Phytochemistry, 1980, Vol. 19, 1963–1965. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0901-1963 $02.00/0

NATURE OF NICKEL COMPLEXES IN *PSYCHOTRIA DOUARREI* AND OTHER NICKEL-ACCUMULATING PLANTS

WILLIAM J. KERSTEN,* ROBERT R. BROOKS,* ROGER D. REEVES* and TANGUY JAFFRÉ†

* Department of Chemistry, Biochemistry and Biophysics, Massey University, Palmerston North, New Zealand; † O.R.S.T.O.M., Abidjan, Ivory Coast

(*Received* 12 *November* 1979)

Key Word Index—*Casaeria*; Flacourticeae; *Psychotria*; Rubiaceae: *Phyllanthus*: Euphorbiaceae; nickel accumulation; nickel–citrate complexes; nickel–malate complexes.

Abstract—The nature of nickel complexes in various Ni accumulating plant species, mainly from New Caledonia, was investigated by techniques including gel chromatography, ion-exchange chromatography, high performance liquid chromatography, and a combination of gas–liquid chromatography and mass spectrometry. *Psychotria douarrei* contained Ni complexed mainly (63%) as a negatively-charged malate complex balanced by a cationic aquo complex. *Phyllanthus serpentinus* had anionic Ni bound as 42% citrate and 40% malate. All other species studied, contained Ni as an anionic citrate complex.

INTRODUCTION

Phytochemical studies on several Ni-accumulating plants (>1000 μg/g dry wt) from New Caledonia [1, 2] have led to the isolation of Ni-containing extracts in which most of the Ni exists as an anionic citrato-nickelate(II) complex. Other workers [3, 4] have found that the Ni complex in the Ni accumulator *Alyssum bertolonii* Desv. involves malic acid. Several workers [5–10] have noted increased synthesis of malic, citric and other organic acids in plants accumulating excessive amounts of various cations and Mathys [11] found large differences in the malic acid content between Zn-resistant and Zn-sensitive plants, the Zn-resistant ecotypes containing much higher amounts of this acid.

Although the New Caledonian 'nickel plant' *Psychotria douarrei* (G. Beauvisage) Däniker, contains citric acid, it has been noted [12, 13] that most of the Ni in extracts of this species is in a previously unidentified form originally supposed to be $Ni(H_2O)_6^{2+}$ from its position in the elution train of a Sephadex G-10 column. Because of this anomalous behaviour of *P. douarrei*, a special investigation was made of the nature of its Ni complex. This work was combined with a study of other Ni hyperaccumulators from Oceania and is reported in this paper. The following species were studied: New Caledonia—*Casearia silvanae* (J. R. et G. Forster) H. Sleumer (Flacourtiaceae) [14]; *Lasiochlamys peltata* H. Sleumer (Flacourtiaceae) [14]; *Phyllanthus serpentinus* Moore (Euphorbiaceae) [15]; *Psychotria douarrei* (G. Beauvisage) Däniker (Rubiaceae) [16]; *Xylosma vincentii* Guillaumin (Flacourtiaceae) [14]; Western Australia—*Hybanthus floribundus* F. Muell. (Violaceae) [17]; New Guinea—*Rinorea bengalensis* (Wall.) O. K. (Violaceae) [18].

RESULTS AND DISCUSSION

Although the eluate of an aqueous extract of leaves of *P. douarrei* passed through a Sephadex G-10 column indicated the presence of a small citrate–complex peak and a single large peak of lower MW [Fig. 1(a)], closer inspection showed a shoulder corresponding to a second peak within the latter. When the eluate was passed through a weakly acidic cation exchanger, the unabsorbed material gave the usual citrate peak and a single sharp peak when passed again through a G-10 column [Fig. 1(b)]. When the absorbed ions were eluted with 1.5 M hydrochloric acid and passed through the G-10 column, another sharp peak corresponding to $Ni(H_2O)_6^{2+}$ [Fig. 1(c)] was obtained. This indicates that a small proportion of the Ni in *P. douarrei* is bound to citrate but a much larger proportion is found: (i) in association with another ligand, and (ii) as the positive aquo complex. The eluate from the cation exchange column was passed through a phosphate-buffered (pH 2.8) HPLC column. A UV absorption detector (220 nm) showed that the major peak corresponded in rentention time to malic acid. This peak was further confirmed as being due to malic acid by adding this acid to the sample and passing it again through the column. No additional peak was observed.

The identity of the organic ligand was finally confirmed by GC–MS. The ligand was methylated and passed through a GC column. The MS of the effluent confirmed the identity of the small peak as citrate and of the large peak as malate, with only minor impurities. These peaks were readily identified from their R_t and from comparison of their MS with those of pure methylated standards. The amounts of Ni passing through the G-10 column in association with malate and citric respectively were in the ratio of *ca* 4:1 [Fig. 1(b)].

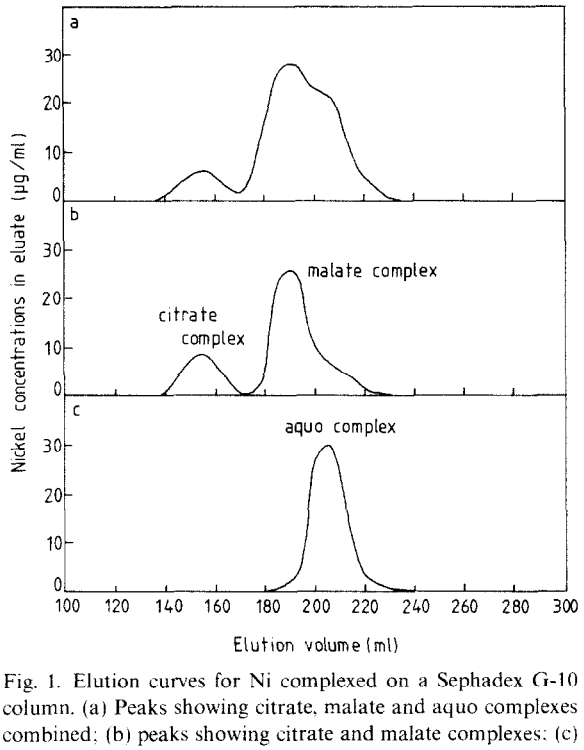

Fig. 1. Elution curves for Ni complexed on a Sephadex G-10 column. (a) Peaks showing citrate, malate and aquo complexes combined; (b) peaks showing citrate and malate complexes; (c) peak showing aquo complex alone.

Table 1 lists the results of studies on *P. douarrei* and other hyperaccumulators of Ni. In all species except *Phyllanthus*, the Ni was bound as a citrate complex. Only in the case of *P. serpentinus* were traces of malic acid found in Ni-containing extracts from the G-10 column. However, in this case, the ratio of Ni in the two fractions was only 1:1 instead of 4:1 as in the case of *P. douarrei*.

Our results confirm that, among the New Caledonian Ni-accumulating plants, binding of Ni as a citrate complex predominated. Including, the present data, the nature of the Ni complexes has now been studied in 24 plants. Of these species, all except four (*Alyssum bertolonii* [2–4], *A. serpyllifolium* s. sp. *lusitanicum* [2], *Pearsonia metallifera* [2], and *Psychotria douarrei*) involve a citrate complex. Aqueous extracts of the remainder (except *P. metallifera*) have contained malate as the major ligand associated with Ni. The Ni-binding ligand in *Pearsonia metallifera* is as yet unidentified. In general Ni hyperaccumulators complexing with citric acid tend to be species from relatively primitive families such as the Flacourtiaceae which contains at least 19 Ni plants [19]. This family is the second most primitive of all flowering plants if the Sporne Index [20] is accepted as a criterion (see Table 1).

Despite the work presented in this paper, much work still remains to be done on the phytochemistry of Ni plants, particularly as regards the site of formation of these organic acid complexes with Ni.

EXPERIMENTAL

Extraction of complexes. Extraction of freeze-dried leaf material was carried out with H_2O at room temp. Some 50–70% of the total Ni could readily be extracted under these conditions. The extracts were shaken with $CHCl_3$–*n*-BuOH (10:1) to remove high MW compounds of low polarity. Negligible amounts of Ni were lost in this process.

Chromatography. The aq. soln was reduced in vol. and passed through a Sephadex G-10 column. H_2O was used to elute the column. Fractions were analysed for Ni by atomic absorption spectrophotometry and were in some cases taken to dryness in a rotary evaporator for further processing. Separation of the negatively charged Ni–malate complex was carried out by passing the aq. extract through a column of Amberlite IRC 50 weak cation exchange. $Ni(H_2O)_6^{2+}$ was removed by elution with 1.5 M HCl. Separation of organic acids was carried out on a Waters HPLC instrument using a tetrabutylammonium phosphate buffer (pH 2.8) and a Microbondapak C-18 column. At pH 2.8, the Ni was separated from the ligand and appeared as a separate peak early in the elution train.

GC–MS. The purified aq. extract was methylated with CH_2N_2 using the method of ref. [21]. A GC instrument (180°, 4 mm × 2.8 m glass column packed with 3% SP 2340 on Supelcoport) was connected to a medium resolution MS with a source temp. of 240°.

Table 1. Nickel complexes in hyperaccumulators of nickel

Species	Tests used S	H	G	M	μg/g Ni (dry wt)	Family	Advancement index [19]	Form of Ni (%) Aquo	citrate	malate
*Alyssum bertolonii**	X	·	X	X	5000	Cruciferae	63	not known		
Casearia silvanae	X	·	X	X	1490	Flacourtiaceae	22	33	67	—
Hybanthus floribundus	·	·	X	X	1300	Violaceae	42	5	95	—
Lasiochlamys peltata	X	·	X	X	1000	Flacourtiaceae	22	33	67	—
Phyllanthus serpentinus	X	·	X	X	38 100	Euphorbiaceae	30	18	42	40
Psychotria douarrei	X	X	X	X	13 400	Rubiaceae	70	21	16	63
Rinorea bengalenis	X	·	X	X	5000	Violaceae	42	7	93	—
Xylosma vincentii	X	·	X	X	3750	Flacourtiaceae	22	36	64	—

S: Sephadex G-10 column.
H: HPLC.
G: GC.
M: MS.
* Not part of this study. Data from ref. [3].

Determination of Ni. Samples of freeze-dried leaf material were ashed at 500° and the residue redissolved in 2 M HCl for analysis by atomic absorption spectrophotometry. Eluate fractions were analysed directly without pretreatment.

Acknowledgements—We gratefully acknowledge the assistance of Mr. C. A. Bishop with HPLC and Dr. K. G. Couchman with MS.

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