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A NEW ANTHRAQUINONE ISOLATED FROM *RUMEX OBTUSIFOLIUS*

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Keywords: *Rumex obtusifolius*, Ferriprotoporphyrine Biocrystallization Inhibition Test, NMR, Malaria, Anthraquinone, structure-activity relationship

ABSTRACT

In this research we studied *Rumex obtusifolius* (*Polygonaceae*) along with other plants used in traditional medicine in relation to the chemical reaction FBIT (Ferriprotoporphyrine Biocrystallization Inhibition Test) which may provide information on a possible action mechanism for presumed antimalarial plants. According to folk medicine *Rumex obtusifolius* has a pronounced detoxifying effect on the liver and is used against jaundice and fever. Following a chemical reaction-guided isolation on *Rumex obtusifolius* we obtained demethylmacrosporine I, an anthraquinone derivative. Its structural determination by one and two dimensional NMR and a proposition of structure-activity relationship are presented./*En este trabajo estudiamos Rumex obtusifolius (Polygonaceae) junto a otras plantas usadas en la medicina tradicional respecto a la reacción química FBIT (Ensayo de Inhibición de la Biocristalización de la Ferriprotoporfirina), ensayo que puede proporcionar información de un posible mecanismo de acción de plantas presumidas antimalaricas. Según la medicina tradicional, Rumex obtusifolius posee un efecto desintoxicante del hígado y es usada contra la ictericia y la fiebre. Siguiendo una separación guiada por la reacción química en Rumex obtusifolius, obtuvimos la demethylmacrosporine I, un derivado antraquinonico. Su determinación estructural por RMN de una y dos dimensiones y una proposición de relación estructura actividad son presentadas.*

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INTRODUCTION

Rumex obtusifolius (*Polygonaceae*) is a plant extensively studied because it is used in traditional medicine in several countries in South America. According to folk medicine this plant's root has a pronounced detoxifying effect on the liver and is used against jaundice, fever and as an anti-anemic tonic. The roots are also laxative. In addition, the leaves of this *Rumex* are used against hepatic, eye and dermatological problems. They are applied for the relief of bruises, furuncles and are also used as disinfectant and as scar healer. [1]. Among the compounds isolated from this plant we can find a glycopyranoside: 6-O-malonyl-β methyl-glucopyranoside [2]. Another work has permitted the isolation and identification of amino acid plastocyanin [3]. Isolation of anthraquinones from many types of *Rumex* has also been reported [4, 5, 6, 7, 8], naphthalene glycosides [9], α- naphtols [10], simple naphtalenes, tannins [11, 12], anthracene derivatives [13], naphtoquinone derivatives [14, 11, 12] and flavonoid glycosides [5].
In this research work we studied *Rumex obtusifolius* along with other plants used in traditional medicine over the chemical reaction FBIT (Ferriprotoporphyrine Biocrystallization Inhibition Test) which may provide a possible action mechanism for presumed antimalarial plants. We also present the chemical reaction-guided isolation of the active compound from *Rumex obtusifolius*, its structure determination by one and two dimensional NMR and a proposition of structure-activity relationship.

EXPERIMENTAL SECTION AND RESULTS

PLANTS COLLECTION AND SAMPLE PREPARATION
The plants were collected in a tropical region called Yungas in the Zongo's Valley a 75Km from the city of La Paz (Bolivia). The collection was done in October 2001 during the Spring dry season. Eight species were collected based on their traditional uses. The species, which belong to four different families, were identified in the Bolivian National Herbarium, La Paz. The air dried species were separated into their organs, ground and extracted with ethanol (100 mg/ml) for 24 hr. The dried extracts were tested in FBIT. Table #1 presents the collected plants, their traditional uses and their FBIT results.

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FBIT

The ability of the extracts and fractions to inhibit ferriprotoporphyrin IX (FPIX) biocrystallization was assessed following the protocols previously reported by Deharo et al. [15]. For the assay, 96 hole plates were used introducing in each hole 50µl of the extract or fraction to be studied (2.5mg/ml final concentration), 50µl of DMSO, 50µl of hemine chloride (0.5mg/ml) and 100µl of sodium acetate (0.5M, pH 4.4). The plates were incubated (37°C, 20 hrs), washed (DMSO) and the crystals formed were dissolved with NaOH (0.1M). The spectroscopic quantification was performed at 405nm with a micro-ELISA reader (Titertek Multiskan MCC/340). The results are presented as percent inhibition of the biocrystallization of FPIX. Hydrochloric quinine was used as natural positive control (IC_{50} = 0.03mg/ml).

ISOLATION OF THE FBIT ACTIVE COMPOUND FROM *RUMEX OBTUSIFOLIUS*

RUMEX OBTUSIFOLIUS

The plant studied belongs to the Caryophyllales order, to the Polygonaceae family, to the *Rumex* genus and to the *Rumex obtusifolius* specie. Its binomial name is *Rumex obtusifolius* L. It is commonly known as broad-leaved dock, bitter dock, bluntleaf dock, dock leaf or confusingly as "butter dock". *Rumex obtusifolius* is a perennial weed, native to Europe but can now be found in America and in many other countries around the world. It is a smooth-face weed with straight stems that resembles those of grass. The foliage of the plant can grow from 40 to 100cm height. The lower leaves are largely petiole with an oval limb, rounded or crown at the base, whole and a little frizzy over the edges, from 150 to 250mm long and 70 to 100mm wide. The upper leaves are oblong or oblong-petiole, smaller with a shorter petiole. The flowers are a peduncle with the calyx-crown both measuring 2mm long with obtuse sepals. Large clusters of racemes contain the green flowers that change to red as they mature. The fruits have ovoid, triangular, valves crown at the base. They are deeply dentate in the lower part of the membranous margin, with the callus poorly developed from 4 to 5mm long. The seeds produced are reddish-brown [16]. Figure #1 shows the *Rumex obtusifolius* specimen.

Figure 1*. Rumex obtusifolius, Polygonaceae.*

ISOLATION OF THE FBIT ACTIVE COMPOUND

General Experimental Procedures.

^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Brucker DRX-400 and 250 using as solvents CD_3OD-$CDCl_3$ and DMSO-d6. Silica gel G-60 (EM Merck) for VLC and (EM Merck, 70- 230 mesh) for CC. Silica gel over aluminum was used as TLC plates (EM, Merck, silica gel 60 F254). TLC spots were visualized by UV light (254 and 365 nm) and by developing reagent (H_2SO_4 25%).

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Table 1*. Collected species, traditional uses, FBIT results*

SPECIE	FAMILY	TRADITIONAL USE	FBIT % INHIBITION[1]
Centropogon gloriosus (Britton) Zahlbr.	*Campanulaceae*	Against intestinal parasites and for heart palpitations.	Leaves: 55 (±5) Stems: 63 (±7) Flowers: 31 (±10)
Siphocampylus cf. *bilabiatus* Zahlbr.	*Campanulaceae*	To heal wounds and for nervous problems.	Leaves: 0 Stems: 5 (±4)
Siphocampylus dubius Zahlbr.	*Campanulaceae*	To heal wounds and for nervous problems.	Leaves: 36 (±6) Stems: 9 (±7) Flowers: 47 (±2)
Siphocampylus sp.	*Campanulaceae*	To heal wounds and for nervous problems.	Leaves: 0 Stems: 6 (±16) Flowers: 11 (±7)
Clusia sp.	*Guttiferae*	In lung diseases, renal pain, sprains and osseous dislocations. As a laxative, vermifuge and disinfectant.	Leaves: 64 (±3) Stems: 60 (±11) Flowers: 63 (±5)
Rumex obtusifolius L.	*Polygonaceae*	For hepatic, eye and dermatitis problems. To heal furuncles and bruises. As a laxative, disinfectant, scar healer and as anti-arthritic and anti-anemic tonic. It is also used against jaundice and fever.	Leaves: 60 (±2)
Rumex sp.	*Polygonaceae*	For hepatic and dermatitis problems. To heal furuncles and bruises. As a laxative, disinfectant, scar healer and as anti-arthritic and anti-anemic tonic. It is also used against jaundice and fever.	Leaves: 42 (±19)
Costus sp.	*Zingiberaceae*	For bruises and fever. To relieve stomach an intestinal pain. As an antiseptic and laxative.	Leaves: 60 (±5) Stems: 0

ISOLATION

The *Rumex obtusifolius* leaves were dried, ground, extracted with petroleum ether and then with ethanol (125g in 600ml for 24 hrs). The ethanolic extract (1.1g) presented 58% of inhibition on FBIT at 2.5mg/ml. The active extract was subjected to a vacuum liquid chromatography (VLC) on SiO_2 using a gradient solvent system (petroleum ether (PE) to PE-CH_2Cl_2 to CH_2Cl_2-MeOH to MeOH). The VLC yielded 16 fractions, where fraction 8 (F8) eluted with CH_2Cl_2 -PE 70% (26.9mg) and fraction 9 (F9) eluted with CH_2Cl_2-PE 75% (44.8mg) were the more active ones. The percent inhibitions on FBIT at 0.25mg/ml for F8 and F9 were 54% and 58%, respectively. The other fractions were less active or negative at the tested dose. Fractions 8 and 9 were joined and chromatographed on a silica gel open column and eluted wit the mixture EtOAc-CH_2Cl_2 3%. This chromatography yielded 11 fractions and three of them were active. The active fractions were fraction 7 (2.7mg; 60% inhibition at 0.125mg/ml), fraction 8 (18.4mg; 75% inhibition at 0.125mg/ml) and fraction 9 (4.1mg; 53% inhibition at 0.125mg/ml). The other fractions were less active or negative at the tested dose. Compound (**1**) was obtained (17.3mg, IC_{50} = 0.071mg/ml) from these fractions and after recrystallization with methanol.

STRUCTURE DETERMINATION OF THE FBIT ACTIVE COMPÒUND

Compound (**1**), an orange crystal, was identified through NMR and MS studies as an anthraquinone derivate with a molecular weight of 270 ($C_{15}H_{10}O_5$, with 11 unsaturations). The ^{1}H-NMR spectra shows two types of protons: four aromatic protons at lower field and 3 protons corresponding to a methyl group at higher field. Two of the aromatic protons are coupled in a meta position while the other two protons presented a coupling constant of a para arrangement. The methyl group, at 2.36ppm, is displaced from its normal region of resonance due to its proximity to an aromatic ring. The ^{13}C-JMOD-NMR of compound (**1**) presents 15 carbons: one CH_3, four CH-aromatics, two aromatic carbonyls, three oxygenated quaternary carbons and five quaternary aromatic carbons. Table #2 shows the ^{1}H-NMR and ^{13}C-NMR spectra data of compound (**1**). The proton-carbon assignment was achieved from the HMQC spectra. Table #3 shows the spectral data of the HMBC experiment showing couplings from two to four bonds. Many of the correlations to four bonds are confirmed by the COSY spectra. In addition, there are publications that show the importance of long range coupling (^{4}J) [17]. Figure #2 shows the HMBC spectra of compound (**1**).

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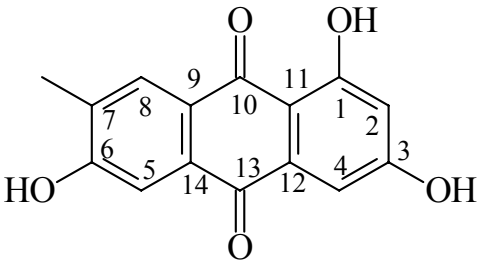
Table 2. *^{13}C-NMR spectra data for compound (**1**), (400MHz, CD_3OD-$CDCl_3$)*



Compound (1)

Atom	δ_H (ppm)	δ_C (ppm)
15	2.36	22.1
2	6.53	108.6
4	7.14	109.6
5	7.50	121.2
8	6.98	124.5
11	-	110.0
14	-	114.0
9	-	133.3
12	-	135.4
7	-	148.3
6	-	163.0
3	-	164.8
1	-	165.6
13	-	182.7
10	-	190.2

Table 3. *^{1}HNMR and HMBC, COSY spectral data for compound (**1**), (400MHz, CD_3OD-$CDCl_3$).*

Atom	δ_H (ppm)	δ_C (ppm)	COSY	HMBC (^{2}J)	HMBC (^{3}J)	HMBC (^{4}J)
15	2.36	22.1	7.50 (H-5) 6.98 (H-8)	148.3 (C-7)	124.5 (C-8)	121.2 (C-5)
2	6.53	108.6	7.14 (H-4)	165.6 (C-1) 164.8 (C-3)	109.6 (C-4)	190.2 (C-10)
4	7.14	109.6	6.53 (H-2)	135.4 (C-12)	108.6 (C-2) 182.7 (C-13)	190.2 (C-10) 165.6 (C-1)
5	7.50	121.2	6.98 (H-8) 2.36 (H-15)	114.0 (C-14)	182.7 (C-13) 190.2 (C-10)	124.5 (C-8) 22.1 (C-15)
8	6.98	124.5	7.50 (H-5) 2.36 (H-15)	-	114.0 (C-14) 163.0 (C-6) 22.1 (C-15)	121.2 (C-5)

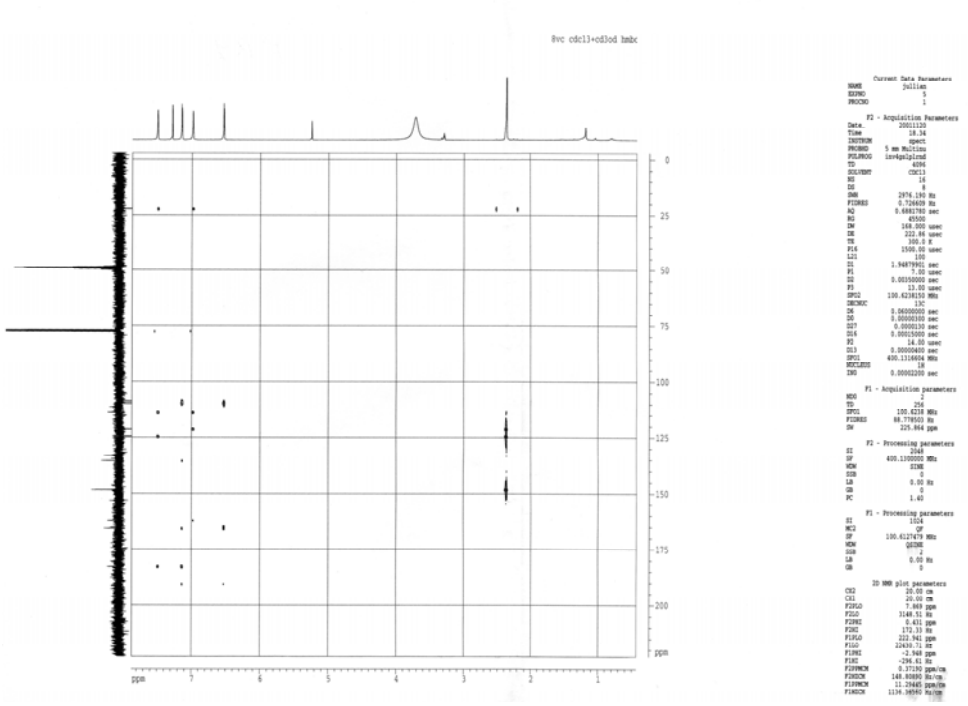


Figure 2. *HMBC spectrum of compound (**1**), 400MHz, CD_3OD-$CDCl_3$.*

All correlations of protons H-15, H-8 and H-5 give us the information of one of the aromatic cycles, which we called cycle A. Figure #3 shows the HMBC correlations on the aromatic cycle A. In this cycle, the following correlations are important to note. The correlation from 2.36ppm (H-15) indicates that the methyl group is present in this cycle that has the para aromatic protons. In addition, one of the correlations of H-8 (6.98ppm) is to C-6 (an oxygenated

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quaternary carbon) which stands for the presence of one alcohol group in this cycle. Finally, the correlation of H-5 (7.50ppm) to the carbonyl groups at C-10 and C-13 confirms he anthraquinone type skeleton.

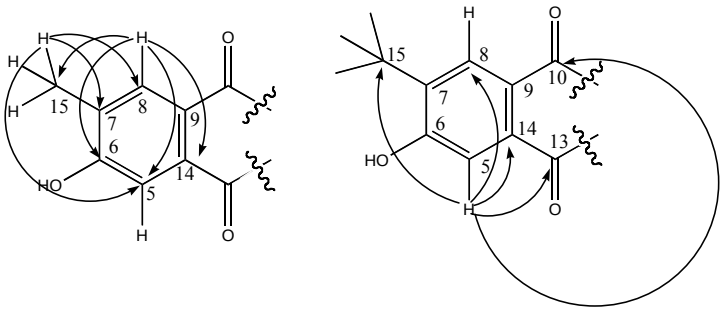

Figure 3. *HMBC correlations in cycle A of compound* ***(1).***

All correlations of protons H-2 and H-4 give us the information of the other aromatic cycle, which will be called cycle B. Figure #4 shows the HMBC correlations on the aromatic cycle B. In this cycle, it is important to remark the following correlations. The correlation from 6.53ppm (H-2) to C-1 and C-3 indicates that two alcohols are found in this meta aromatic cycle. The presence of anthraquinone is also confirmed by the correlations of H-2 (6.53ppm) to the carbonyl signal C-10 and from H-4 (7.14ppm) to both carbonyl signals C-10 andC-13.

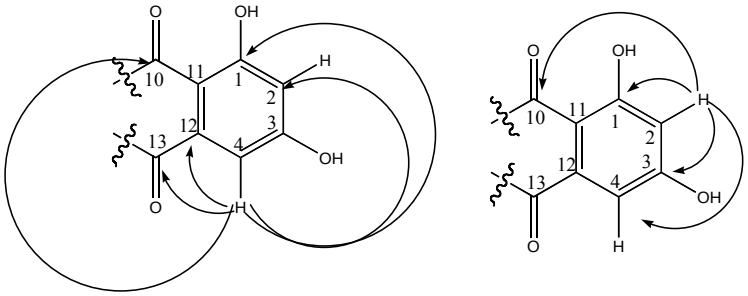

Figure 4. *HMBC correlations in cycle B of compound (1).*

It is significant to emphasize the formation of a "peri" system in the molecule thanks to the chelation of the hydroxyl in C-1 and the carbonyl at C-10. In this "peri" system we appreciate carbon C-11 very shielded compared to the other quaternary carbons. The same effect counts for the resonance of C-1 at low field compared to the other quaternary carbons bearing an alcohol group.

In addition, it is important to indicate that the anthraquinone presented for compound (**1**) has an isomer that can not be distinguished by NMR techniques. Figure #5 shows both isomers named isomer 1 and isomer 2. The possible biosynthesis of this type of anthraquinones confirms the existence of both isomers. Isomer 1 may be biosynthesized following the classic procedures from an eight carbons polyketide through the biosynthesis of emodine. Isomer 2 may be biosynthesized through an adaptation of the biosynthetic path of norsolorinic acid [18, 19].

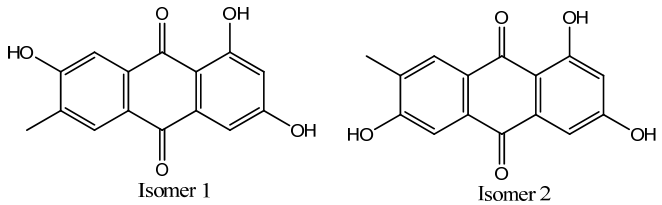

Figure 5. *Isomeric forms of the anthraquinone compound* ***(1)***

Compound (**1**): Orange crystals, ^{1}H-NMR and ^{13}C-NMR data see Tables #1 and #2. EIMS, 70 eV: m/z (intesity ratio, fragmentation): 270 (100, $M^{+\bullet}$:$C_{15}H_{10}O_5$); 242 (6.4, $M^{+\bullet}$- CO); 241 (8.2, 242-$H^{\bullet}$); 214 (4.5, 242-CO); 213 (8.8, 214-$H^{\bullet}$); 185 (4.0, $M^{+\bullet}$- $C_4H_4O_2$); 184 (4.0, 214-CHO); 164 ($M^{+\bullet}$- C_7H_6O); 158 (214- C_3H_4O); 149 (14.9, 184-$2H_2O$+ H); 139 (12.6, 158- H_2O-H); 128 (8.1, 164- $2H_2O$); 84 (6.6, $M^{+\bullet}$,- 185); 69 (15.1, C_4H_5O); 57 (10.5, C_3H_4O);

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43 (10.3, C_2H_2O) [18].

STRUCTURE-ACTIVITY RELATIONSHIP PROPOSAL

Portela et al. have demonstrated that quinolic and xanthonic type molecules interact with hemine chloride and stabilize it thanks to large range interactions determined by their complementary electrostatic profiles [20 Portela]. The electronic centers of the active compound will interact with the opposite poles found in the hematine (hemine chloride crystal). In the hemine chloride, the most negative potential is found in the position occupied by the iron in the tetrapyrrolic system while the most positive site is found at the propionic groups. One of the necessary characteristics that active compounds should have is the presence of a null or positive potential all along the molecule or the presence of an aromatic cycle. Based on Portela's work we presented a structure-activity relationship proposal to understand the fashion in which compound (**1**) interacts with hemine chloride. If we assign the electrostatic center in compound (**1**), we find that this anthraquinone has more than one negatively charged center (ketone and alcohol groups) and a positive pole in the aromatic centers. Among the two ketones, which carry out the negative charge, the ketone beta to an hydroxyl group will have a bigger negative pole. Figure #6 shows (A) the electronic stabilization of hemine chloride by compound (**1**) and (B) the complex formed by hemine chloride-compound (**1**). Figure #7 shows (A) the electronic stabilization of hemine chloride by quinine and (B) the complex formed by hemine chloride-quinine.

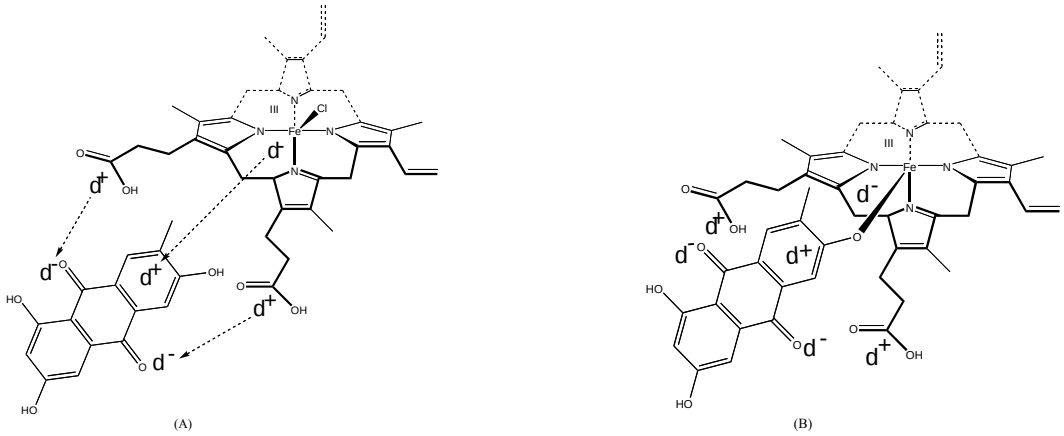

Figure 6*. A) Stabilization of hemine chloride by compound (**1**). (B) Complex of hemine chloride-compound (**1**).*

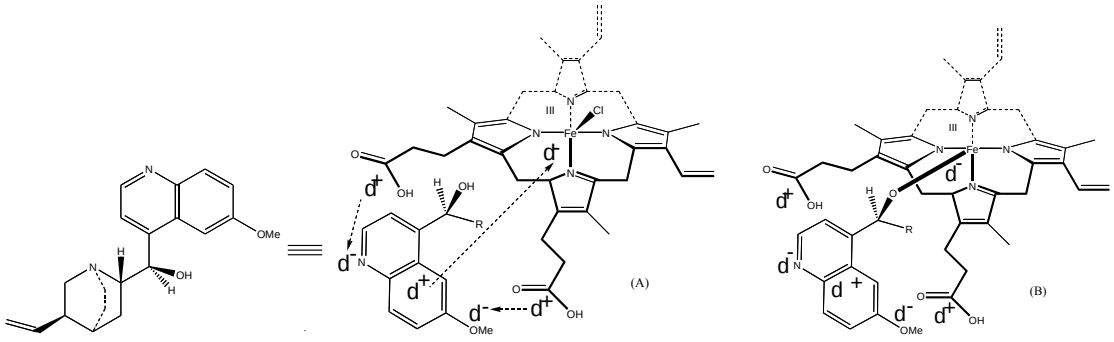

Figure 7*. A) Stabilization of hemine chloride by quinine. (B) Complex of hemine chloride-quinine.*

DISCUSSION AND CONCLUSION

PLANTS COLLECTION AND SAMPLE PREPARATION

Our research group works with antimalarial natural compounds and one of the assays used to study the possible

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mechanism of action of the active compounds is the FBIT. For this reason we have used the FBIT to find compounds that inhibit the hematine crystal formation. From the eight collected species, three of them caught our attention *Centropogon gloriosus*, *Clusia* sp., *Rumex obtusifolius* and *Costus* sp. The traditionally used *Clusia* and *Costus* species were not chosen in this study since their complete identification was not possible. In addition, *Centropogon gloriosus* which was more active than *Rumex obtusifolius* was not selected since it was allergenic. For the above reasons *Rumex obtusifolius* was submitted to a FBIT-guided isolation.

ISOLATION AND STRUCTURE DETERMINATION OF THE FBIT ACTIVE COMPÒUND

After a FBIT guided-isolation using different chromatographic techniques, 17.3mg of an orange crystal were purified. The NMR and MS studies have permitted its identification as an anthraquinone derivate (compound **1**) which has an isomer, demethylmacrosporine, previously reported from *Dichotomophthora lutea* [21]. Comparing the ^{1}H-NMR spectra data of demethylmacrosporine and compound (**1**) we conclude that our molecule is the isomer of the previously reported structure and we named compound (**1**) as demethylmacrosporine I. Table #4 shows the ^{1}H-NMR spectra data of demethylmacrosporine and that of compound **1** (demethylmacrosporine I). To complete our work in the assignment of the isomers we used the X-ray diffraction technique (XRD); unfortunately, compound (**1**) crystallizes in a very fine fan conformation, crystal structure inadequate for the XRD technique. Further studies with molecular modeling in NMR will be carried out in order to complete the isomeric determination. Thanks to this technique the chemical shift of the para proton may be obtained giving us another tool to differentiate among these anthraquinone isomers.

This is the first time that an anthraquinone of the substitution pattern presented is reported from *Rumex*. Most of the reported anthraquinones from plants are dihydroxy-1, 8 anthraquinone derivates. In this paper we are presenting a trihidroxy- 1, 3, 6 anthraquinone derivate which is usually found in mushrooms. At this point it is crucial to emphasis that our work has been carried out with all the required care to avoid bacterial or fungi contamination.

***Table 4**. Comparison of ^{1}H-NMR spectra data of compound **1** (demethylmacrosporine I) and demethylmacrosporine, (250MHz, DMSO-d6).*

Atom	δ_H (ppm) of compound (**1**)	δ_C (ppm) of demethylmacrosporine
15	2.4	2.3
2	6.5	6.6
4	7.1	7.1
5	7.5	7.9
8	7.0	7.6
OH-1	12.1	12.8

STRUCTURE-ACTIVITY RELATIONSHIP PROPOSAL

Demethylmacrosporine I (compound **1**) shows an important activity on FBIT (IC_{50} = 0.071mg/ml) which is comparable to that of hydrochloric quinine (IC_{50} = 0.03mg/ml). A possible mechanism of action has been proposed based on the work of Portela [20]. The difference between the observed IC_{50} values may be due to the electrostatic behavior in the active molecule and the distance among the different electronic poles in the active compound and in the hemine chloride. To complete this proposal, a molecular modeling study is needed specially to calculate the distance between the three electrostatic potentials that form a triangle in the formed complex and the distance between the two zones with negative potentials. This type of work is being carried out in our group.

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REFERENCES

1. L. Girault; “Kallawaya. Guérisseurs itinérants des Andes”. Ed. de l’ORSTOM- Institut Français de recherche scientitique pour le développement en coopération. Paris, 1984.
2. T. Kasai; M. Okuda; S. Sakamura; Phytochemistry 1981, 20, 1131- 1132.
3. B. G. Haslett ; C. J. Bailey ; J. A. M. Ramshaw; M. D. Scawen; D. Boulter; Phytochemistry 1978, 17, 615-617.

Received: 10/09/09 Approved: 21/10/09 Published: 2/12/09

4. L. Ö. Demirezer; A. Kuruüzüm-Uz; I. Bergere; H. –J. Schiewe; A. Zeeck; Phytochemistry 2001, 58, 1213-1217.
5. A. Hasan; I. Ahmed; M. Jay; B. Voirin; Phytochemistry 1995, 39, 1211- 1213.
6. J. O. Midiwo; G. M. Rukunga; Phytochemistry 1985, 24, 1390- 1391.
7. R. M. Munavu; L. O. Mudamba; J. A. Ogur; Planta Med. 1984, 50, 111.
8. J. B. Harborne; N. Mokhtari; Phytochemistry 1977, 16, 1314- 1315.
9. A. Kuruüzüm-Uz; L. Ö. Demirezer;; I. Bergere; A. Zeeck; Phytochemistry 2001, 56, 399- 402.
10. H- J. Bauch; R. P. Labadie; E. Leistner; J. Chemical Science Pekin 1975, 689- 692.
11. L. Ö. Demirezer; A. Kuruüzüm-Uz; J. Pharmaceutical Science 1997, 22, 153- 158.
12. J. L. Suri; K. L. Dhar; C. K. Atal; J. Indian Chemical Society 1978, 55, 292- 293.
13. Y. Fassil; A. Bezabeh; B. Abegaz; B. Botta; G. Delle Monache; F. Delle Monache; J. Nat. Prod. 1985, 48-148.
14. M. Sharma; P. Sharma; S. Rangaswami; Indian J. Chemistry 1977, 544- 545.
15. E. Deharo; R. N. García; P. Oporto; A. Jiménez; M. Sauvain; V. Julian; H. Ginsburg; Exp. Parasitol. 2002, 100, 252-256.
16. A. L. Cabrera; Flora de la provincia de Buenos Aires. Colección científica del I.N.T.A. Buenos Aires , 1967.
17. R. Araya-Maturana; T. Delgado-Castro; W. Cardona; B. E. Weíss-Lopéz; Current Organic Chemistry 2001, 5, 253- 263.
18. S. L. Ibáñez-Calero. Ph.D. Thesis, ″Application du test d'inhibition de la biocristallisation de l'hème a la caractérisation de composés antipaludiques isolés de la flore de Bolivie. Université Paul Sabatier, France, 2005.
19. J. Mann; "Secondary metabolism". Oxford Science Publications, second edition. Oxford, New York, Toronto, Dehli, Bombay, Calcuta, Madras, Karachi, Petaling, Jaya, Singapure, Hong Kong, Tokyo, Nairoby, Dar es Salaam, Cape Town, Melbourne, Auckland, Berlin, Ibadan, 1992.
20. C. Portela; C. M. Afonso; M. M. M. Pinto; M. J. Ramos; Bioorganic & Medicinal Chemistry 2004, 12, 3313- 3321.
21. T. Hosoe; K. Nozawa; S-I. Udagawa; S. Nakajima; K-I. Kawai; Phytochemistry 1990, 29, 997- 999.