Antioxidant Activity of an Unusual 3-Hydroxyindole Derivative Isolated from Fruits of *Aristotelia chilensis* (Molina) Stuntz

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3-Hydroxyindole was isolated from the EtOH extract of fruits of *Aristotelia chilensis* during analyses by HPLC/MS and GC/MS and identified by its mass fragmentation pattern and spectrophotometric data. Additionally, this extract showed an interesting antioxidant activity in DPPH, crocin and TBARS assays. The presence of this type of compound in this fruit species permits us to explain its strong antioxidant activity and its important part in the biosynthetic pathway of phenolic and alkaloid compounds in this plant. Therefore this compound could be useful for the development of future nutraceutical and antioxidant protective agents.

Key words: Hydroxyindole, Antioxidant Activity, *Aristotelia chilensis*

Introduction

Anthocyanins, flavonoids and phenolic acids are compounds which occur very often in many plant families. Their different biological activities, including antioxidant, antimicrobial, carcinogenic, cytotoxic, anti-inflammatory and mutagenic properties, make them interesting objects of research (Middleton and Kandaswami, 1993). Recently, attenuation has been payed to the dietary indole amines melatonin and serotonin in different plant foods, including grapes and berries, thus further supporting the hypothesis that health benefits associated with Mediterranean dietary style are due to plant food chemical diversity (Iriti and Faoro, 2008). The presence of hydroxyindole in fruits tissues is relevant for their important biological activities. Additionally, the importance of indole moieties in the biosynthesis of phenolics is documented (Applewhite *et al.*, 1994).

We have under continuous study different extracts of edible fruits of southern Chile. Recently, we have published the antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean black berry (Céspedes *et al.*, 2008), which have traditionally been consumed for the treatment of diarrhea and dysentery. Previously, we have reported the alkaloid composition of the leaves of *Aristotelia chilensis* (Céspedes *et al.*, 1993; Silva *et al.*, 1997). There are some reports about the antioxidant activity and a partial composition of anthocyanidins (Miranda-Rottman *et al.*, 2002; Escribano-Bailon *et al.*, 2006).

In continuation of our search for more specific antioxidant activities, in the present paper we report the antioxidant activity of an unusual 3-hydroxyindole derivative and of a very interesting fraction obtained during a metabolomic analysis of fruits of *A. chilensis*. The occurrence of 3-hydroxyindole helps us to explain the antioxidant, chemopreventive, cardiopreventive, and anti-inflammatory activities of this fruit.

Material and Methods

Plant material

Plants and fruits of *Aristotelia chilensis* (Molina) Stuntz (Elaeocarpaceae) were collected from fields at the foothills of Los Andes Mountains (900 m, 38°38'57'' S, 71°52'32'' W) on a track at the shore of "Quepe" lake, very near to "Conguillio" National Park, Cherquenco district, near Temuco City, Chile, in January 2006. The samples were identified botanically by Professor Fernando Perich, M. Sc. (Department of Chemistry Sciences, University of La Frontera, Temuco, Chile) and voucher specimens (voucher: R. Rodríguez and C. Marticorena) were deposited at the herbarium (CONC) of Departamento de Botánica, Facultad de Ciencias Naturales y Oceanograficas, Universidad de Concepción, Concepción, Chile. The collected plants were air-dried and prepared for extraction.

Isolation procedures

Fruits were separated in their main morphological parts (seed and pulp); the pulp was dried and

then milled and extracted with ethanol (containing 0.1% HCl). The EtOH extract was partitioned with acetone and ethyl acetate according to Céspedes *et al.* (2008). By flash-column chromatography three main fractions (*n*-hexane, CH_2Cl_2, and ethyl acetate) were obtained. The ethyl acetate fraction was analyzed by TLC using an antioxidant bioautographic assay against DPPH (Domínguez *et al*., 2005). From this fraction eleven active subfractions were isolated by HPLC. Then, the most active fraction (F-11) was analyzed by HPLC and GC/MS from which an active compound was obtained that was identified from its fragmentation patterns as 3-hydroxyindole. Its retention times in HPLC and GC, and spectroscopic data (MS, ^{1}H NMR, ^{13}C NMR and UV) compared with those of an authentic sample.

3-Hydroxyindole: ^{1}H NMR (MeOD): δ = 6.36 (d, H-2), 6.84 (dd, H-7), 6.86 (dd, H-7), 7.19 (t, H-5), 7.16 (t, H-5), 7.17 (t, H-5), 7.29 (t, H-6), 7.31 (t, H-6), 7.32 (t, H-6), 7.81 (t, H-4), 7.83 (t, H-4), 8.59 (s, broad signal, OH and NH). – MS: *m/z* (rel. int.) = 133 (21) $[M]^{+\cdot}$, 127.9 (18), 113.9 (27), 103 (30), 101.9 (37), 84 (46), 72.9 (44), 57 (100), 44 (94), 32 (85).

Antioxidant activity

3-Hydroxyindole, extracts and fractions of *A. chilensis* were examined for their antioxidant effects according to Céspedes *et al.* (2008) and Domínguez *et al*. (2005).

Results and Discussion

The electron impact fragmentations of indole, hydroxyindole and oxindol have been extensively investigated and documented (Powers, 1968; Marchelli *et al*., 1971; Evans *et al*., 1990). The fragmentation pattern shows the importance of the *m/z* 133 $[M]^{+\cdot}$ peak for these compound types and their following signals at *m/z* 131 ($[M]^{+\cdot}$ – 2H), *m/z* 103 (131 – CO), *m/z* 76 (103 – HCN), *m/z* 114 (132 – H_2O), *m/z* 84 (114 – H_2N^+=CH_2). Interestingly, *m/z* 114 and *m/z* 115 (132 – H_2O and 133 – H_2O, respectively) indicate that the water loss must be necessarily in an 3-hydroxyindole to produce $[C_8H_5N]^{+\cdot}$ (*m/z* 115) (Evans *et al*., 1990), after *m/z* (114 – HCN) gives *m/z* 87 and the fragment $-C_2H_2$ to produce the fragment *m/z* 61. Furthermore, *m/z* 57 ($[C_2H_4NO]^{+\cdot}$) (100%) is generated by the fragmentation of (C_2H_3NO), *i.e.* the loss of –NH–CH=CHOH. Thus *m/z* (133 – 57) yields *m/z* 76 ($[C_6H_4]^{+\cdot}$), and *m/z* (131 – 57) yields *m/z* 74. Furthermore, the fragments *m/z* 29 ($[H_2N{=}CH]^{+\cdot}$), *m/z* 30 ($[H_2N{=}CH_2]^{+\cdot}$), *m/z* 31 ($[CH_2{=}OH]^{+\cdot}$), and *m/z* 44 ($[CH_2NHCH_3]^{+\cdot}$) correspond to typical fragmentations of these indole compounds. Finally, the study of these fragmentations, UV and NMR spectral data and comparison with those of an authentic sample led us to a 3-hydroxyindole. All fragmentations are shown in Fig. 1.

The DPPH radical scavenging assay was first used as a screen for antioxidant components within the primary extracts (Céspedes *et al.*, 2008). After HPLC analyses of the ethyl acetate extract fraction F-11 obtained at 24.5 min was collected and evaluated using the antioxidant assays. F-11 exhibited a concentration-dependent radical scavenging activity, particularly it showed a high activity (>86.8% inhibition) at 5.9 ppm with IC_{50} values of 1.27 and 1.98 ppm against DPPH and crocin, respectively. On the other hand, 3-hydroxyindole showed lower IC_{50} value than α-tocopherol, which at 31.6 ppm caused only 53.8% quenching against DPPH. This fraction was further analyzed by GC/MS where 3-hydroxyindole was identified with IC_{50} values of 0.87, 0.67 and 1.29 ppm against DPPH, crocin and TBARS, respectively.

Of the many biological macromolecules, including carbohydrates, lipids, proteins, and DNA, that can undergo oxidative damage in the presence of reactive oxygen species (ROS), membrane lipids are especially sensitive to oxidation by physiological processes. For this reason, brain homogenate was used for the investigation of lipid peroxidation as an assessment of oxidative stress (Ko *et al*., 1998). F-11 was more effective than an MeOH extract, used as pattern solution, and quercetin or tocopherol in inhibiting lipid peroxidation (TBARS) showing an IC_{50} value of 1.77 ppm. Thus, this fraction reduced lipid peroxidation in a dose-dependent manner, and proved to be an excellent antioxidant, reflected by its low IC_{50} values when analyzed by both TBARS and DPPH assays.

Halliwell and Aruoma (1991) have defined antioxidants as substances that, when present at low concentrations compared with an oxidizable compound (*e.g.* DNA, protein, lipid, or carbohydrate), delay or prevent oxidative damage due to the presence of ROS. These ROS can undergo a redox reaction with phenolics, such that the oxidant activity is inhibited in a concentration-

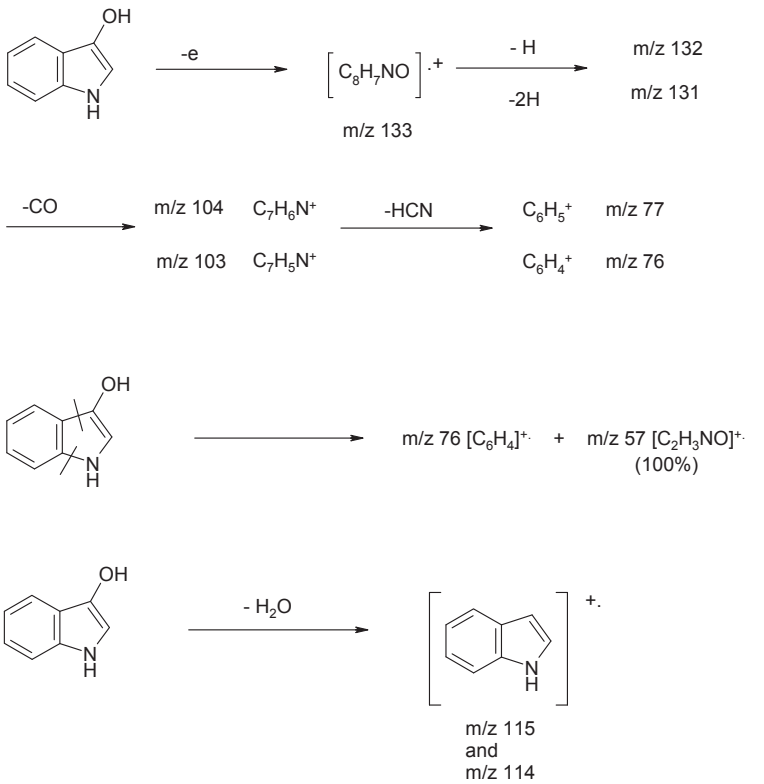

Fig. 1. Mass fragmentations of 3-hydroxyindole.

dependent manner. In the presence of low concentrations of phenolics or other antioxidants, the breaking of chain reactions is considered to be the predominant mechanism (Pokorny *et al.*, 1988), and phenolics have been suggested to be the most active substances from natural sources (Rice-Evans, 2000). Thus, we measured the total phenolic content of the fraction, which had the greatest DPPH and TBARS activities. We found that it had a significantly higher phenolic content than other fractions [(18.987 ± 755.9) μmol catechin equiv./g extract] (Céspedes *et al.*, 2009). These findings correlate well with our last findings (Céspedes *et al.*, 2008). Since this fraction had the greatest activity against TBARS formation, this can also be attributed to the occurrence of the 3-hydroxyindole derivative in this fruit.

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