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Two novel betaine derivatives from Kancolla seeds (Chenopodiaceae)

Irene Dini, Gian Carlo Tenore, Elena Trimarco, Antonio Dini *

Universita Federico II, Facolta di Farmacia Universita, Dipartimento di Chimica delle Sostanze Naturali, via Domenko Montesano, 49, Napoli 80131, Italy

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Abstract

Analysis of the polar extracts from kancolla seeds led to the isolation of five betaines: glycine betaine, trigonelline, trigonelline methylester, trigonelline glucosylester and 3-carboxy-1-(2-sulfoethyl)-pyridinium, the last two of which have not previously been reported in the literature. All structures were elucidated from spectroscopic [NMR (^{1}H, ^{13}C, COSY, HOHAHA, HMQC, HMBC)] and mass spectrometric data (ESI-MS).

Keywords: Kancolla; *Chenopodium quinoa*; Betaines; Trigonelline; Trigonelline methylester; Trigonelline glucosylester; 3-Carboxy-1-(2-sulfoethyl)-pyridinium

1. Introduction

Kancolla is a sweet variety of *Chenopodium quinoa* Willd. (quinoa) (Chenopodiaceae family), used as a food plant, principally in the same way as wheat and rice. It is a highly nutritious food and the main edible parts are the seeds (Koziol, 1992). Kancolla is known as a pseudo-cereal, recently rediscovered by agricultural researchers of industrialized societies (Popenoe, King, Leòn, & Sumarkaunowsk, 1989; Schlick & Bubenheim, 1993) and selected for its tolerance to heat, cold, and resistance to disease. In this study, we have surveyed the betaines in kancolla seeds, because these compounds are widely accumulated in stressed plants. Particularly, we have evaluated the presence of glycine betaine, trigonelline and their derivatives.

In mammals, glycine betaine acts as an osmolyte in the inner medulla of the kidney, preserving osmotic equilibrium, while also maintaining the tertiary structure of macromolecules (Yancey & Burg, 1990; Yancey & Somero, 1979). In humans, glycine betaine can be readily absorbed through dietary intake or endogenously synthesised through the catabolism of choline in the liver (Flower, Pollitt, Sanford, & Smyth, 1972). The concentration of glycine betaine in human plasma is highly regulated (Chambers & Lever, 1996), although concentrations are lower in patients with renal disease, and urinary excretion is elevated in patients with diabetes mellitus (Dellow, Chambers, Lever, Lunt, & Robson, 1999). Glycine betaine is also an important source of methyl groups, required for the formation of methionine and *S*-adenosylmethionine (Barak, Beckenhauer, & Tuma, 1996; Chambers & Lever, 1996). Glycine betaine intake can lower plasma homocysteine levels in patients suffering from homocystinuria (Wilken, Wilken, Dudman, & Tyrrell, 1983), and in chronic renal failure patients with hyperhomocysteinemia (McGregor et al., 2002), as well as in healthy subjects (Brouwer, Verhoef, & Urgert, 2000). Homocysteine is derived through the metabolism of methionine, and has been recognised as an independent risk factor for the development of vascular disease (Hankey & Eikelboom, 1999; Wilken & Gupta, 1979). In a reaction catalysed by betaine–homocysteine methyltransferase, a methyl group is transferred from glycine betaine to homocysteine, pro-

* Corresponding author. Tel.: +39 81 678535; fax: +39 81 678552.
E-mail address: andini@unina.it (A. Dini).

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ducing methionine and *N*,*N*-dimethylglycine (Malinow, 1994). Trigonelline (nicotinic acid betaine), instead, is the *N*-methyl conjugate of nicotinic acid. Trigonelline is considered to be an important multifunctional natural plant hormone with potential taxonomic value, generally present in herbaceous species of saline and dry habitats. It has a function as a cell cycle regulator during the early growth of many legume root meristems (Tramontano & Jouve, 1997), and it is also one of the secondary messengers in plant cells under stress, preventing oxidative stress caused by UV-B light (Kalbin, Ohlsson, Berglund, Rydstrom, & Strid, 1997) or preventing water loss (Tramontano & Jouve, 1997) and has been shown to stabilize enzyme activity in vitro (Shomer-Ilan, Jones, & Paleg, 1991).

In recent years, as the market for functional ingredients and foods has grown, betaines have been actively investigated for their health-promoting potential. They may have a role as a possible therapy in lowering the plasma concentration of homocysteine in humans, which at high levels has been shown to increase incidence of vascular disease (Hankey & Eikelboom, 1999). Initial clinical studies, conducted on patients with elevated serum homocysteine levels, revealed that glycine betaine successfully lowers serum homocysteine (Wilken et al., 1983). In addition, several human clinical studies are available, to date, which discuss the benefits of glycine betaine supplementation at levels ranging from 2 to 20 g/day, typically at 6 g/day. Other investigators studied glycine betaine metabolism in animals, including its role in the methionine cycle and the potential of glycine betaine to protect the liver from ethanol-induced liver injury and non-alcoholic steatohepatitis (Barak et al., 1996). For consumer safety, it is also important to determine the presence of this compound in new foods and to evaluate the potential hazard for human health. In fact, the toxicological effects of trigonelline have not yet been studied and, considering its multiple activities in plants, there is a need to investigate its potential effects on human health (Rozan, Kuo, & Lambein, 2000), and also because cooking seems to have no appreciable influence on the betaine content of foods. No significant losses were observed during baking, microwaving and frying, and only small to medium losses were observed during steaming although, during boiling, large losses occurred (60–80%) because the betaines pass into water. Glycine betaine, particularly, was found to be heat-stable at 220 °C, and only small losses occurred (<14%) after 30 min at 250 °C (de Zwart et al., 2003). Some special diets are also likely to exclude major sources of betaines. For example, patients with coeliac disease are likely to have a lower than average glycine betaine intake because of the lack of wheat products in their diet, while vegetarians are likely to have higher than average intakes of trigonelline because they generally consume higher amounts of legumes (chickpeas, lentils).

2. Materials and methods

2.1. Plant material

Kancolla seeds were collected in Peru and identified by Dr. S.E. Jacobsen of the International Potato Centre (CIP), Lima, Peru. A sample used has been deposited in the Herbarium Neapolitanum of the Dipartimento di Biologia Vegetale Università degli Studi "Federico II" of Naples. The collection number was NAP # A. C. 002.

2.2. Extraction and isolation of betaine analogues

2.2.1. General

The whole flour from the seeds (709 g) was extracted with MeOH. The MeOH extract (49.0 g) was partitioned between *n*-BuOH and H_2O. The *n*-BuOH extract (26.2 g) was evaporated and defatted with $CHCl_3$. The residual fraction (10 g) was chromatographed on a Sephadex LH-20 column (100×5 cm), with MeOH as eluent. Fractions (9 mL) were collected and checked by TLC [Si-gel plates in *n*-BuOH/HOAc/H_2O (60:15:25)]. Fractions 1–36 (1.21 g) were further separated on a silica gel column using $CHCl_3$/MeOH/H_2O (15:5:0.6) as eluent. Fractions 41–50 (28.5 mg) and fractions 86–189 (62.6 mg) were chromatographed by reverse phase HPLC with MeOH/H_2O (40:60) to yield the pure compounds glycine betaine (17.8 mg; $R_t = 3.8$ min), trigonelline (9.0 mg; $R_t = 5.4$ min), trigonelline methylester (3 mg; $R_t = 5.4$ min), trigonelline glucosylester (3 mg; $R_t = 6$ min) and 3-carboxy-1-(2-sulfoethyl)- pyridinium (4.5 mg; $R_t = 6.2$ min).

2.2.2. High performance liquid chromatography (HPLC)

HPLC separations were performed on a Hewlett–Packard HP 1050 series apparatus with a Varian RI-4 refractive index detector, equipped with a Waters μ–Bondapack C-18 column (7.8×300 mm), flow rate 2.5 ml/min.

2.3. Characterization of betaine analogues

2.3.1. General

All structures were elucidated by spectroscopic [NMR (^1H, ^{13}C, COSY, HOHAHA, HMQC, HMBC), IR and UV] and mass spectrometric methods.

2.3.2. NMR

^1H and ^{13}C NMR spectra were recorded at 500 MHz, on a Bruker AMX-500 spectrometer. Chemical shifts were referred to the residual solvent signal (CD_3OD: δ H 3.34, δ C 49.0). The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. The DEPT experiments were performed