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Flavonoids and rotenoids in Lonchocarpus genus: Rotenoids from Lonchocarpus urucu and Lonchocarpus sp. (Uaicà)

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Chimica organica. — *Flavonoids and rotenoids in* Lonchocarpus *genus: Rotenoids from* Lonchocarpus urucu *and* Lonchocarpus sp. (*Uaicà*) (*). Nota VII di DORA SIALER DE ZAPATA (**), FRANCO DELLE MONACHE, GLADYS CAIRO VALERA (***) e GIOVANNI BATTISTA MARINI-BETTOLO, presentata (****) dal Corrisp. G. B. MARINI-BETTOLO.

RIASSUNTO. — Nel quadro delle ricerche sui principi attivi presenti nelle piante del genere *Lonchocarpus* (Leguminose) che hanno permesso mettere in evidenza la presenza di una serie di flavoni, calconi, isoflavoni 3-aril-4-metossi-cumarine prenilati oltre che di rotenoidi sono state studiate con nuove tecniche i principi attivi di *L. urucu* e *L.* sp.

È stato possibile separare e identificare quattro componenti, precedentemente ottenuti solo in miscela, e precisamente rotenone, deguelina, cis-*a*-12-idrossi-rotenone e tefrosina mentre non sono presenti flavonoidi.

The recent detection of substances with new molecular structures in the genus *Lonchocarpus* and *Derris*, i.e., prenylated chalcones, flavones, isoflavones and stilbenes and the 3-aryl-4-hydroxycoumarins (fig. 1), has once more drawn the attention of chemists to the biological and biochemical implications of these substances in the secondary metabolism of these plants [1].

In past years the ichtyotoxic properties of the extracts of these plants, which are directly related to the insecticidal activity, were studied in particular.

Research in this field has led to the isolation of rotenone mainly from *Lonchocarpus nicou* (Aubl) DC and Derris root (*Derris elliptica* [Wall] Benth. and *Derris malaccensis* Prain) and from *Lonchocarpus* root. (*Lonchocarpus utilis* A. C. Smith (often referred to erroneously in chemical literature as *L. nicou* (Aubl) DC.), and *L. urucu* Killip & Smith). The first, commonly referred to as ' Cube ' or ' Barbasco ', is exported from the Amazonian Peru, whereas the second—usually referred to as ' Timbo urucu ' or ' Timbo vermelho ', is exported from the Amazonian Brazil).

Interest in rotenone-containing plants has, for a certain number of years, been focused on the analytical determination of rotenone in *Lonchocarpus* and *Derris* extracts in order to find species varieties and clones with a high yield of rotenoids with insecticidal properties, without considering the other compounds which may also be of some interest.

Therefore, in connection with the International Conference on Legumes, to be held in London in 1978, and in collaboration with the New York

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(****) Nella seduta del 23 giugno 1977.

Botanical Garden and the Department of Plant Sciences, King's College, University of London, we have begun to study a more convenient methodology for the separation and identification of all the active principles of *Lonchocarpus* and *Derris*.

The results of a systematic investigation of the biologically active products present in *Lonchocarpus* and *Derris* may be useful both for their biological implications and for their chemotaxonomic significance, especially as these are two very closely related genera.

The nomenclature of South American spp. referred to here are in accordance with Krukoff, B. A. & A. C. Smith's paper, Rotenone-yielding plants of South America [16].

On the basis of the literature we may consider the phenolic fraction of *Lonchocarpus* and *Derris* to be constituted by five main types of structures:

1) chalcones and flavones (aurones);
2) isoflavones (pterocarpans);
3) 3-aryl-4-hydroxy-coumarins;
4) rotenoids;
5) stilbenes.

These products generally contain prenyl chains, which can also be cyclized to form chromano (and chromene) and furan rings.

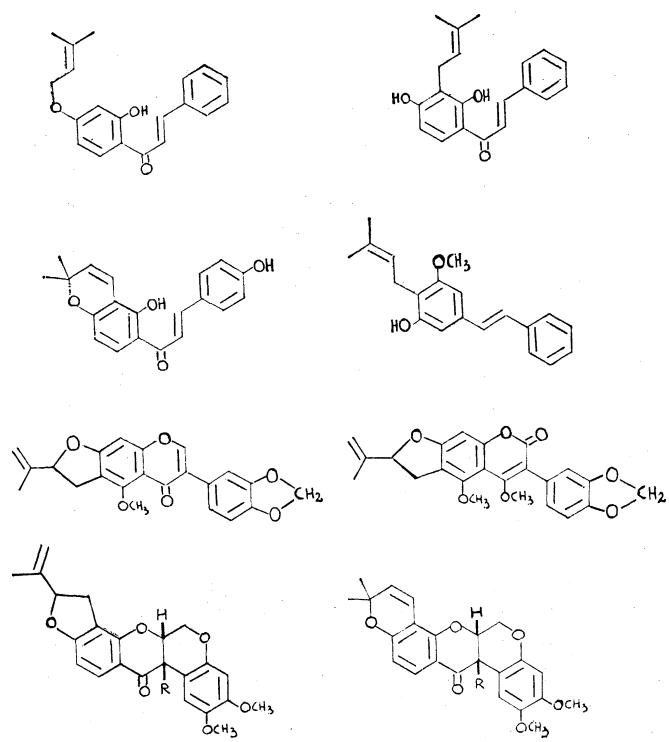

Fig. 1.

Undoubtedly chalcones, flavones, isoflavones, 3-aryl-coumarins and iotenois are connected by a common biogenetic pattern if we consider their structure (fig. 1).

Little is known of the synthesis of these substances in plants and the yearly distribution of the various components.

As mentioned above, in the species of *Lonchocarpus* and *Derris* so far studied only a limited number contain rotenoids, namely *L. urucu* and *L. utilis*. Other species contain prenylated flavones or isoflavones; 3 aryl-coumarins are also present. All these substances can occur together in the same plant, whereas very seldom do the rotenoid-rich plants contain the C–15 products reported above (Table I).

TABLE I

Plant	Lit.	Chalcones (*)	Flavones	Isoflavones(**)	Coumarins (***)	Rotenoids	Stilbenes
D. rariflora (****) .	[5]	+					+
D. malaccensis . .	[6]			+		+	
D. elliptica	[4]					+	
D. robusta	[7]			+	+		
D. scandens	[8]			+	+		
D. glabrescens . . .	[9]			+	+		
D. chirensis	[10]					+	
D. mollis	[11]		+				
D. sericea	[12]	+	+				
D. floribunda . . .	[3]	+					+
D. amazonica (****)	[3]			+			
D. nicou = *L. urucu*	[3, 4]					+	
D. obtusa = *L. obtusus*	[13]	+	+				
L. utilis	[10]					+	
L. laxiflorus . . .	[14]			+			
L. longistylus . . .	[15]						+
L. neuroscapha . .	[1]	+					

(*) Chalcones: including derived products such as flavanones and aurones.
(**) Isoflavanones; including isoflavanes and pterocarpans.
(***) 3-phenyl-4-hydroxy-coumarins.
(****) This plant reported as *Derris* must be considered *Lonchocarpus* according to [16].

The quantitative relations of these products could be important in order to establish a biogenetic relationship among them.

In the present paper we have examined two plants. One is *Lonchocarpus urucu*, collected in the area of Rio Purus (Amazonas, Brazil) and used by Yamamadi Indians. This plant is well known from cultivated sources as the plant used for the manufacturing of rotenone. The second plant is *Lonchocarpus*, undertermined, known as *Uaicà* collected at Itacoitiara (Amazonas, Brazil).

Lonchocarpus urucu known as *timbò* in Brazil contains rotenone and deguelin, according to Clark [2].

Only recently have Gottlieb and coworkers examining a sample of *Derris* (?) *urucu*, shown the presence of three more substances, i.e., a pterocarpan, 12–*a*–hydroxy-rotenone, and tephrosin, but without succeeding in separating the latter two [3].

The samples examined by us contain only four rotenoids; no flavonoids were detected and have the same qualitative composition.

The separation on SiO_2 of the extracts gave rise to two main fractions.

The first one shows the presence of two products which can be visualized by TLC using SiO_2 impregnated with $AgNO_3$.

The separation of the two products can be obtained by column chromatography (SiO_2, eluent benzene: $CHCl_3$ 1 : 1).

1 g of extract of *L. urucu* yields 150 mg deguelin in the head fraction and 120 mg of rotenone in the tail fraction.

The second fraction is also formed by two products; in this case the separation is extremely tedious and difficult. It was accomplished using a SiO_2 column and as eluant: benzene ethylacetate 95 : 5. 3 g of the mixture yield 670 mg of 12 *a*-hydroxyrotenone (70% pure) and 600 mg tephrosin (70% pure).

The separation was followed, checking the various sub-fractions by NMR spectroscopy.

Re-chromatography of the single products in the same conditions yields 200 mg cis 12-*a*-hydroxyrotenone (95 % pure) and 150 mg tephrosin (95 % pure), respectively.

In Table II are reported the yields of *L. urucu* and *Lonchocarpus* sp. Uaicà. Both contain four rotenoids: rotenone, deguelin, 12-α-hydroxyrotenone and tephrosin, but in different percentages.

The identification of these compounds has been established by physicochemical (m.p., $[\alpha]_D$) and spectroscopical data (U.V., I.R., N.M.R. and M.S.) and confirmed by TLC in comparison with authentic samples.

From the above results it appears that both *Lonchocarpus urucu* and Uaicà contain only four rotenoids: rotenone, deguelin, cis-*a*-12-hydroxyrotenone and tephrosin.

L. urucu gives a higher yield of rotenone, about 2 % of the weight of the root, and Uaicà (1 %).

The relative proportion of the four rotenoids is practically identical in both plants.

TABLE II.

Composition and yields of rotenoids in the extracts and fractions of L. urucu *and L. sp. (Uaicà).*

Plant	$CHCl_3$-extract	Fraction 1	Fraction 2
L. urucu			
72 g	8.2 g	2.4 g	0.8 g
	(11%)	(3.4%)	(1.1%)
		Rotenone 62%	cis-12-*a*-hydroxy-rotenone 60%
		Deguelin 38%	tephrosin 40%
L. sp. Uaicà . . .	10.4 g	3 g	2.5 g
	(4%)	(1.2%)	(1%)
250 g		Rotenone 55%	cis-12-*a*-hydroxy-rotenone 60%
			tephrosin 40 %

The ratios rotenone/deguelin and 12-hydroxy-rotenone/tephrosin were determined by NMR spectroscopy on the basis of the approximate integral plot in the methyl region. In effect rotenone has a methyl group adjacent to a double bond $=C—CH_3$, whereas degueline presents two saturated methyl groups $\diagup\!\!>C—CH_3$ which give signals in different regions.

The same applies to 12-*a*-hydroxy-rotenone and tephrosin.

These results indicate that the quantitative composition in rotenoids of *L. urucu* and *L.* sp., Uaicà, is identical. Quantitatively there is a clear difference in their content although the ratios between the components are mantained.

EXPERIMENTAL

Plants.

Lonchocarpus urucu roots were collected in the basin of the Rio Purus (Amazonas) from a plant cultivated by the Yamamadi indians.

The material is used by the Yamamadi for killing fish. Collected by Prance (Prance, n. 13930). A voucher sample is deposited in the Herbarium of the New York Botanical Garden.

Lonchocarpus sp. is called Uaicà by the Indians. Roots were collected by Prance on Itacoatiara Road (Amazonas) (Prance n. 13601). We are indebted to Dr. B. A. Krukoff for this material. A voucher sample is deposited in the Herbarium of the New York Botanical Garden.

Extraction.

The dried plant was pulverized and extracted in Soxleth with methanol, for 20 hours. The methanol extract was evaporated to dryness under vacuum and the residue dissolved in $CHCl_3$.

The solvent dissolves the rotenoids and the flavonoids; the insoluble fraction is mainly constituted by amino-acids.

This method applies to the extraction from various parts of the plants of the main components of *Lonchocarpus* and *Derris*.

Separation.

The rotenoids and flavonoids can be fractionated and purified as above reported, by SiO_2 column chromatography, using different solvent systems. Purification of the single products can be obtained by successive chromatography. Results of the separation are reported in Table II.

Thin layer Chromatography.

For detecting the different rotenoids, SiO_2 prepared with silver nitrate was used according to the method of Delfel and Tallent [4].

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