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4-HYDROXY-*N*-METHYLPROLINE ANALOGUES IN *MELALEUCA* SPP.

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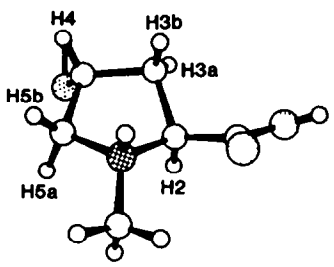
Key Word Index—*Melaleuca*; Myrtaceae; (−)-(2*S*, 4*R*)-1-methyl-4-hydroxy-pyrrolidine-2-carboxylic acid; 4-hydroxy-*N*-methylproline; (−)-(2*S*, 4*R*)-1,1′-dimethyl-4-hydroxypyrrolidine-2-carboxylic acid; 4-hydroxy-*N*,*N*′-dimethylproline; betonicine.

Abstract—4-hydroxy-*N*-methylproline and 4-hydroxy-*N*,*N*′-dimethylproline have been identified in the aqueous extracts of *Melaleuca* spp. Characterization of the two compounds is discussed.

Whilst using ^{1}H NMR to measure the accumulation of glycinebetaine in a range of native South Australia flora [1] it was observed that a specimen of *Melaleuca lanceolata* contained a high concentration of an *N*-methyl hydroxyproline derivative. Subsequent studies have shown that a number of species belonging to the genus *Melaleuca* accumulated this compound and another hydroxyproline analogue. The levels of these compounds in plant tissue respond to environmental stress (Naidu, B. P., Jones, G. P., Paleg, L. G. and Poljakoff-Mayber, A., unpublished data) suggesting that they may be important in determining the drought and/or salinity resistance of these species. Furthermore, the occurrence of these compounds together with proline and *N*-methylproline, at different levels in different *Melaleuca* species holds promise for their use in chemotaxonomic studies. The compounds were isolated by ion-exchange chromatography and identified as (−)-(2*S*, 4*R*)-1-methyl-4-hydroxy-pyrrolidine-2-carboxylic acid (4-hydroxy-*N*-methylproline, **1**) and (−)-(2*S*, 4*R*)-1,1′-dimethyl-4-hydroxy-pyrrolidine-2-carboxylic acid (4-hydroxy-*N*,*N*′-dimethylproline, betonicine, **2**) by ^{1}H and ^{13}C NMR and by comparison with authentic compounds. X-ray crystallographic analyses of the two compounds enabled unambiguous confirmation of their identities. The conformations of **1** and **2** determined by X-ray diffraction studies of their respective hydrochlorides are shown below. Oxygen and nitrogen atoms are lightly and heavily shaded respectively. The orientation of the *N*-methyl group with respect to the carboxyl group in **1** is the same as that predicted by an earlier ^{1}H NMR study [5].

1 and **2** have both been found in higher plants [2–4] and algae [5–7] at levels less than 0.5% dry weight. In the present case levels of **1** of >4% dry weight were measured in *M. lanceolata* and > 1.4% dry weight of both **1** and **2** in leaves of *M. uncinata*. ^{1}H and ^{13}C NMR spectral data for **1** are consistent with that reported previously [5]. The ^{1}H NMR spectrum of **2**, as the hydrochloride (90 MHz, D_2O, pH 1.0), closely resembled that of **1** and showed peaks at δ3.19 (3H, N−Me) and δ3.47 (3H, N–Me). A complex multiplet at δ2.25–3.00 (2H) was attributed to H-3a and H-3b. H-5a and H-5b each gave a doublet of doublets centred at δ3.62 and δ4.11 respectively as part of an AMX pattern involving H-4 where $J_{5a,5b}$ = 13.0 Hz, $J_{5a,4}$ = 4.4 Hz and $J_{5b,4}$ = 6.0 Hz. A multiplet at approximately δ4.7, which was significantly obscured by the solvent peak, was ascribed to H-2 and H-4. When the pH was adjusted to 4, a part of this multiplet moved upfield to form a broad doublet at δ4.36 (1H), J = 11 Hz which was ascribed to H-2.

The ^{13}C NMR spectrum of **2** (22.5 MHz, D_2O, pH 1.0) shows a singlet at δ171.0 (–COOH), two doublets at δ77.5 and δ76.8 (C-2, C-4), two triplets at δ69.1 (C-5) and δ38.1 (C-3) and two quartets at δ57.6 and δ52.0 (*N*–Me, *N*–′Me). At pH 4.0 the carboxyl signal moved downfield to δ173.0 and the signals associated with C-2 and C-4 merged to form a single doublet at δ76.9.



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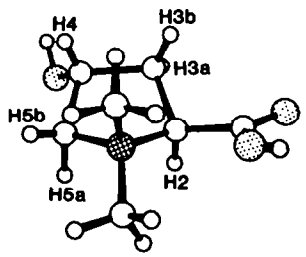

2

It is noted that the melting points of the hydrochlorides for both **1** and **2** are significantly lower than those reported previously [2, 5] and this is attributed to the formation of different crystalline modifications.

EXPERIMENTAL

Extraction and isolation. Leaves of *M. lanceolata* (450 g fr. wt) were homogenized and extracted with 70% aq MeOH (3 × 1). The extracts were filtered through muslin and combined. The filtrate was then clarified by centrifugation, concd in vacuo and again centrifuged. The supernatant was applied to a column of Dowex 50W (H^+ form, 50–100 mesh, column size 3 × 65 cm) and after washing the resin with water (2l) to remove neutral species, the amino acid fraction containing **1** was eluted with 4 M HCl (1l). The eluent was taken to dryness *in vacuo*. The reddish-brown solid was taken up in hot dry EtOH (60 ml) to which was added dry Et_2O to ppt. the hydrochloride of **1** as pale pink crystals (yield 7.5 g). A small sample of this material (200 mg) was recrystallized from MeOH–Et_2O to give colourless prisms suitable for X-ray crystallographic studies with mp 160–161° (lit. 183–184° (dec) [5]), $[\alpha]_D^{25}$ −59.8° (H_2O; *c* 1.1) (lit. −58.9°: H_2O; *c* 1.2) [5]. A sample of the hydrochloride of **1** (200 mg) was converted to the free amino acid on a Dowex 50 W $[H]^+$ column by elution with 2 M NH_4OH. The elute was dried *in vacuo* and the colourless residual solid was recrystallized from MeOH–Et_2O giving **1** as colourless needles (120 mg), mp 239° (dec) (lit. 238° (dec) [5]), $[\alpha]_D^{25}$ −82.2° (H_2O; *c* 1.0) (lit. −82.7°: H_2O; *c* 1.1 [5]), FAB-MS $(M+H)^+$ 146.

M. uncinata leaf tissue (350 g) was extracted in a similar manner. After the initial ion exchange chromatography stage, however, **1** and **2** were sepd on a second Dowex 50W column (50–100 mesh; H^+, 3 × 65 cm) with a stepwise gradient of HCl from 0 to 1 M in 0.3 M steps (2l) collecting 100 ml fractions. The separation was monitored by TLC analysis (precoated silica gel plates using EtOH–conc NH_4OH, 85:15) using iodine and Dragendorff's reagent for visualizing the spots. **1** was eluted at the end of the 0.6 M HCl step whereas **2** was eluted with 0.9 M HCl. Fractions containing either **1** or **2** were pooled, dried *in vacuo* and recrystallized from MeOH–Et_2O yielding 650 mg of **1** and 620 mg of **2** as the hydrochlorides. The physical properties of **1** were the same as the material isolated from *M. lanceolata*. Crystals of **2** as the hydrochloride, suitable for X-ray crystallographic analysis (X-ray crystallographic data for the two compounds have been deposited with the Cambridge Crystallographic Data Centre.) are colourless prisms, mp 204–205° (lit 224° (dec) [2]), $[\alpha]_D^{25}$ −26.4° H_2O; *c* 1.1) (lit. −24.8°: H_2O; *c* 3.6 [2]), FABMS $(M+H)^+$ 160.

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