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Note

Quantitative high-performance liquid chromatographic analysis of the bitter quassinoid compounds from *Simaruba glauca* seeds

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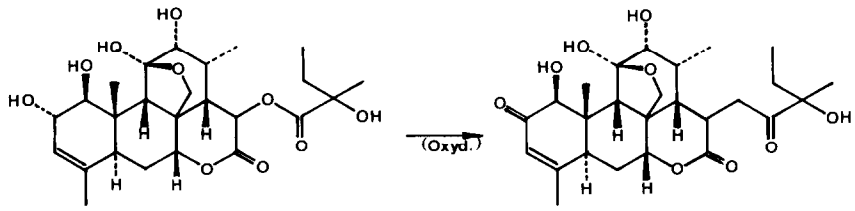
For several years, the "Centre d'Informatique Appliquée au Développement et à l'Agriculture Tropicale" (CIDAT, Tervuren, Belgium) has helped the Republic of Burundi to combat desertification by transplanting the tree *Simaruba glauca* in some regions of the country. The seeds of *S. glauca* are also very rich in edible fat, up to 60% (w/w)[1,2].

This edible fat is used for cooking in tropical countries[1,3]. The cake from oil extraction is rich in proteins[1,2] but cannot be used for cattle feed unless the toxic and bitter compounds are removed. The most efficient and least expensive method of removal is extraction with hot water[2], improved by use of 0.01 *N* acetic acid for the third extraction step[3].

The major compounds extracted from the seeds of *S. glauca* are glaucarubine[3–5] and glaucarubinone, which is derived from glaucarubine by oxidation (Fig. 1)[6].



glaucarubine glaucarubinone

Fig. 1. Oxidation of glaucarubine. Oxidation with MnO_2 gives glaucarubinone in 50% yield[7].

As well as edible fat[7], the seeds contain amoebicide molecules[8] that are used in tropical countries. Glaucarubinone has also been claimed to exhibit antileukaemic activity[9].

In this work, an analytical method to detect traces of the toxic and bitter quassinoids was developed. The most efficient analysis technique was shown to be an aqueous extraction followed by reversed-phase high-performance liquid chromatography for the quantitation of these natural products.

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EXPERIMENTAL

Products

The seeds of *S. glauca* were imported from Burundi. Other sources of Simarubaceae were obtained from Costa Rica, Belize and Senegal. The bitter compounds, glaucarubine and glaucarubinone, were extracted and crystallized until they were chromatographically pure. The ultraviolet, infrared and mass spectral characteristics of these compounds were found to be identical with those given in the literature[3–6].

Pure methanol was purchased from SBA Chimie (Belgium) and filtered on a 0.45-μm Millipore filter before use. Water was distilled twice and then filtered on a Norganic system from Millipore.

Instrumentation

The modular high-performance liquid chromatographic system used was equipped with a 6000 A pump, a U6K injector and a R401 refractometer, all from Waters Assoc.

The column used was a R-Sil C_{18} HL/D stainless-steel column (250 × 4.7 mm I.D., particle size 10 μm) modified following Verzele and Dewaele[10] and purchased from Altech Europe. The results are recorded on a HP 3388 A terminal from Hewlett-Packard.

Calibration

Fig. 2 shows the calibration curves obtained by diluting 50 mg of each compound in 20 ml of methanol (2.5 μg/μl), and adding successively 80, 60, 50, 40, 30, 20 and 10 μl of these alcoholic solutions to 10 μl of internal standard (phenylacetone). The mobile phase was added to obtain a final volume of 100 μl in each case.

RESULTS AND DISCUSSION

The mobile phase was selected in order to obtain good selectivity in a relatively short time. The best isocratic separation was obtained with water–methanol (52.5:47.5) as the mobile phase. Other compositions were tested but gave inferior results. The flow-rate was 0.7 ml/min. A plot of capacity factors *versus* the composition of the mobile phase is shown in Fig. 3, which reveals that glaucarubine and glaucarubinone both have a very high capacity factor in water and a very low one in methanol. Aqueous extraction enabled us to use this fact to develop an analytical method based on preconcentration C_{18} cartridges.

In practice, the aqueous fractions obtained from the extraction steps were filtered and cooled to 20°C. Then an aliquot was filtered on a preconditioned C_{18} cartridge and washed twice with water. The bitter compounds were eluted from the cartridge with methanol, which can either be used directly or concentrated to the volume necessary for preparation of the injection volume. The recovery of both compounds was quantitative. The detection limit was 0.3 μg.

This time-saving method has the following advantages: a lower detection limit (a ratio of 100 was observed), better protection of the column, and a small amount of seeds required even when the content of bitter compounds is low.

Fig. 4 shows a typical chromatogram of a hot-water extraction of *S. glauca*

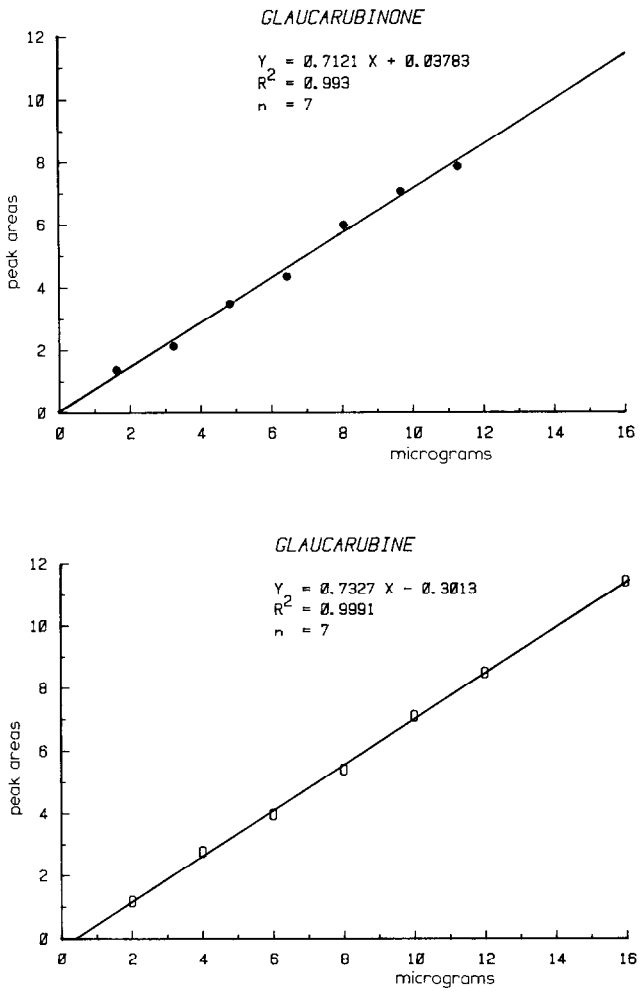

Fig. 2. Calibration curves used for quantitative determination of the concentration of the glaucarubine (○) and glaucarubinone (●) in crude water extracts. The peak areas are linearly correlated with the amount of injected quassinoid.

seeds with water–methanol (52.5:47.5) as mobile phase. In order to quantify the concentration of the quassinoid derivatives, calibration curves were made and, as shown in Fig. 2, very good linear correlations were found between the peak areas and the amount of the bitter compounds injected. The standard deviation was 0.15. Table I lists the amounts extracts with each successive extraction.

The preconcentration C_{18} cartridges were used to analyse the glauca content of some crops received from Belize, Costa Rica, Senegal and Burundi. The results are shown in Table II, which reveals that the concentrations of glaucarubine and glaucarubinone vary with the origin of the crop. The sample from Senegal was a crop from *Hannoa undulata*, and besides the very low content of glaucarubine and glaucarubinone, it is rich in undulatone and ailanthinone as described by Wani[11]. The un-

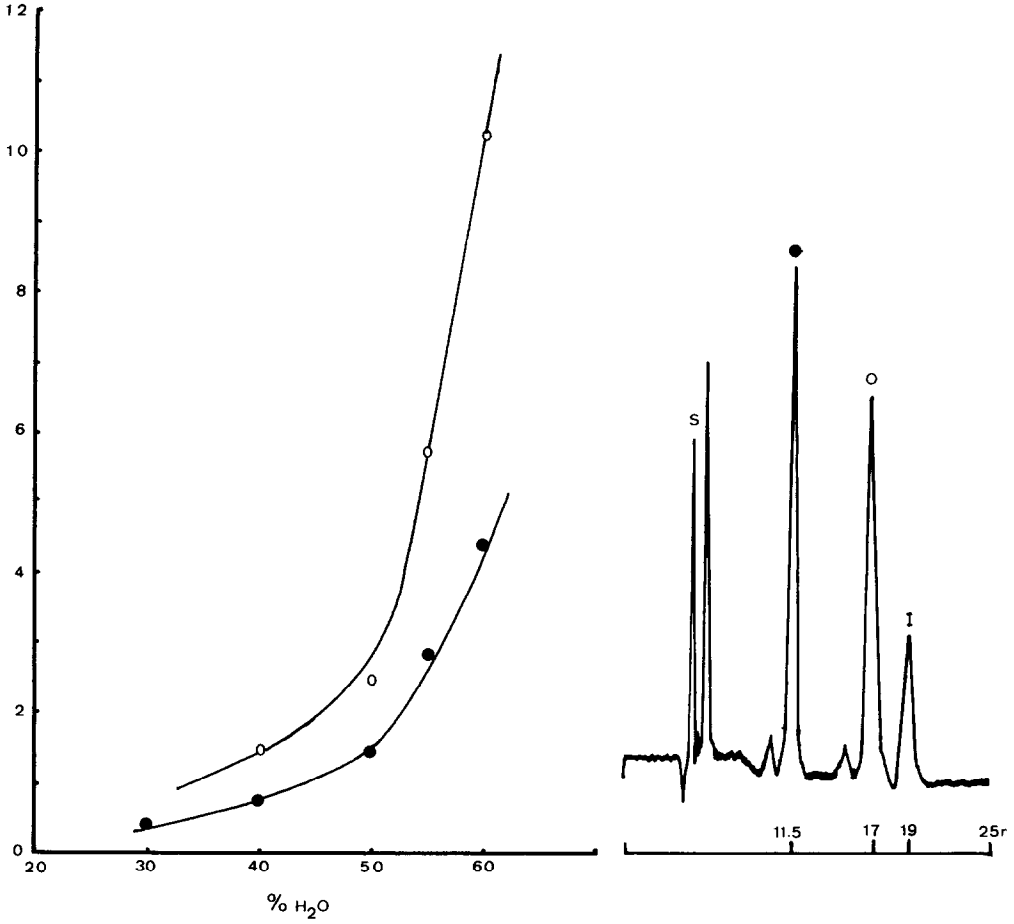

Fig. 3. Capacity factors (k') of glaucarubine (○) and glaucarubinone (●) as a function of the amount of water in the mobile phase.

Fig. 4. Typical separation of the hot-water extraction of the *S. glauca* seeds using water–methanol (52.5–47.5) as mobile phase. Injection of 10 μl of filtered crude water extracts. Flow-rate, 0.7 ml/min. Peaks: S = solvent; ● = glaucarubinone; ○ = glaucarubine; I = internal standard (phenylacetone).

TABLE I

GLAUCA CONTENTS OF *SIMARUBA GLAUCA* SEEDS FROM BURUNDI

The values, which are averages from three determinations, are given following the successive extractions. The amounts, calculated from the calibration curves shown in Fig. 2, are reported in grams per 100 g dry cakes from oil extraction.

Compound	*Amount extracted (g/100 g)*			
	Step 1	*Step 2*	*Step 3*	*Total*
Glaucarubinone	0.932	0.177	0.01	1.119
Glaucarubine	1.112	0.305	0.005	1.422

TABLE II

AMOUNTS OF GLAUCARUBINE AND GLAUCARUBINONE IN SIMARUBACEAE FROM VARIOUS SOURCES

The amounts of glaucarubine (A) and glaucarubinone (B) given are those found in crude water extracts following the method based on the preconcentration with C_{18} cartridges. Results, reported in g/100 g dry cakes from oil extraction, are given following the successive extractions.

Sample	*Origin*	*Quassinoid*	*Amount extracted (g/100 g)*		
			Step 1	*Step 2*	*Step 3*
SCR 1	Costa Rica	A	0.2	–	–
		B	0.63	0.039	$257.5 \cdot 10^{6}$
SCR 2	Costa Rica	A	1.081	0.037	$76.3 \cdot 10^{-6}$
		B	0.988	0.021	$46.3 \cdot 10^{-6}$
SCR 4	Costa Rica	A	0.974	–	–
		B	0.884	–	–
HS	Senegal	A	0.126	–	–
		B	0.055	–	–
B5	Belize	A	1.596	0.407	0.041
		B	1.45	0.34	0.03

known compounds found in the sample SCR 4 from Costa Rica are under investigation and will be reported later.

The analytical method described can identify and quantify the toxic and bitter compounds present in the seeds before their use as a protein source for cattle feed. The analysis and the quantification of these compounds become easier and the detection limit is increased by at least two orders of magnitude by the use of preconcentration C_{18} cartridges.

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