PRODUCTION OF GLUCOBRASSICIN, A PHYTOCHEMICAL OF MAJOR INTEREST, THROUGH JASMONIC ACID TREATMENT OF WOAD (*Isatis tinctoria L.*) LEAVES.

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Glucobrassicin (GBS) is an indol-type glucosinolate (GL) contained in all the *Brassica* vegetables (broccoli, Brussels sprouts, etc) and whose dietary intake is associated with health benefits. GBS (Figure 1) is a precursor to a complex mixture of compounds, derived from enzymatic hydrolysis, which exhibit uncertain or questionable anti-cancer properties (1).
Up to now, the biological effects of pure GBS have not been studied because of difficulties in purifying the molecule from from vegetable extracts, since those usually contain complex GLs mixtures. On the other hand, the chemical synthesis of GBS is difficult and rather costly (2).
HERE WE REPORT THE PRODUCTION OF PURE GBS STARTING FROM WOAD FREEZE-DRIED LEAVES TREATED WITH JASMONIC ACID.

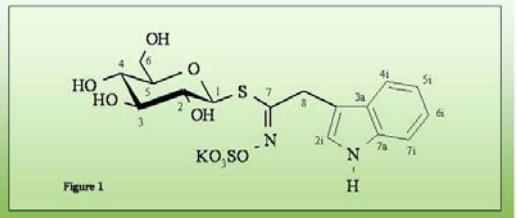


Figure 1

It was reported in a previous work (3) that treatment with jasmonic acid increased (by about five times) the GBS content in leaves of an Italian accession of woad (*Isatis tinctoria* L. – *Brassicaceae* family, Figure 2) which contains this GL exclusively.


Figure 2 – Leaves of one-month-old plants

Treatment with
5 mM jasmonic acid
in 0.1% triton X-100

Table 1 - GLs content in freeze-dried leaves of woad

SAMPLE	GLs µmol g^{-1} (d.w.)	
	GBS	Other GLs
Control	17.6 +/- 1.7	absent
Treated	85.5 +/- 8.3	absent

PURIFICATION SCHEME

FREEZE-DRIED TREATED LEAVES
↓
EXTRACTION
↓
ANION-EXCHANGE CHROMATOGRAPHY
↓
DRYING AND SOLUBILIZATION OF GBS IN HOT METHANOL
↓
REPLACE METHANOL BY WATER AND FREEZE-DRY
↓
SOLID GBS

CHARACTERISATION

GBS was analysed by a Hewlett-Packard (Model 1100) HPLC equipped with a diode array detector (229 nm) and a Inertsil ODS3 (250 x 3 mm, 5 µm) Chrompack column, according to the ISO 9167-1 method and using sinigrin as internal standard (Figure 3).

GBS was unambiguously characterized using ^1H and ^{13}C NMR spectrometry (Table 2).

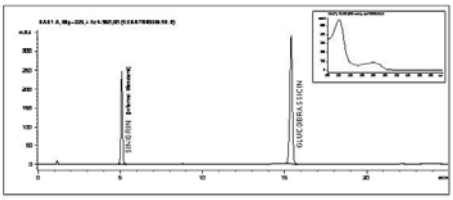
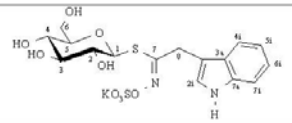


Figure 3 – HPLC chromatogramm of purified GBS and UV spectrum of desulfated GBS.

Table 2 – ^1H and ^{13}C NMR chemical shift assignments of GBS in D_2O.



POSITION	1H(δ in ppm ; J in Hz)	13C(ppm)
1	4.83, d, $J_{1,2}$ = 9.3	81.8
2	3.29, t, $J_{1,2}$ = $J_{2,3}$ = 9.3	72.1
3	3.19, t, $J_{2,3}$ = $J_{3,4}$ = 9.3	77.2
4	3.35, t, $J_{3,4}$ = $J_{4,5}$ = 9.3	68.9
5	2.94, dt, $J_{4,5}$ = 9.3, $J_{5,6}$ = 3.6	80.2
6	3.57,d, $J_{5,6}$ = 3.6	60.5
7	-	163.5
8a et 8b	4.30, d, $J_{A,B}$ = 16.4, 4.19, d, $J_{A,B}$ = 16.4	29.7
3a	-	126.5
7a	-	136.6
2i	7.36, s	124.5
3i	-	108.5
4i	7.77, d, $J_{4i,5i}$ = 7.9	119.9
5i	7.21, t, $J_{4i,5i}$ = 7.9, $J_{5i,6i}$ = 8.1	122.6
6i	7.29, t, $J_{6i,7i}$ = 8.1, $J_{6i,7i}$ = 8.1	118.9
7i	7.55, d, $J_{6i,7i}$ = 8.1	112.4

RESULTS

Yield: • 80% of the total amount of GBS present in the vegetable;

• 3% on the basis of dry matter weight.

Purity: about 88% on the basis of weight.

CONCLUSION

The method that we set up allowed us to obtain GBS with a high grade of purity and a high yield. GBS availability at low cost should allow further studies to be performed with a view to determining pharmacokinetic and antioxidant properties, and to remove any doubts about the possible risks versus benefits of GBS-derived indolyl degradation products, using actually ingested GBS as part of the human diet.

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