmatine and N-acetylated forms which are seldom reported in plants while being predominant in animals.[8−10] Aromatic amines such as dopamine and tyramine are also present at high levels in proportions that depend on temperature conditions.[8,10] While the total amount of polar lipids in *Pringlea* is high, as is characteristic of chilling-resistant plants, the polar lipid composition and the fatty acid pattern of leaves in this plant are similar to that of temperate plant species.[11]

The antiscorbutic nature of Kerguelen cabbage was confirmed by the detection of high levels of ascorbic and dehydroascorbic acids and catechol derivatives.[4,12] As in every member of the Brassicaceae family, *P. antiscorbutica* contains glucosinolates (GL), that is, sulfur-containing secondary metabolites; however, no systematic evaluation of the plant GL profile has been performed since two representatives, sinigrin (**2**) and glucotropaeolin (**5**), were detected in *P. antiscorbutica* leaves.[12] As precursors of bioactive isothiocyanates (ITCs), GLs are considered to be responsible for the beneficial effect that is attributed to consumption of *Brassica* vegetables. Evidence from epidemiological studies shows that GLs may play a role in the prevention of human cancer, a claim supported by mechanistic data from in vitro and in vivo studies.[13] This is the first study to carry out a systematic identification of GLs and their relative abundance in *P. antiscorbutica*.

The ISO 9167-1 method[14] based on the HPLC of desulfo-GLs (DS-GLs) resulting from removal of the sulfate group by sulfatase-catalyzed hydrolysis was used to determine the GL content in Kerguelen cabbage leaves (Figure S1, Supporting Information) and seeds (Figure S2, Supporting Information). The results showed that the same compounds were present in the leaves and the seeds from *P. antiscorbutica* except for one, 4-methylthiobutyl GL (glucoerucin **6**), which was found only in seeds. The identification of five out of six HPLC peaks present in samples was performed by comparing their UV spectra and retention time with those of injected DS-GL standards.[15] Although 18 pure certified DS-GLs characterized by ^{1}H- and ^{13}C NMR spectroscopy are available in our laboratory, the lack of the appropriate standard DS-GL made it necessary to employ HPLC−MS for the identification of *n*-butyl GL (**4**) (Figure S3, Supporting Information). The concentration of GLs was higher in the seeds than in the leaves (Table 1), in accordance with data obtained for other Brassicaceae.[16] The amounts of 2-propenyl GL (sinigrin **2**), 3-butenyl GL (gluconapin **3**), and *n*-butyl GL (**4**) in the seeds were about twice those measured in the leaves, whereas 4-methylsulfinylbutyl GL (glucoraphanin **1**) was about the same in both plant parts. The only arylaliphatic GL present in both vegetable parts was benzyl GL (glucotropaeolin **5**), which was almost 4-fold more abundant in the seeds than the leaves. It is worth observing that *P. antiscorbutica* leaves and seeds contain appreciable amounts of *n*-butyl GL (**4**), a very unusual constituent of Brassicaceae, according to the phytochemical literature.[17] Not all *Brassica* vegetables contain equal amounts of GLs, and both the quantity and the individual GL profile have been shown to vary between countries and between vegetable subspecies and varieties

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Table 1. GL Content in Samples of *Pringlea antiscorbutica* from the Kerguelen Archipelago[a]

	glucosinolates (μmol/g d.w.)						
sample	**1**	**2**	**3**	**4**	**5**	**6**	total
leaves	5.4 ± 0.2	2.9 ± 0.1	26.1 ± 0.1	5.6 ± 0.2	12.9 ± 0.4	n.p.	52.9
seeds	3.9 ± 0.3	6.4 ± 0.1	66.2 ± 0.2	11.8 ± 0.1	50.3 ± 0.5	10.1 ± 0.2	148.7

[a] **1**: glucoraphanin; **2**: sinigrin; **3**: gluconapin; **4**: *n*-butyl glucosinolate; **5**: glucotropaeolin; **6**: glucoerucin. Values are means ±SD of three determinations.

within subspecies. A recent review indicates that the median difference between the minimum and maximum values of GLs for the same kind of vegetable was 5−8-fold in different studies.[18] It is well-known that different growing conditions, such as soil, climate, and cultivation practices can indeed considerably influence the GL content. In this study, the leaves and seeds from *P. antiscorbutica* were collected from the same group of monitored plants but in different years. Thus, it will have to be verified if the observed differences in the GL compositions of seed and leaf parts from *Pringlea* may not be attributed to different climatic conditions in these two years; however, year-to-year climatic variations at Kerguelen are relatively slight. Considering that the range of GL contents for the main edible, *Brassica oleracea*, is from 10 μmol g^{-1} (d.w.) in cabbage to 25 μmol g^{-1} (d.w.) in brussels sprouts,[13] Kerguelen cabbage, in having more than 50 μmol g^{-1} (d.w.) in the leaves, may be considered a good dietary source of GLs.

Brassica vegetables, which are commonly consumed throughout the world, can have a very broad impact on human health notably by virtue of their GL content. The importance of Brassicaceae in nutrition is related to the alleged capability of these vegetables to protect against a number of types of cancer, reducing the incidence of cancer in populations that are large and regular consumers of *Brassica* species.[13] The beneficial effect is widely attributed to the isothiocyanates (ITCs), specific phytochemicals that distinguish this family of vegetables from others. However, ITCs are not present as such in plants but they are derived via myrosinase hydrolysis from GLs, their thioglucosidic precursors. This hydrolytic cleavage occurs either directly by endogenous enzymes, when raw vegetables are chopped or chewed, or indirectly, by intestinal microflora, when cooked vegetables are ingested.[19] In addition to the total content of GLs, the relative amount of each compound in leaves is a fundamental aspect of the potentially healthy properties of this vegetable. In particular, the Kerguelen cabbage contains high levels of **1**, the precursor of sulforaphane, which is recognized as one of the most active health-promoting GL derivatives.[20] This compound is believed to be capable of protecting against carcinogenesis by increasing the expression of antioxidative proteins and detoxication enzymes. Compound **1** usually reaches 7 μmol g^{-1} (d.w.) in broccoli, the major dietary source of this GL, whereas the content usually ranges from 0.1 to 1.0 μmol g^{-1} (d.w.) among subspecies of *Brassica oleracea.*[13] In contrast to common *Brassica* vegetables, *P. antiscorbutica* does not contain the indole-type GL, glucobrassicin (GBS). Myrosinase hydrolysis of GBS produces an unstable ITC that is spontaneously transformed into indole-3-carbinol. As with sulforaphane and phenylethyl ITC, this compound was included among the 40 most promising substances evaluated at the National Cancer Institute as chemopreventive agents,[21] but its ability to promote and enhance carcinogenesis, in some experimental conditions,[22] requires further detailed investigation.

Of particular interest is the absence in *P. antiscorbutica* of GLs bearing a β-hydroxylated aliphatic side chain, whose breakdown products exert antinutritional effects and are responsible for unhealthy implications on thyroid metabolism.[23] For example, progoitrin (2-hydroxy-3-butenyl GL) occurs in a 0.2−2.4 μmol g^{-1} (d.w.) range in edible tissues of many Brassicaceae, such as broccoli, brussels sprouts, cabbage, and cauliflower,[13] whereas it could be found in neither the leaves nor seeds of *P. antiscorbutica*.

In *Brassica* species, GLs always occur with myrosinase and form a system that is involved in a range of biological activities.[24] Generally, the GL/myrosinase system is physiologically used by plants as a defense against environmental stress or pathogens.[25] The high level of GLs in *P. antiscorbutica* thus appears to be rather unjustified because the plant grows in extreme environmental conditions that do not favor pest proliferation and the only known pathogen of *P. antiscorbutica* is the recently introduced fungus, *Albugo candida.*[26] A phytophagous fly, *Calycopteryx moseleyi* Eaton, found in all mature *P. antiscorbutica* plants, could also be involved by laying its eggs upon *P. antiscorbutica* leaves; the larvae dig into the parenchyma that they feed on, and the pupal stages are found in the decaying leaves.[27] Moreover, the total myrosinase activity[28] in the leaves, responsible for the GL degradation, was estimated as 2.5 ± 0.1 U g^{-1} (d.w.), whereas the soluble myrosinase activity was found in trace amounts. Thus, the rather low activity of endogenous myrosinase might suggest, in *P. antiscorbutica*, a further physiological role of GLs in addition to protection against herbivore attacks, since this requires hydrolysis of GLs into ITCs.

We conclude that the Kerguelen cabbage, an edible Brassicaceae, is to be considered a good dietary source of GLs, conferring on this remote species, in addition to a known high content of vitamin C, a high nutritional value.

Experimental Section

General Experimental Procedures. HPLC separations of DS-GLs were performed on an Agilent model 1100 equipped with an automatic injector (20 μL) and a diode array detector.

Plant Material. Seeds and leaves of *P. antiscorbutica* were sampled from a group of 10 monitored plants from a population of *P. antiscorbutica* at Ile Australia, Kerguelen Archipelago. This population stood in a well-watered herbfield on deep organic soil and was protected from salt spray. *Pringlea* plants were found as dense and well-growing, large cabbages and were at the ripe seed stage at the time of sampling. Nine leaves (4.5 g), each from one individual plant of this group, were collected at mid-day in February 2001 and instantly nitrogen-frozen. They were freeze-dried and reduced to powder before mixing. Seeds (2 g) were collected in February 2002 as a mix from this group of plants and kept dry at 4 °C with silica gel until use except for 7 days post delay. The samples were compared with a voucher specimen of *P. antiscorbutica* R. Br. (48-1984, collected by J. C. Jolinon in February, 1984 at Kerguelen) deposited at the Muséum National d'Histoire Naturelle in Paris.

Extraction and Isolation. GLs were extracted twice from a weighed amount of dried sample (around 300 mg of seeds and 700 mg of leaves) using an U-Turrax homogenizer in a total volume of 10 mL of warm ethanol−water (70:30 v/v). The extraction was performed at a constant temperature of 75 °C to provoke a quick deactivation of endogenous myrosinase and thus to extract intact compounds. Each *P. antiscorbutica*

extract (1 mL) was loaded onto a mini-column filled with 0.6 mL of DEAE-Sephadex A-25 (Amersham Biosciences) conditioned with 25 mM acetate buffer pH 5.6. After being washed with 3 mL of buffer, 100 μL (0.5 U/mL) of prepurified sulfatase[15] was loaded onto the mini-column and left overnight at room temperature. The resulting DS-GLs were then eluted with 3 mL of distilled water and finally injected into HPLC.

HPLC Separation. HPLC analyses were carried out on a Inertsil ODS-3 (5 μm, 250 × 3.0 mm) column eluted at 1 mL/min flow rate with aqueous acetonitrile (solvent A: water; solvent B: acetonitrile) at 30 °C following the program: 1 min 1% B; 22 min linear gradient up to 22% B; 3 min linear gradient down to 1% B. To have a better separation of **5** and **6** in seed samples, the HPLC elution program was modified as follows: 1 min 1% B; 38 min linear gradient up to 22% B; 3 min linear gradient down to 1% B. The GL content was determined by HPLC according to the ISO9167-1 method,[14] but the use of sinigrin as internal standard was avoided because of the presence of this GL in the samples being analyzed. The availability of several pure standard DS-GLs,[15] which had been previously characterized by ^{1}H- and ^{13}C NMR spectroscopy, allowed the identification of five out of six GLs in extracts, and the last one, *n*-butyl GL **4**, was identified by HPLC−MS, as reported below.

The GL content was determined using a calibration curve of the HPLC peak area of pure desulfo-sinigrin solutions (range from 0.07 to 0.7 mM) and the response factor of each individual DS-GL.[14] The amount of **4**, whose response factor is unknown, was estimated considering the value of one referred to sinigrin. GL levels are expressed in μmol g^{-1} d.w., and each value represents the mean ±SD of three determinations.

HPLC−MS Identification of 4. HPLC was performed as described above using a Thermo Separation P1000 pump/UV 1000 detector/AS 3000 Autosampler and Kromasil column (5 μm, 250 × 2 mm), using a water−acetonitrile gradient at 0.2 mL/min flow rate.

MS Experimental Conditions. Thermo Finnigan LCQ ion-trap mass spectrometer, negative electrospray ionization (needle, −4.8 kV; heated capillary, 200 °C/−4 V; tube lens offset, 20 V). Acquisition was effected under automatic gain control conditions for MS and MS-MS. The trap damping gas and collision gas for MS-MS was helium. The negative ion mode fragmentation pattern of DS-*n*-butyl GL displayed *m/z* 293.7 (M^-), 194.9 (1-thioglucose anion), and 132.1 (valerothiohydroximate anion), and a simultaneous injection experiment with the dehydroanalogue DS-GNA (*m/z* 291.7, 194.9, 130.2) removed any doubts on the structure of **4**.

Myrosinase Activity. Total and soluble endogenous myrosinase activities in leaves of *P. antiscorbutica* were determined by the pH-Stat Assay (pHSA) and direct spectrophotometric assay (DSA) methods, respectively.[28]

pHSA. The composition of the standard mixture was 5 mM sinigrin and 80 mM NaCl in a total assay volume, before titration, of 10 mL.[28] The solution was introduced into a thermostatically controlled cell at 37 °C and gently stirred magnetically, and then the pH was adjusted to 6.5. After 50 mg of leaves was introduced, the myrosinase activity was determined by measuring the acid release rate by titrating with 0.01 N NaOH using a Mettler Toledo titrator model DL50. The total endogenous myrosinase activity is reported in U g^{-1} d.w. and represents the mean of three measurements. One myrosinase unit was defined as the amount of enzyme able to hydrolyze 1 μmol of sinigrin min^{-1}.

DSA. This enzyme extract was obtained by homogenizing 200 mg of leaves with 50 mM phosphate buffer (1:50 w/v) with an Ultra-Turrax, removing the insoluble material by centrifugation, and filtering the solution through a 0.22-μm filter. The activity was determined by measuring the decomposition of the sinigrin substrate at 37 °C by following the decrease in absorbance at 227 nm with quartz cells with 0.5-cm path length in a model 219 Cary recording spectrophotometer. The standard reaction mixture contained 0.5 mM sinigrin, 33 mM phosphate, buffer (pH 6.5), and 50 μL of crude extract in a total volume of 1.5 mL.

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Supporting Information Available: HPLC traces for GL constituents in Kerguelen cabbage leaves and seeds and mass spectra of compounds **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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