Analysis and Quantitation of Rotenoids and Flavonoids in *Derris* (*Lonchocarpus urucu*) by High-Temperature High-Resolution Gas Chromatography

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Abstract

A glass capillary column coated with PS-086 (15% phenyl–80% methylpolysiloxane, 15 m × 0.30-mm i.d., 0.1-µm film thickness) is used to analyze extracts from *Lonchocarpus urucu* (*Derris urucu*). Several secondary metabolites (8 flavonoids, 10 rotenoids) are characterized without derivatization, and the rotenoids are quantitated by high-temperature high-resolution gas chromatography (HTHRGC) and HTHRGC coupled with mass spectrometry (HTHRGC–MS). The limit of detection in flame ionization detection of rotenone is approximately 0.5 µg/mL, and the limit of quantitation was 2 µg/mL. *Derris urucu* bark is an excellent source of rotenone isomers (80 mg/g), deguelin (30 mg/g), and rotenolone (26 mg/g). Single solvent extractions (hexane, methylene dichloride, acetone, or methanol) are not able to fully extract the flavonoids and rotenoids. Complete extraction is achieved using a mixture of methanol–methylene dichloride (1:1), indicating a complex association of these compounds with the plant tissue. HTHRGC and HTHRGC–MS are shown to be quick and informative tools for the rapid analysis of crude extracts without the need for prior derivatization and fractionation.

Introduction

Plants containing rotenone have been used since antiquity (1) in China, where the genus *Derris* occurs naturally. In South America, plants known commercially as *Derris* are classified in the Index Kewensis as *Lonchocarpus*, but for commercial purposes, they retain the names *Derris urucu* and *Derris nicou* for the two principal species used for insecticide production. The importance of this trade for Brazil led the Instituto Agronômico do Norte (IAN) in Belém (belonging to the Brazilian Ministry of Agriculture) to perform a survey in the 1940s of the occurrence of this genus in the Amazon region and analyze the rotenone content in several plants. The original survey of the locations where the plants were encountered by Lima and Mors (Núcleo de Pesquisas de Produtos Naturais-Universidale Federal do Rio de Janeiro), who had made the original survey, repeated during 1985–1995, and the plants were registered in a publication of the Brazilian Agricultural Company EMBRAPA-CPATU (2), the successor of IAN.

Rotenone is the principal insecticide present in the plant but not the only compound present with this type of chemical structure. At the same time, the role of other present substances, such as the *Derris* saponins, should be investigated, because these may also contribute to the insecticidal activity. Commercially, the finely powdered bark is frequently found as the active component of insecticidal formulations, or crude extracts that contain major amounts of other substances are sold. Rotenoids comprise the chemical class of compounds that include rotenone and which are usually both insecticidal and ictiotoxic. The use of rotenoids in agriculture showed their importance in the control of insects such as aphids, homoptera, lepidoptera, and hemiptera (3). Rotenone was the first rotenoid to be identified and was used as an insecticide before the advent of organosynthetic insecticides (4). Rotenone is currently considered the most powerful insecticidal compound of the rotenoid class among almost 100 known compounds. A review of the biological activity of the rotenoids may be found in *Naturally Occurring Insecticides* (4).

Of the naturally occurring insecticides that were known and industrialized before 1950, after which synthetic insecticides dominated the market, *Derris* is the most important one that is still marketed. The insecticide is rapidly biodegraded and may be used on market garden produce for short-term consumption.

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Other important classes of secondary metabolites of plants are the flavonoids; some flavonoids were shown to inhibit the replication of picornaviruses such as poliomyelitis and rhinoviruses. Compounds such as 4'-ethoxy-2'-hydroxy-4,6'-dimethoxychalcone interact directly with specific sites on the viral capsid proteins, thereby producing an uncoating of the virus with consequent liberation of viral RNA (5).

The characterization of rotenoids, flavonoids, and other classes of aromatic natural products is usually accomplished by classical phytochemical techniques comprised of several isolation steps (column chromatography, thin-layer chromatography, or high-performance liquid chromatography) before identification by the usual spectroscopic methods (ultraviolet, infrared, mass psectrometry, or nuclear magnetic resonance). As a result, the identification of multiple components by classical phytochemistry is slow.

Previous reports on the application of high-temperature high-resolution gas chromatography (HTHRGC) and HTHRGC coupled with mass spectrometry (HTHRGC–MS) for the analysis of high-molecular-weight compounds showed that this general analytical procedure could easily be applied to problems in synthetic organic chemistry, organic geochemistry, and environmental chemistry (6). The use of the cold on-column injection technique proved appropriate for sample introduction during analysis and characterization of high-molecular-weight and thermolabile compounds. These techniques may be excellent alternatives to the classical phytochemical analysis procedures for the systematic study of rotenoids, flavonoids, and possibly several other important classes of aromatic natural products in plant extracts (7–8).

Experimental

Samples

Material

Lonchocarpus urucu (*Derris urucu*) roots were collected from nature in March 1995 in Santana de Ajudante, Mazagão Velho, Amapá, Brazil. A voucher was deposited at the herbarium of the IAN in Belém, Pará, Brazil.

Standards

Rotenone was obtained from Sigma Chemical (St. Louis, MO).

Fractionation of extracts

Extraction of the dried powdered bark (3.0 g) was performed with 10 mL (× 3) for each solvent using ultrasonic agitation for 30-min periods. Solvents followed this sequence: hexane, methylene dichloride, acetone, and methanol. Solvent evaporation of the total extract for each solvent gave 35.5, 65.0, 312.3, and 122.3 mg of crude extracts, respectively. The solvent extracts were evaporated using a stream of filtered nitrogen gas.

A 1.0-mg aliquot of each crude extract was transferred to a vial and diluted with 500 µL of the extraction solvent.

Chromatography

Columns

GC was performed on borosilicate capillary columns (15 m × 0.30-mm i.d., Duran-50, Vidrolex, Brazil) coated with 0.1 µm of PS-086 (15% phenyl–80% methylpolysiloxane, Petrarch Systems, Bristol, PA). The columns were prepared in the laboratory according to a procedure in the literature (9).

For HRGC–MS analyses, the capillary column was connected to a 2-m piece of an empty capillary (GC–MS interface) prepared from 0.25-mm-i.d. high-temperature fused silica (HTFS, J&W Scientific, Folsom, CA). The HTFS was purged with hydrogen at 180°C for 15 min and deactivated by flushing with HMDS–DPTMDS (1:1), sealing the capillary, and heating at 400°C for 12 h. The tubing was then rinsed with hexane, methanol, and diethyl ether.

Column performance was checked by the Grob test prior to use (10,11).

Chromatographic conditions

An on-column injector (Carlo Erba, Rodano, Italy) was mounted on a Hewlett-Packard (Palo Alto, CA) model 5890-II GC. Column temperature was maintained at 40°C, increased to 250°C at 40°C/min, and increased to 370°C at 5°C/min. The flame ionization detector (FID) and the on-column injector were operated at 380°C and room temperature, respectively.

Hydrogen was used as the carrier gas at a linear velocity of 50 cm/s (40°C), and the sample volume injected was 0.2 µL. GC data were acquired and processed with an HP 3396-II integrator.

Calibration curve

The calibration curve was prepared with five standard solutions of rotenone with 7, 14, 24, 35, and 70 ng/µL prepared by successive dilutions of the standard stock solution (10 mL, 1.0 mg/mL). The quantitation curve was prepared after five injections of each solution (injected volume, 0.2 µL).

MS conditions

HTHRGC–MS analyses were carried out on an HP-5890-II GC coupled with an HP 5972 spectrometer (Hewlett-Packard) under electron impact ionization (70 eV). The GC operating conditions were the same as described previously, and He was the carrier gas. The on-column injector and the transfer line temperatures were set at 40 and 380°C, respectively (MS scan range, 50–700 amu).

Results and Discussion

Characterization

In the analysis of extracts of *Lonchocarpus urucu* by HTHRGC and HTHRGC–MS (Figure 1), mass spectral data show the presence of eight flavones, ten rotenoids, and three acids: hexadecanoic (palmitic) acid (retention time t_R = 6.1 min), 9-octadecenoic (oleic) acid (t_R = 6.8 min), and octadecanoic (stearic) acid (t_R = 6.9 min). These compounds are shown with their respective retention times in Table I. The mass spectral characteristics of two of these compounds are presented in Figure 2 and indicate the presence of positional isomers of a dimethoxy-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one.

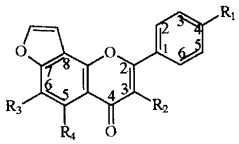
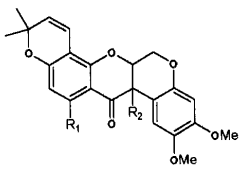
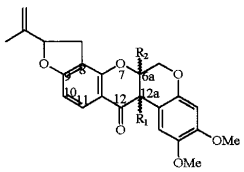
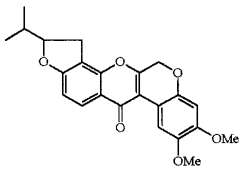
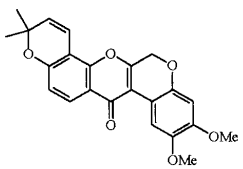
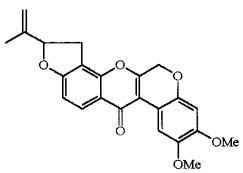
Compared with the direct introduction mass spectra available in the literature (12–19), the HTHRGC–MS spectra show similar results with no significant differences in relative intensities of the mass spectral peaks. The MS data of all analyzed flavonoids

and rotenoids are shown in Table I.

The majority of the mass spectra of rotenoids show a base peak resulting from a Retro Diels–Alder (RDA) type of rearrangement ion or the molecular ion.

The mass spectra show the presence of the molecular ions at *m/z* 410, 394, and 392. Considering the rotenone skeleton (Table II, structure 12), which presents an ion at *m/z* 394, it is possible to suggest the following structural features for them: monohydroxylated-, nonhydroxylated-, and 6*a*,12*a*-dehydro-rotenone, respectively.

Table I. Structure, Names, and GC Retention Times of the Compounds Characterized in Crude *Derris* Root Bark Extract

Structure	Number*	Name	Substituent				t_R (min)*	Molecular weight (daltons)
			R_1	R_2	R_3	R_4		
	1	3-Methoxy-2-(4-methoxyphenyl)-4H-furo[2,3-h]-1-benzopyran-4-one	OMe	OMe	H	H	8.87	322
	2	3-Hydroxy-2-(4-methoxyphenyl)-4H-furo[2,3-h]-1-benzopyran-4-one	OMe	OH	H	H	9.35	308
	3	3,6-Dimethoxy-2-(4-methoxyphenyl)-4H-furo[2,3-h]-1-benzopyran-4-one	OMe	OMe	OMe	H	9.69	352
	4	3,6-Dihydroxy-2-(4-methoxyphenyl)-4H-furo[2,3-h]-1-benzopyran-4-one	OMe	OH	OH	H	9.85	324
	5	3,6-Dimethoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one	H	OMe	OMe	H	11.80	322
	6	3-Hydroxy-6-methoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one	H	OH	OMe	H	12.02	308
	7	3,5-Dihydroxy-6-methoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one	H	OH	OMe	OH	12.70	324
	8	3,5,6-Trimethoxy-2-pheny-4H-furo[2,3-h]-1-benzopyran-4-one	H	OMe	OMe	OMe	12.98	352
	9	Tephrosin	H	OH			13.93	410
	10	Deguelin	H	H			15.02	394
	11	12*a*-Hydroxy-rotenone (rotenolone)	OH	H			15.12	410
	12	Rotenone (isomer)	H	H			15.34	394
	13	Rotenone (isomer)	H	H			16.10	394
	14	Rotenone (isomer)	H	H			16.60	394
	15	6*a*-hydroxy-rotenone	H	OH			18.21	410
	16	1',2'-Dihydro-6*a*,12*a*-dehydrorotenone					15.67	394
	17	6*a*,12*a*-Dehydrodeguelin					18.96	392
	18	6*a*,12*a*-Dehydrorotenone					19.74	392

* Peak numbering and retention times are shown in Figure 1.

The flavonoids comprise a large family of secondary metabolites that derive part of their structures from the shikimate and part from the polyketide biogenetic pathways. The identification of this and other classes of natural products by mass spectral analysis alone is rather difficult because of the number of isomers and, in several cases, minor differences between their mass spectra. Usually, only probable structures can be advanced using the mass spectral data together with biogenetic arguments. For compounds 1–4 (Table I), the presence of a 4'-methoxyphenyl moiety seems likely, based on the biogenetic formation of the flavonoids from shikimic acid (20). For a discussion of the fragmentation characteristics of flavonoids, see Porter and Baldas (21) and Takayama et al. (22).

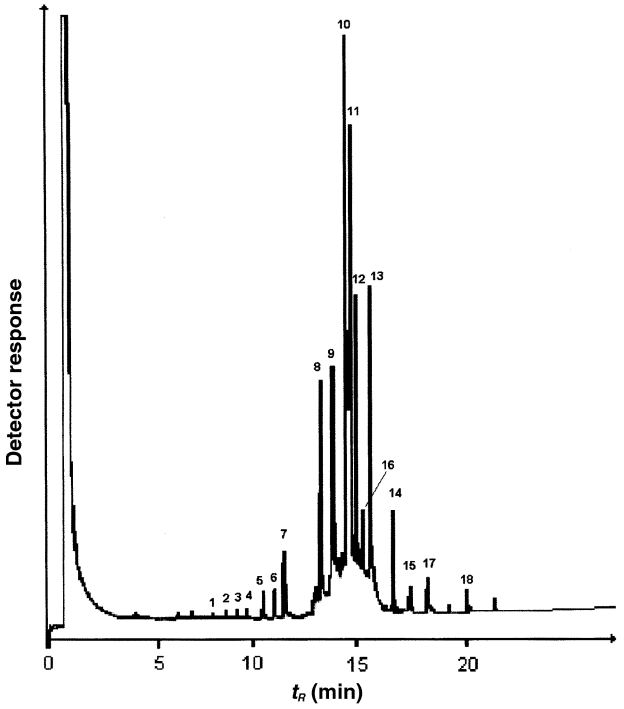


Figure 1. Chromatogram of the CH_2Cl_2–methanol (1:1) crude extract of *Derris urucu*. For HT–HRGC conditions, see the Experimental section.

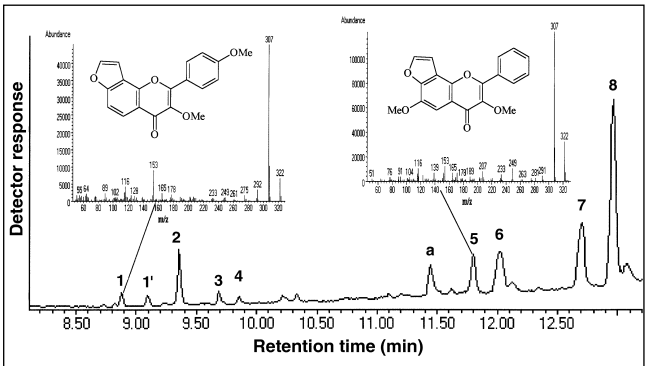


Figure 2. Partial chromatogram of the methanol crude extract of *Derris urucu* and mass spectra of two dimethoxy-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one isomers. For mass spectrometric conditions, see the Experimental section. Peak 1′, phthalate.

Quantitation

The calibration equation was $y = 632.20x - 1880.56$, $r^2 = 0.9867$, where y is the area and x is the concentration (7–70 ng/µL). The limit of detection (LOD) in FID of rotenone (considering the splitting of "rotenone" in three chromatographic peaks) was approximately 0.5 µg/mL, and the limit of quantitation (LOQ) was 2 µg/mL. The values of LOD and LOQ were rela-

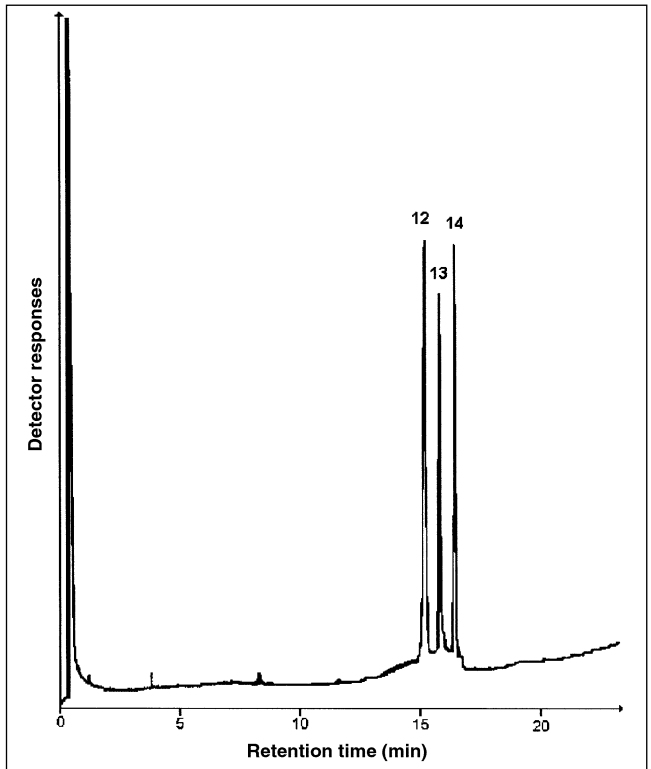


Figure 3. Chromatogram of the standard solution of rotenone with a concentration of 24 ng/µL. For HT–HRGC conditions, see the Experimental section.

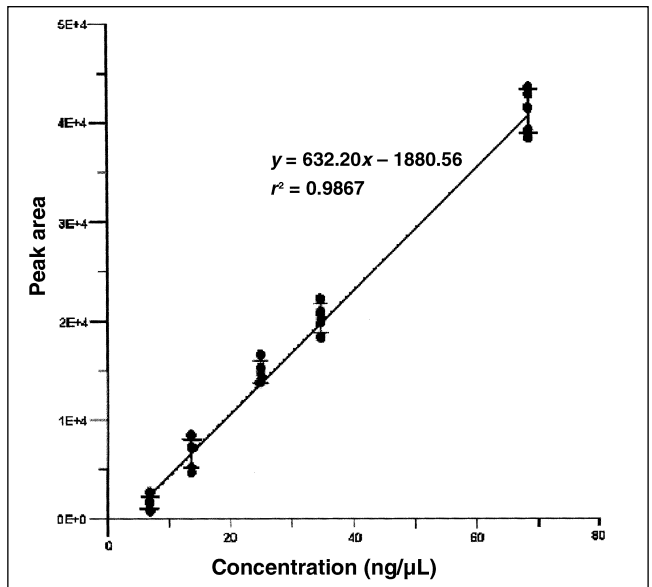


Figure 4. The calibration curve for rotenone. The area values used for calibration (Figure 3) were the sum of the three peak areas. The LOD in FID of rotenone was approximately 0.5 µg/mL, and the LOQ was 2 µg/mL.

Table II. MS Data of the Flavonoids and Rotenoids Found in Crude Root Bark Extract of *Derris urucu*

Compound	Principal fragments *m/z* (relative abundance)
3-Methoxy-2-(4-methoxy-phenyl)-4H-furo[2,3-h]-1-benzopyran-4-one **(1)**	323 **(M + 1)** 3.8, 322 **($M^{+\bullet}$)** 13.6, 308 **(323 – 15)** 21.4, 307 **(322 – 15)** 100.0, 293 **(308 – 15)** 1.9, 292 **(307 – 15)** 8.3, 275 **(292 – 17)** 5.0, 153 **(RDA)** 16.6 Other important ions: 165 **(6.9%)**, 116 **(8.0%)**, 91 **(7.8%)**, 77 **(17.0%)**
3-Hydroxy-2-(4-methoxy-phenyl)-4H-furo[2,3-h]-1-benzopyran-4-one **(2)**	309 **(M + 1)** 3.1, 308 **($M^{+\bullet}$)** 13.3, 294 **(309 – 15)** 21.0, 293 **(308 – 15)** 100.0, 280 **(308 – 28)** 1.0, 278 **(309 – 31)** 1.0, 277 **(308 – 31)** 4.5, 263 **(278 – 15)** 1.0, 262 **(277 – 15)** 2.8 Other important ions: 146 **(15.2%)**, 91 **(3.1%)**, 77 **(5.3%)**.
3,6-Dimethoxy-2-(4-methoxy-phenyl)-4H-furo[2,3-h]-1-benzopyran-4-one **(3)**	353 **(M + 1)** 10.2, 352 **($M^{+\bullet}$)** 49.9, 338 **(353 – 15)** 22.0, 337 **(352 – 15)** 100.0, 323 **(338 – 15)** 7.4, 321 **(352 – 31)** 6.7, 307 **(338 – 31)** 7.4 Other important ions: 169 **(23.1%)**, 104 **(24.8%)**, 91 **(9.2%)**, 77 **(5.8%)**
3,6-Dihydroxy-2-(4-methoxy-phenyl)-4H-furo[2,3-h]-1-benzopyran-4-one **(4)**	325 **(M + 1)** 22.8, 324 **($M^{+\bullet}$)** 96.4, 310 **(325 – 15)** 22.0, 309 **(324 – 15)** 100.0, 293 **(310 – 17)** 8.1 Other important ions: 152 **(27.8%),** 105 **(16.9%)**, 91 **(16.7%)**, 77 **(15.0%)**
3,6-Dimethoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one **(5)**	323 **(M + 1)** 6.5, 322 **($M^{+\bullet}$)** 26.2, 308 **(323 – 15)** 22.1, 307 **(322 – 15)** 100.0, 292 **(307 – 15)** 1.7, 291 **(322 – 31)** 35.5, 276 **(307 – 31)** 1.1, 275 **(292 – 17)** 2.6, 153 **(RDA)** 9.7 Other important ions: 165 **(5.7%)**, 116 **(8.7%)**, 91 **(3.0%)**, 77 **(3.0%)**
3-Hydroxy-6-methoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one **(6)**	309 **(M + 1)** 3.3, 308 **($M^{+\bullet}$)** 15.3, 294 **(309 – 15)** 22.1, 293 **(308 – 15)** 100.0, 281 **(309 – 28)** 3.7, 278 **(308 – 31)** 1.0, 277 **(308 – 31)** 0.4, 263 **(278 – 15)** 0.1, 262 **(293 – 31)** 1.0 Other important ions: 261 **(12.2%)**, 165 **(4.0%)**, 146 **(20.5%)**, 91 **(2.0%)**, 77 **(4.0%)**
3,5-Dihydroxy-6-methoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one **(7)**	325 **(M + 1)** 24.2, 324 **($M^{+\bullet}$)** 100, 310 **(325 – 15)** 19.8, 309 **(324 – 15)** 89.0, 294 **(325 – 31)** 5.8, 293 **(310 – 17)** 9.5 Other important ions: 165 **(18.7%)**, 152 **(12.9%)**, 107 **(31.2%)**, 91 **(12.6%)**, 77 **(12.3%)**
3,5,6-Trimethoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one **(8)**	353 **(M + 1)** 10.2, 352 **($M^{+\bullet}$)** 49.9, 338 **(353 – 15)** 22.0, 337 **(352 – 15)** 100.0, 323 **(338 – 15)** 7.4, 321 **(352 – 31)** 6.7, 307 **(338 – 31)** 7.4 Other important ions: 169 **(23.1%)**, 104 **(24.8%)**, 91 **(9.2%)**, 77 **(5.8%)**
Tephrosin **(9)**	411 **(M + 1)** 3.1, 410 **($M^{+\bullet}$)** 11.8, 395 **(M – 15)** 0.3, 392 **(M – 18)** 0.6, 208 **(RDA)** 100.0, 207 **(208 – 1)** 43.4, 203 **(M – 207)** 9.1, 193 **(208 – 15)** 5.8, 191 **(208 – 17)** 2.9, 180 **(208 – 28)** 1.3, 177 **(208 – 31)** 1.0, 165 **(191 – 26)** 11.2, 162 **(190 – 30)** 0.7, 149 **(180 – 31)** 1.0, 147 **(177 – 30)** 1.0
Deguelin **(10)**	395 **(M + 1)** 6.3, 394 **($M^{+\bullet}$)** 24.3, 380 **(395 – 15)** 1.6, 379 **(M – 15)** 6.2, 351 **(379 – 28)** 1.8, 192 **(RDA)** 100.0, 191 **(192 – 1)** 37.3, 177 **(192 – 15)** 15.6, 147 **(177 – 30)** 3.3
12*a*-Hydroxy-rotenone (rotenolone) **(11)**	411 **(M + 1)** 3.4, 410 **($M^{+\bullet}$)** 12.2, 208 **(RDA)** 100, 207 **(208 – 1)** 47.6, 203 **(M – 207)** 8.7, 180 **(208 – 28)** 1.2, 165 **(180 – 15)** 11.8, 163 **(203 – 40)** 1.4, 149 **(180 – 31)** 1.0
Rotenone (isomers) **(12–14)**	395 **(M + 1)** 2.8, 394 **($M^{+\bullet}$)** 10.8, 192 **(RDA)** 100.0, 191 **(192 – 1)** 34.6, 177 **(192 – 15)** 16.5, 175 **(203 – 28)** 1.3, 161 **(192 – 31)** 1.8, 147 **(177 – 30)** 3.0, 131 **(161 – 30)** 2.9, 105 **(131 – 26)** 2.0
6*a*-Hydroxy-rotenone **(15)**	411 **(M + 1)** 5.0, 410 **($M^{+\bullet}$)** 16.9, 392 **(410 – 18)** 1.7, 208 **(RDA)** 100, 207 **(208 – 1)** 35.7, 203 **(M – 207)** 12.6, 180 **(208 – 28)** 11.3, 165 **(180 – 15)** 13.6, 149 **(180 – 31)** 2.3
1',2'-Dihydro-6*a*,12*a*-dehydrorotenone **(16)**	395 **(M + 1)** 26.4, 394 **($M^{+\bullet}$)** 100, 380 **(395 – 15)** 6.2, 379 **(M – 15)** 24.4, 365 **(380 – 15)** 6.2, 363 **(380 – 17)** 4.2, 351 **(379 – 28)** 8.1, 333 **(351 – 18)** 1.7, 307 **(333 – 26)** 1.6, 279 **(307 – 28)** 1.3 Other important ions: 191 **(36.8%)**, 190 **(13.5%)**, 175 **(5.6%)**, 161 **(13.3%)**, 149 **(7.8%)**, 147 **(14.2%)**, 105 **(8.9%)**
6*a*,12*a*-Dehydrodeguelin **(17)**	393 **(M + 1)** 27.2, 392 **($M^{+\bullet}$)** 100, 378 **(393 – 15)** 12.9, 377 **(392 – 15)** 53.0, 350 **(378 – 28)** 5.4, 349 **(377 – 28)** 23.4 Other important ions: 189 **(21.9%)**, 187 **(13.4%)**, 167 **(6.3%)**, 132 **(6.0%)**, 131 **(8.5%)**
6*a*,12*a*-Dehydrorotenone **(18)**	393 **(M + 1)** 26.7, 392 **($M^{+\bullet}$)** 100, 378 **(393 – 15)** 2.0, 377 **(392 – 15)** 8.5, 350 **(378 – 28)** 1.3, 349 **(377 – 28)** 6.0, 346 **(377 – 36)** 4.9 Other important ions: 345 **(17.8%)**, 290 **(4.8%)**, 233 **(5.3%)**, 189 **(9.5%)**, 132 **(4.7%)**, 104 **(6.5%)**, 77 **(6.0%)**

Table III. Concentration (mg/g) of the Compounds Characterized in the Crude Extracts of the Dried Root Bark of *Derris urucu*

Compound	Extraction A*	Extraction B	Extraction C
3-Methoxy-2-(4-methoxy-phenyl)-4H-furo[2,3-h]-1-benzopyran-4-one **(1)**	< 0.1	< 0.1	< 0.1
3-Hydroxy-2-(4-methoxy- phenyl)-4H-furo[2,3-h]-1-benzopyran-4-one **(2)**	< 0.1	< 0.1	< 0.1
3,6-Dimethoxy-2-(4-methoxy-phenyl)-4H-furo [2,3-h]-1-benzopyran-4-one **(3)**	0.2	0.2	0.1
3,6-Dihydroxy-2-(4-methoxy-phenyl)-4H-furo[2,3-h]-1-benzopyran-4-one **(4)**	0.3	0.4	0.3
3,6-Dimethoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one **(5)**	0.5	0.6	0.5
3-Hydroxy-6-methoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one **(6)**	0.8	0.8	0.5
3,5-Dihydroxy-6-methoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one **(7)**	1.5	1.6	1.4
3,5,6-Trimethoxy-2-phenyl-4H-furo[2,3-h]-1-benzopyran-4-one **(8)**	8.0	8.0	7.9
Tephrosin **(9)**	18.1	17.5	17.8
Deguelin **(10)**	28.5	29.1	30.1
12*a*-Hydroxy-rotenone (Rotenolone) **(11)**	24.7	24.8	26.0
Rotenone (isomers) **(12–14)**§	79.1	80.0	80.1
6*a*-Hydroxy-rotenone **(15)**	0.5	0.6	0.4
1',2'-Dihydro-6*a*,12*a*-dehydrorotenone **(16)**	0.3	0.3	0.3
6*a*,12*a*-Dehydrodeguelin **(17)**	0.3	0.2	0.2
6*a*,12*a*-Dehydrorotenone **(18)**	0.2	0.1	0.1
n-Hexadecanoic (palmitic) acid	0.1	< 0.1	< 0.1
n-9-Octadecenoic (oleic) acid	< 0.1	< 0.1	< 0.1
n-Octadecanoic (stearic) acid	< 0.1	< 0.1	< 0.1

* Extraction of 3 g powdered dried bark three times (using ultrasonic agitation for 30-min periods each solvent) with 10 mL of hexane, dichloromethane, acetone, and methanol.
† Extraction of 3 g powdered dried bark six times (using ultrasonic agitation for 30-min periods each solvent) with 10 mL of hexane, dichloromethane, acetone, and methanol.
‡ Extraction of 100 mg powdered dried bark five times (with ultrasonic agitation for 30-min periods) with 250 mL of methanol–CH_2Cl_2 (1:1).
§ Concentration based on the sum of the three peak isomer areas.

tively poor because of the presence of four isomers (αα, αβ, βα, and ββ, with resolution in three peaks in Figure 3), reducing the signal-to-noise ratio (S/N). The area values used for calibration (Figure 4) were the sum of the three peak areas with a standard deviation of 4.9% (70 ng/µL) to 35.7% (7 ng/µL).

Rotenoids in *Derris*

A dispersion of all compounds in all solvent fractions with practically the same relative concentration was observed. The dispersal of these compounds in all fractions could be due to the interaction of the compounds with the matrix needing polar solvents such as methanol to disentangle them, or else due to the high concentration of rotenoids (approximately 20%, w/w) in *Derris urucu*. Initially, it was theorized that this effect was caused by an insufficient volume of solvent used in the extractions. However, the same also happened with the triple amount of all solvents and the same sample weight, extracting to exhaustion with each solvent (Table III). Much better extraction yields were obtained with methanol–CH_2Cl_2 solvent mixtures.

Another important observation was the presence of other natural products classes in low concentrations. Compounds such as acids and alkanes are in trace concentrations or simply are not observed in the chromatograms. Remarkably, alcohols, terpenoids, and other compounds ordinarily present in plants were not detected.

Recovery of the rotenoids from the bark was studied using many different extraction conditions. The larger percentage extracted from 3 g powdered dried bark six times (by each solvent) using ultrasonic agitation for 30-min periods each with 10 mL of hexane, dichlorometane, acetone, and methanol, respectively (see Table III). The small procedure for the rapid quantitation of the flavonoids and rotenoids in *Derris* proposed here, that 100 mg of powdered dried bark extracted with ultrasonic agitation for 30-min periods five times with 250 µL of methanol–CH_2Cl_2 (1:1), gave similar rotenone yields (Table III).

Conclusion

The technique evaluated allows the rapid qualitative analysis (e.g., profiling) of rotenoids and other secondary metabolites and is a viable alternative with many advantages over classical natural product chemistry methodology. As a result of the capacity to handle crude extracts, the technique is ideal as a screening method for the systematic quantitative study of rotenoids.

Derris urucu bark is an excellent source of rotenone isomers (80 mg/g), deguelin (30 mg/g), and rotenolone (26 mg/g). Many other secondary metabolites (8 flavonoids and 10 rotenoids) were detected. HTHRGC and HTHRGC–MS proved to be powerful tools for the characterization and quantitation of these compounds without the need for isolation or extensive clean-up. The possibility of analyzing crude extracts can be extremely useful for the systematic rapid screening study of medicinal plants and other sources of biologically active compounds, which could guide subsequent natural product chemistry work.

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