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Two novel betaine derivatives from Kancolla seeds (Chenopodiaceae)

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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-

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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

with a pulse of 135° to obtain positive signals for CH and CH_3 and negative signals for CH_2; an average CH coupling constant of 135 Hz was assumed. ^1H connectivities were determined by using COSY and HOHAHA experiments; the 2D HOHAHA experiments were performed in the phase-sensitive mode (TPPI) using the MLEV-17 (mixing time 125 ms) sequence for mixing. One-bond heteronuclear ^1H–^{13}C connectivities were determined with 2D HMQC experiments, with a BIRD pulse 0.5 s before each scan to suppress the signal from protons not directly bonded to ^{13}C. The interpulse delays were adjusted for an average ^1J CH of 125 Hz. Two and three bond heteronuclear ^1H–^{13}C connectivities were determined with 2D HMBC experiments, optimized for ^{2-3}J CH of 8 Hz. Nuclear Overhauser effect (NOE) measurements were performed by 2D ROESY experiments.

2.3.3. FTIR analysis

The FTIR spectra were obtained on a Bruker IFS-48 spectrophotometer using a KBr matrix.

2.3.4. UV analysis

UV spectra were obtained on a Beckman DU70 spectrophotometer in MeOH solution.

2.3.5. Mass analysis

Electrospray ionization ESI-MS spectra were recorded in CH_3OH on an AB Applied Biosystems mass spectrometer API 2000 MS/MS system. Operational parameters were as follows: vaporizer, 350 °C; heated capillary, 150–200 °C; carrier gas, nitrogen at a sheath gas pressure of 70 psi and auxiliary gas at 30 psi to assist in nebulization; ions were decomposed in the collision cell at 0.8 mTorr by using an optimized collision energy of 55.0 eV.

3. Results and discussion

3.1. General

The study of betaines follows our previous studies on the phytochemical content of kancolla seeds in which we found five flavonoid glycosides, vanillic acid (Dini, Tenore, & Dini, 2004) and seven triterpenoid saponins (Dini, Tenore, & Dini, 2002).

3.2. Betaines analysis

The whole flour from the seeds was exhaustively extracted with MeOH. The MeOH extract was partitioned between *n*-BuOH and H_2O. The buthanol extract was evaporated and defatted with $CHCl_3$. The organic layer was subjected to Sephadex LH-20 chromatography, followed by silica gel chromatography and reverse phase HPLC to give betaines. The ESI-MS mass spectral and NMR data of compounds **1**, **2** and **3**, are in accordance with the results reported in the literature for glycine betaine (**1**), trigonelline (**2**) and trigonelline methylester (**3**) (see Fig. 1) (Lynn et al., 1978).

The structure of compound **4** was readily elucidated on the basis of its considerable similarities to compound **2**. The ESI-MS mass spectrum of compound **4** exhibited a quasi-molecular ion peak at *m/z* 301 $[M + H]^+$, indicating the molecular formula $C_{13}H_{18}NO_7$ in accordance with ^{13}C and ^{13}C DEPT NMR data. Moreover, the ESI-MS mass spectrum showed a fragment ion peak at *m/z* 137, indicating the loss of one hexose. The ^1H and ^{13}C NMR resonances of **4** were superimposable on those obtained for **2**, with the exception of an additional sugar group signal in each of the ^1H NMR and ^{13}C NMR spectra. The nature of the monosaccharide was determined by combined analysis of the COSY, HOHAHA,

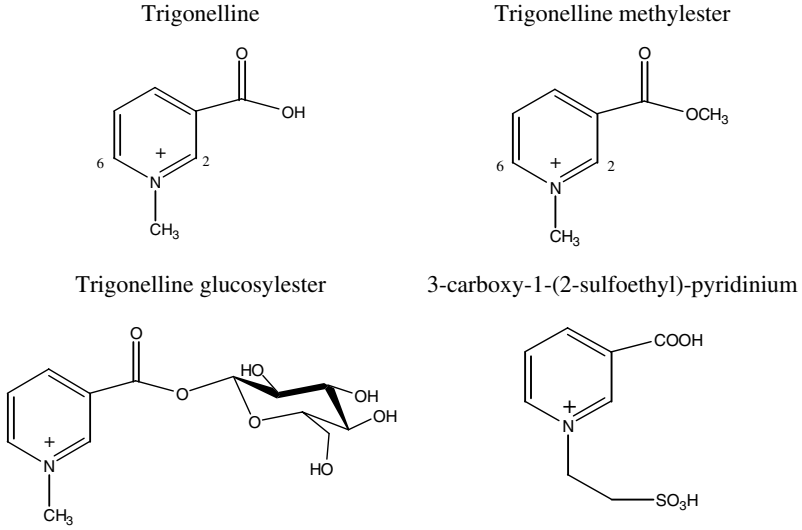

Fig. 1. Betaine derivatives in Kankolla seeds.

and HMQC and shown to be glucose. The pattern of ^{13}C NMR resonances confirmed this assignment. This residue was linked to the carboxy group of the aglycone, as indicated by the HMBC correlation peak between the anomeric proton Glc-1 (δ 5.41) and the upfield-shifted (ppm 167.0) carboxy group carbon of the aglycone. From all the evidence mentioned above, the structure of **4** was deduced to be trigonelline glucosylester (see Fig. 1).

The ESI-MS mass spectrum of compound **5** exhibited a quasi-molecular ion peak at *m/z* 233 $[M + H]^+$, indicating the molecular formula $C_8H_{10}NO_5S$, in accordance with ^{13}C and ^{13}C DEPT NMR data. The molecular formula, and the IR spectrum (KBr), which showed two absorptions at ν_{max} 1360 and 1175 cm^{-1}, suggested the presence of an alkylsulfonate moiety (taurine-like) as the portion linked to the pyridinium nitrogen. The ^1H NMR spectrum exhibited signals at δ 9.25 (1H, s, H-2), δ 8.93 (1H, d, $J = 7.2$ Hz, H-4), δ 8.10 (1H, t, $J = 7.2$ Hz, H-5), δ 8.90 (1H, d, $J = 7.2$ Hz, H-6), δ 5.05 (2H, t, $J = 5.2$ Hz, H-7), δ 3.87 (2H, t, $J = 5.2$ Hz, H-8). The ^{13}C NMR spectrum exhibited signals at 147.5 (C-2), 139.3 (C-3), 146.8 (C-4), 128.5 (C-5), 146.0 (C-6), 67.0 (C-7), 53.7 (C-8), 168.8 (COOH). Comparison of the ^1H and ^{13}C NMR spectra with those of trigonelline, showed evidence for a β-substituted pyridinium nucleus with a $–CH_2CH_2–$ moiety linked to the nitrogen atom for compound **5**. Two coupled triplets at δ 5.05 and 3.87 in the ^1H NMR spectrum suggested that, in compound **5**, there is a dimethylene portion. In particular, the methylene resonating at δ 5.05 must be that linked to the nitrogen atom, as demonstrated by its strong NOE effect with the doublet at δ 9.25 (proton of pyridine). As a consequence, the second triplet must link the sulfonate function, and this conclusion is further supported by the chemical shift of C-8 at δ 53.7 in the ^{13}C NMR spectrum. The presence of a carboxylate in the structure of **5** was inferred by IR (KBr) absorption bands at ν_{max} 1645 and HMBC correlation peaks of the carboxylate carbon (C-9) with H-2 and H-4. This agrees with the molecular formula deduced by mass spectral data and ^{13}C and ^{13}C DEPT NMR. These facts indicated that compound **5** was 3-carboxy-1-(2-sulfoethyl)-pyridinium (see Fig. 1).

In the Chenopodiaceae (Adrian-Romero et al., 1998) only glycine betaine has been reported and, therefore the presence of trigonelline and its derivatives and 3-carboxy-1-(2-sulfoethyl)-pyridinium in Kancolla seeds could be used as taxonomic markers.

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