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Psychotria douarrei and *Geissois pruinosa*, novel resources for the plant-based catalytic chemistry

Claire Grison,^a Vincent Escande,^{b,c} Eddy Petit,^a Laetitia Garoux,^d Clotilde Boulanger^d and Claude Grison^{*b}

Psychotria douarrei and *Geissois pruinosa* are known as a hypernickelophore plants. The study of their chemical characteristics was revisited to demonstrate a novel potential of this natural resource for the Green Chemistry. *P. douarrei* showed a unique composition, which led to a novel concept of plant-based catalytic chemistry. The supported Biginelli reaction illustrated an interest of this concept for green organic synthesis.

Introduction

15 Because of their immobility, plants grow on environments which they cannot escape. Therefore some plants develop very specific biological mechanisms to withstand abiotic or biotic constraints. The heavy-metal content of soil is one of the most important edaphic factors impacting the vegetation composition. Plants only
20 survive by adapting their physiological processes. Metallophytes have known to survive on metal-rich soils. Wide-ranging identifications of hyperaccumulators of Ni from tropical soils of ultramafic origin were reported. For example, in New Caledonia, the soil is derived from ultramafic rocks, which is naturally
25 enriched in Nickel [1]. Around 40 nickel-tolerant species have adapted to this natural nickel-toxicity [2-4]. Species were classified into four categories of Ni accumulation ability: non-accumulator (<100 mg Ni/ kg), accumulators (100–1000 mg Ni/kg), hyperaccumulators (1000–10,000 mg Ni/kg), and
30 hypernickelophores (>10,000 mg Ni/kg) [5]. Nine are hypernickelophores and belong to *Geissois* genus (Cunoniaceae), *Homalium* (Silicaceae), *Hybanthus* (Violaceae), *Phyllanthus* (Phyllanthaceae), *Psychotria* (Rubiaceae) and *Sebertia* (Sapotaceae). Among these metallophytes, *Psychotria douarrei*
35 and *Pycnanandra acuminata* present exceptional tolerance to the nickel enriched soils. They are the both strongest nickel-hyperaccumulators [2, 5]. Besides, *P. douarrei* is characterized by the ability to accumulate very high concentrations of nickel, up to 4.7% of Ni in its shoots. The hypernickelophore *Geissois*
40 *pruinosa* had a relatively high Cr content (430 ppm). The potential of *G. pruinosa* and *P. douarrei* had been identified for the ecological restoration and phytoremediation [6]. Therefore we have recently developed a large scale ecological restoration of the Thio Caledonian mining site [7,8] introducing a large number of
45 Ni metallophytes, especially *P. douarrei* and *G. pruinosa*. For the first time, it is possible to propose credible outlets to dispose of nickel-enriched biomass. A fundamental question is addressed: What potential do Ni hyperaccumulating plants offer as a

resource base for the Green Chemistry? Taking the advantage of
50 the capacity of these plants to concentrate Ni into shoots, we address the direct use of Ni as Lewis acid catalysts for a modern organic synthesis. The use of nickel-enriched biomass to produce catalysts used in organic chemistry could bring valorization for the development of phytoextraction in New Caledonia. In this
55 article, we investigate the potential of *P. douarrei* biomass as an alternative source of nickel, which is used in the synthesis of a promising antimitotic compound, but difficult to access, the dihydrothiopyrimidinone, named monastrol.

The present study was conducted with two aims: (a) revisiting
60 the inorganic composition of *P. douarrei* and *G. pruinosa* extract and the derived catalyst with specific analytical techniques and (b) identifying their abnormal compositions as an inspiration for plant-based and environmental friendly chemistry.

65 Results and discussion

Leaves of *P. douarrei* and *G. pruinosa* were harvested in the South province of New Caledonia. 560 samples were collected for three years, two times a year, from two different sites: on Thio-Plateau Mining Site and Mont Koghis near Noumea.

70 In order to determine the mineral composition of *P. douarrei* an appropriate treatment of the shoots was necessary. The first step was a thermic treatment of leaves at 400°C to destroy the organic matter. The addition of HCl (1M) led to a complex mixture of metallic species of this plant. XRF was used to determine the
75 composition of the plant catalysts obtained. Detailed results are presented in Table 1.

In Table 1, for 1 g of catalyst, *P. douarrei* furnished 3.3 mmol of Ni, while *G. pruinosa* gave 1.6 mmol of Ni. Significant amounts of Mn and Si were also noticed in *P. douarrei*, while
80 alkaline-earth Ca and Mg were highest in *G. pruinosa*. Moreover solid derived from *P. douarrei* led to the richest mixture in transition metals thus the most interesting catalyst in organic

Table 1. Mineral composition of nickel-hyperaccumulating plant catalysts. Data were compared to *G. pruinosa*, another endemic Caledonian nickel-hyperaccumulating plant which grows in a similar ecological niche, as control.

Catalysts	Mass %													
	O	Na	Mg	Al	Si	P	S	Cl	K	Ca	Mn	Fe	Ni	
<i>P. douarrei</i> extract	17.7	1.2	2.55	<0.32	0.86	<0.39	2.41	41.56	7.7	7.71	0.22	0.28	19.86	
<i>G. pruinosa</i> extract	21	1.5	11.9	<0.15	<0.07	<0.38	1.64	42.33	4.13	10.6	<0.15	0.62	9.35	

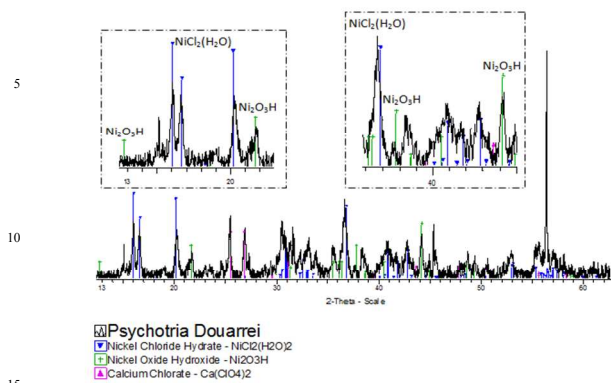


Figure 1. XRD pattern of a *P. douarrei* catalyst (Radiation used: Cu K α ; $\lambda=1.54056 \text{ \AA}$).

Table 2. Major anionic species of *G. pruinosa* and *P. douarrei*

	Phosphate (mg.g ⁻¹)	Sulfate (mg.g ⁻¹)
<i>G. pruinosa</i>	39.8	58.5
<i>P. douarrei</i>	28.3	70.4

Table 3. Sulfate and Phosphate amount from *P. douarrei* lipid membrane according to the extraction solvents

	CHCl ₃	CH ₂ Cl ₂ -MeOH	i-PrOH	H ₂ O-MeOH
Phosphate (mg.L ⁻¹)	0	0	0	12.3
Sulfate (mg.L ⁻¹)	10.6	13.1	58.7	14

synthesis. Finally these XRF analyses confirmed the great ability of *P. douarrei* to concentrate Ni in its shoots.

The ratio Ni/Cl corresponded to partially hydrated NiCl₂, but could not explain the complex structure of *P. douarrei* catalyst. That is why XRD analyses were used to identify the crystallized mineral compounds in the catalyst. In Figure 1, the formation of Ni^{II}Cl₂(H₂O)₂ was confirmed. Very surprisingly, but very interestingly Ni₂O₃H was observed. This unusual result with two oxidation states of Nickel (+2 and higher: +3) should be noted [10, 11, 12]. This observation had never been found in a living organism; and the oxidation degree +3 was exceptionally observed in [NiF₆]³⁻ or in drastic conditions [10]. As well, Ni^{IV} complexes had rarely been detected [11].

The same treatment of leaves was performed on *P. douarrei* and *G. pruinosa*. Ni^{III} had not been found in *G. pruinosa*.

Therefore Ni^{III} had not been formed by the chemical and thermic treatment. Ni^{III} was naturally present in *P. douarrei*.

G. pruinosa XRD analyses provided more predictable results as KNiCl₃.

Furthermore in table 1, the P total level should be noted. It was consistent with the composition of serpentine soil in New-Caledonia, derived from Fe- and Mg-rich ultramafic rocks and also deficient in available phosphorus. According to certain authors [13], symbiosis with arbuscular mycorrhizal fungi could help the host to overcome phosphorus lack. However, for Ni-hyperaccumulators, especially for *P. douarrei*, a high ratio S / P was remarkable. To better understand the form of sulfur assimilation, a purification of 50 catalysts was carried out by anion-exchange chromatography. The results are presented in Table 2.

In Table 2, the amount of sulfate was much higher than the amount of phosphate, which is rare for vascular plants. Comparing Tables 1 and 2, the high level of sulfur resulted in significant amounts of sulfate, and vice-versa, a poor level of phosphorus could be correlated to small amounts of phosphate. *P. douarrei* is the most demonstrative example. We wondered how the abnormal high percentage of sulfate could be explained by *P. douarrei* physiology.

The sulfur assimilation pathway had been recently investigated [13]. Sulfate derived from environment uptakes into plants is reduced to sulfide through a serie of enzymatic catalyzed transformations [14, 15]. The first step is the formation of APS (5'-adenylsulfate) by a reaction between sulfate and ATP. The following step is the reduction of the sulfate group into sulfite, then the reduction of sulfite into sulfide which is used for the construction of cysteine. Cysteine serves as the precursor of all cellular compounds containing sulfur: methionine, glutathione and proteins. Therefore it was interesting to examine whether the sulfate derived from *P. douarrei* was the result of the sulfur amino acid decomposition during the thermic treatment, which could explain the abnormal high level of sulfates. It had been reported that cysteine, methionine, glutathione and proteins are thermally decomposed, at 400°C, in various gases such as H₂O, H₂S, CO₂, NO₂, SO₂, and N₂O₃ [16, 17]. That is why we concluded that the excessive amount of sulfate measured was not a consequence of an unusual sulfur amino acid contained in the plant.

Sulfate could originate from chemically activated forms of sulfate as 5'-adenylsulfate, [13] or from other biological structures, where the S atom replaces the P atom. Phosphorus is a part of the chemical backbone of the phospholipids that form every cell membrane.

Recently, Okaneneko et al. [18] had reported that plants could use sulfate instead of phosphate in their cell membranes. They have many sulfatolipids instead of phospholipids.

Considering this hypothesis, we had launched experiences aimed at the extraction of membrane lipids. The Folch and Bligh and Dyer methods [19,20] were developed as rapid but effective methods for determining the total membrane lipid content in *P. douarrei*. We analyzed the methanol/water phase system, and many organic phases derived from washings of the crude extract with CHCl_3 , CH_2Cl_2 -MeOH, H_2O -MeOH and *i*PrOH. The first analyses were realized by anion-exchange chromatography. Regardless the treatment, the chromatogram (Table 3) showed that sulfate was the major anion. Isopropanol was the best extraction solvent of the sulfate derivatives. In principle, alkali and alkaline earth sulfate are insoluble in organic mixtures. No traces of nickel sulfate, sulfite or sulfide were detected. These observations suggested that the knowledge of the sulfate entity structure had to be improved.

Subsequent analyses by mass spectrometry (ESI-MS, HR-ESI-MS and HR-MS-MS) were performed of the sample, diluted in methanol. Details can be found in supplementary information. Interestingly, a new compound has been identified as a sulfatolipid, 3,4-dihydroxy-tridecanesulfate (figure 2).

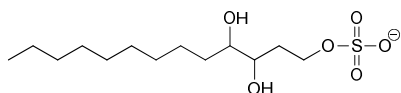
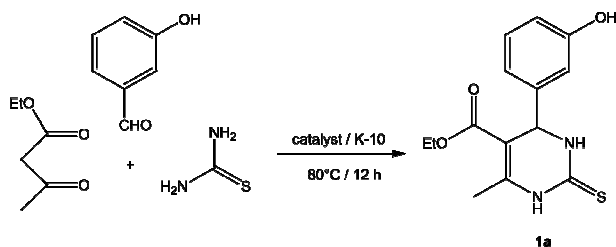


Figure 2. The sulfatolipid structure



Scheme 1. Comparative study of catalysts in Biginelli reaction

Table 4. Biginelli reaction tested with the plant catalysts and commercial NiCl_2 applied to Monastrol model example

Catalyst	Ratio Ni/ArCHO	Ratio (Al + Mn + Fe)/ArCHO	Conversion %	Yield %
NiCl_2	0.25	-	30	11
<i>G. pruinosa</i> catalyst	0.25	0.014	92	72
<i>P. douarrei</i> catalyst	0.25	0.014	95	83

Currently, these data constituted unusual observations and complementary information about sulfate assimilation with the

discovery of a new sulfatolipid in vascular plants, the 3,4-dihydroxy-tridecanesulfate (figure 2).

Application in green organic synthesis

The original composition of the *P. douarrei* catalyst prompted to us to investigate how this mixture could initiate Lewis acid catalyzed reactions. We wish to give an illustrative example, which culminated in a three-component reaction, the Biginelli reaction leading to dihydropyrimidinones. Recently dihydropyrimidinones have been the object of an increased interest, as these molecules exhibit exciting biological features like modulating calcium channels, selectively inhibiting α_{1a} adrenoreceptor and targeting the mitotic machinery [21,22].

Under optimized conditions, the plant catalyst was dispersed on montmorillonite K10. The amount of Ni in the final solid reached a maximum value of 9.05 wt% and Cl:Ni molar ratio of 1:4.

The supported catalyst (25% mol Ni /aldehyde), the substrate and the reagent were mixed thoroughly and stirred at 80°C under solvent-free conditions for 12 h. According to our proposal, the *P. douarrei* catalyst promoted the reaction between 3-hydroxybenzaldehyde, ethyl 3-ketopentanoate and thiourea in a one-pot protocol. After recrystallization, the pure expected heterocycle (ethyl 6-methyl-4-(3-hydroxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (monastrol) [22] was obtained with a high yield (83%) (Scheme 1).

The same reaction was carried out with *G. pruinosa* catalyst dispersed on montmorillonite K10 and commercial NiCl_2 as catalysts.

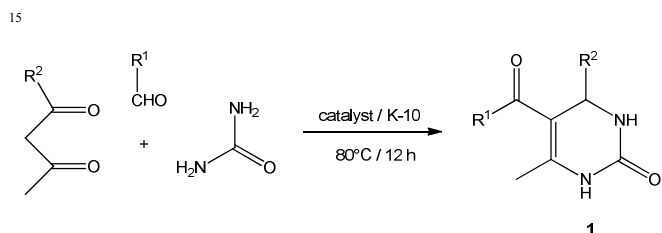
As expected, the use of plant catalysts was more efficient than the use of commercial NiCl_2 . An efficient catalysis of Biginelli MCR was observed with an over 90% conversion rate, while NiCl_2 showed a low catalytic efficiency (30%). Since experimental conditions and mole ratio Ni/ArCHO were identical, it could be concluded that the efficiency difference is due to the polymetallic composition of the plant catalysts. More interestingly, yields were also higher with the plant catalysts. It clearly led to lower yields 11% and the purification of monastrol was very difficult because of the strong association between Ni^{II} and sulfur of the dihydrothiopyrimidinone. This issue was not observed with *P. douarrei* or *G. pruinosa*. The conversion was total and the heterocycle crystallized easily and gave pure crystals.

G. pruinosa catalyst gave good yield although lower than *P. douarrei* catalyst. As can be seen in Table 4, the Ni / ArCHO mole ratio and the (Al + Mn + Fe) / ArCHO mole ratio used in the Biginelli reaction were similar. The only difference was the presence of Ni^{III} in *P. douarrei* catalyst. Therefore it can be assumed that Ni^{III} might be a better catalyst than Ni^{II} in this MultiComponent Reaction.

We assumed that in Ni-hyperaccumulating plant catalysts, a small amount of Ni^{II} formed NiCl_2 , while the major amount of Ni^{II} constituted other associations such as KNiCl_3 (see paragraph 2). These unique associations allowed a slow release of NiCl_2 , which limited the concurrent association with the sulfur of the dihydrothiopyrimidinone.

To check the applicability of these catalysts, the reaction was extended to other substrates with the best plant catalyst, *P. douarrei* catalyst (Scheme 2). Results are presented in table 5. To

demonstrate the general applicability of *P. douarrei* catalyst, a range of components had been studied. The yields were high in all cases tested. *P. douarrei* catalyst promoted Biginelli reaction with β -ketoester and β -dione, thiourea and urea. Additionally, high yields were obtained with aromatic as well as aliphatic aldehydes. In contrast with literature [32], it should be noted that good results were obtained with aliphatic aldehydes. The usual side reaction, such as enolization and aldol reaction of these substrates were limited with *P. douarrei* catalyst. This catalyst was composed of a small amount of NiCl₂; KNiCl₃ was the major form of Ni salt, which is a Lewis acid storage catalytic converter in solution. This result reflected the softness of *P. douarrei* catalyst.



Scheme 2. Biginelli reaction catalyzed by *P. douarrei* catalyst

Table 5. Synthetic Potential of *P. douarrei* catalyst through Biginelli reaction

R ¹	R ²	Product	1 Yield %	Ref. ^a
	EtO		87	[23]
	EtO		83	[23]
	EtO		91	[24]
	EtO		75	[25]
	EtO		72	[26]
	Me		79	[23]
	Me		71	[27]
	Me		81	[28]
H	OEt		61	[29]
(CH ₃) ₂ CHCH ₂	OEt		96	[23]
CH ₃ (CH ₂) ₈	OEt		76	[30]
	OEt		95	[31]
	OEt		74	this work

^a ¹H and ¹³C NMR characterisations were consistent with data published in cited references.

P. douarrei and *G. pruinosa* based catalysis constituted a rapid and simple synthesis of dihydrothiopyrimidinone and dihydropyrimidinone. The reaction is general and has been extended to various substrates. To our knowledge, this was a cutting-edge example for a greener sustainable chemistry. With these experimental results, we could show the relevance of endemic plants. Their efficiency as catalysts in organic synthesis therefore justifies the development of their culture in phyto restoration. Developing the culture of these rare plants in order to use them in organic synthesis would contribute to their safeguard. Green Chemistry is thus an opportunity to preserve biodiversity, valuing rare species.

Experimental

Procedure for chemical analysis

X-ray diffraction (XRD) data measurements on the samples dried at 110°C for 2 hours were performed by using a BRUKER diffractometer (D8 advance, with a Cu K α radiation $\lambda=1.54086$ Å) equipped with a Lynxeyes detector.

Chemical analysis of the plant catalyst samples after calcinations (1000°C for 3 h) was performed by X-Ray Fluorescence spectrometry (XRF) using a BRUKER AXS S4 Explorer wavelength-dispersive spectrometer. The quantitative analysis of major and expected elements was performed on beaded samples for overcoming problems of particle size variation as well as mineralogy effects: the powdered sample is mixed with a Li₂B₄O₇ flux with a flux / sample ratio equal to 8, heated in a crucible between 900-1200 °C, then cast in a platinum dish to produce a homogeneous glass-like bead.

Extractions of lipids had been carried out according to Folch *et al.* [19,20].

The anion exchange chromatography was carried out in the following conditions. The samples were prepared by dissolution of *Geissois pruinosa* extract (25.7 mg) and of *Psychotria douarrei* extract (26.4 mg) in ultrapure water (18.2MW) and 50 μ L HNO₃. A complete dissolution was obtained after ultrasonic activation. This solution is completed to 250 mL with ultrapure water. The analysis was performed with 882 Compact IC Metrohm apparatus equipped with a chemical suppressor, CO₂ suppressor and a conductivity detector.

Conditions: Metrosep A Supp 5 - 250/4.0 column; Elution: Na₂CO₃ (3.2mM) / NaHCO₃ (1mM), rate of flow: 0.7 ml.min⁻¹; calibration: standard solution standard of Alfa Aesar (reference 041693) F⁻, Cl⁻, Br⁻, NO₃⁻, PO₄³⁻, SO₄²⁻ (100 μ g.mL⁻¹). Concentrations were calculated from peak areas.

Electrospray ionization mass spectrometry (ESI-MS) was performed with a Waters Alliance e2695 Chain coupled to a Quattro Micro mass spectrometer and a PDA 996.

High resolution electrospray ionization mass spectrometry (HR-ESI-MS) was acquired in negative ion mode and recorded on a hybrid quadrupole-time of flight instrument Micromass Q-TOF (Waters) by direct infusion of the sample diluted in methanol, with a syringe pump at a flow rate of 1 mL/min. Conditions: capillary voltage 3000 V; dry gas temperature, 120 °C; dry gas

flow, 400 L.h⁻¹ and nitrogen as nebulizer gas. 0.1% phosphoric acid was used as standard for internal calibration.

IR spectra were recorded on a PerkinElmer Spectrum 100 FT-IR spectrometer, in ATR mode. NMR spectra were recorded on a BRÜKER Avance 300, at room temperature.

Procedure for the synthesis of ethyl 6-methyl-4-(3-hydroxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (monastrol).

A mixture of ethyl acetoacetate (781 mg, 6.0 mmol), 3-hydrobenzaldehyde (488 mg, 4.0 mmol), thiourea (457 mg, 6.0 mmol) and *P. douarrei* crude catalyst (295 mg, amount corresponding to 1.0 mmol of nickel following previous dosing), supported on montmorillonite K10 (300 mg) was placed in a 10 mL sealed tube. The tube was heated to 80°C in oil bath, under magnetic stirring for 12 h. The mixture was then extracted with hot ethanol (10 mL, 70°C) and filtered in order to remove the catalyst, which was reactivated by heating (150°C). The solution was poured into crushed ice (20 g) and stirred for 20 min. The solid separated was filtered under suction, washed with cold water (30 mL) and recrystallized from hot ethanol, affording pure product, as colorless crystals (973 mg, 83%). The same procedure was followed with *G. pruinosa* catalyst and commercial NiCl₂. Mp 185-186°C (lit. [22] 184-186°C); IR 3298, 3181, 3115, 2982, 1663, 1617, 1573 cm⁻¹; ¹H NMR (DMSO-d₆, 300 MHz) δ : 1.14 (t, *J*=7.4 Hz, 3H), 2.29 (s, 3H), 4.04 (q, *J*=7.4 Hz, 2H), 5.11 (d, *J*=3.5 Hz, 1H), 6.60-6.71 (m, 3H), 7.06-7.15 (m, 1H), 9.42 (brs, 1H), 9.62 (brs, 1H), 10.29 (brs, 1H); ¹³C NMR (DMSO-d₆, 75 MHz) δ : 14.0, 17.1, 54.2, 59.6, 100.8, 113.0, 114.4, 117.0, 129.3, 144.8, 144.9, 157.4, 165.4, 174.2. MS (EI+) calculated for C₁₄H₁₆N₂O₃S [M]⁺ 292.1, found 293.1 [M+H]⁺.

Conclusion

The inorganic composition of the extract and catalyst derived from the best Ni hyperaccumulating plant, *P. douarrei*, was revisited by specific analytical techniques. XRF analyses confirmed the exceptional ability of *P. douarrei* to store Ni in its shoots. XRD analyses revealed the presence of a rare Ni oxidation state, Ni^{III} or Ni^{IV}, for the first time in a living organism. XRF analyses followed by an anion-exchange chromatography disclosed a lack of phosphorus linked to a lack of phosphates filled by an excess of sulfur linked to an excess of sulfates in *P. douarrei* catalysts. The excess of sulfates was found in the membrane lipids of *P. douarrei* as sulfatolipids. Among the membrane lipid extract, a new sulfatolipid had been discovered, the 3,4-dihydroxy-tridecanesulfate.

From this exceptional composition, *P. douarrei* was used as a new catalyst in a MultiComponent Reaction of increasing importance in organic and medicinal chemistry, the Biginelli reaction. This plant-based catalyst led to higher yields in greener conditions than commercial NiCl₂.

Metallophytes can be the starting point of a novel plant-inspired metallo-catalytic platform for synthesis of biologically interesting molecules, and finally should contribute to develop a new concept of phytochemistry.

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Notes and references

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