Phytochemistry, Vol. 24, No 7, pp. 1620–1622, 1985
Printed in Great Britain

0031-9422/85 \$3.00 + 0.00

2-HYDROXYMETHYL-3,4-DIHYDROXYPYRROLIDINE IN FRUITS OF *ANGYLOCALYX BOUTIQUEANUS*

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(*Received* 13 *November* 1984)

Key Word Index—*Angylocalyx boutiqueanus*, Leguminosae; 2-hydroxymethyl-3,4-dihydroxypyrrolidine.

Abstract—A new pyrrolidine derivative, 2-hydroxymethyl-3,4-dihydroxypyrrolidine, has been isolated from *Angylocalyx boutiqueanus*.

INTRODUCTION

The genus *Angylocalyx* comprises 12 tropical African tree and shrub species, none of which is reported to be poisonous. In a study of the distribution of alkaloids in the Leguminosae, it was observed that seeds and fruits of six *Angylocalyx* spp. (*A. boutiqueanus* Touss., *A. oligophyllus* E. G. Baker, *A. zeucheri* Harms., *A. braunii* Harms., *A. pynaertii* De Wild. and *A. vermeulenii* De Wild) contained a basic compound which, on ionophoresis papers, gave an immediate yellow spot on development with ninhydrin and a blue spot with nitroprusside reagent. These colour reactions are similar to those given by the 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine, reported to be present in leaves of the legume *Derris elliptica* [1], but the ionic mobilities of the two compounds were not the same. The compound was isolated and characterized as 2-hydroxymethyl-3,4-dihydroxypyrrolidine (**1**).

RESULTS AND DISCUSSION

The new compound was isolated from aqueous ethanol extracts of *Angylocalyx* fruit by chromatography on a cation exchange resin. Its elemental composition was found to be $C_5H_{11}NO_3$ by high resolution mass spectrometry; the mass of the $[M+1]^+$ ion was measured, this being the most intense ion detected in the chemical ionization mass spectra using both ammonia and methane for ionization. The ^{13}C NMR spectrum showed that the five carbons present were of the type of $-CH_2N-$, $-CH_2O-$, $-CHN-$ and two $-CHO-$. The 300 MHz ^1H NMR spectrum of a deuterium oxide solution contained signals for seven different protons, implying that four exchangeable protons were present. Irradiation of the low field proton ($\delta 4.01$) showed that it was vicinally coupled to the protons of the $-CH_2N-$ group and to the proton at $\delta 3.70$. Irradiation of the latter proton showed that it was also coupled to the methine proton adjacent to nitrogen. The observed and simulated* proton spectra are shown in Fig. 1. The only structure consistent with this data was 2-hydroxymethyl-3,4-dihydroxypyrrolidine (**1**). This was supported by the electron impact mass spectrum in which the base peak was m/z 102. This corresponded to loss of CH_2OH from the molecular ion to form the fragment ion **2**; loss of water from the latter gave an ion of mass 84.

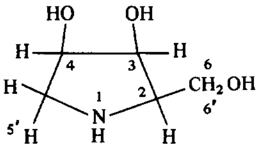
The stereochemistry could not be deduced from the vicinal coupling constants ($J_{2,3} = 5.3$ Hz, $J_{3,4} = 3.7$ Hz); both the relative and absolute configurations will be determined by comparisons with the ribo-, arabino-, xylo- and lyxo-diastereoisomers which are currently being synthesized.

The results of studies of the biological activity of **1** will be reported separately.

EXPERIMENTAL

^1H NMR spectra of D_2O solns were measured at 300 MHz on a Bruker WM 300 spectrometer at a probe temp. of 20° and were referenced with respect to HOD at $\delta 4.82$. ^{13}C NMR spectra were measured on the same instrument at 75.5 MHz and were referenced with respect to dioxane at $\delta 67.4$. Spin simulations were carried out using the SIMEQ-II programme of C. W. F. Kort and M. J. A. Van de Bie on a Varian 620 L100 computer. Chemical ionization MS were measured on a VG 7070 spectrometer and electron impact MS were recorded at 70 eV on AEI MS9 and MS902 spectrometers.

Analysis of extracts Finely ground seeds and fruits of *Angylocalyx* spp. were extracted overnight in 75% EtOH



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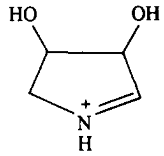

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*Two long-range couplings were used ($J_{3,5'} = 0.3$ Hz, $J_{2,5'} = 0.5$ Hz) but these have not been refined.

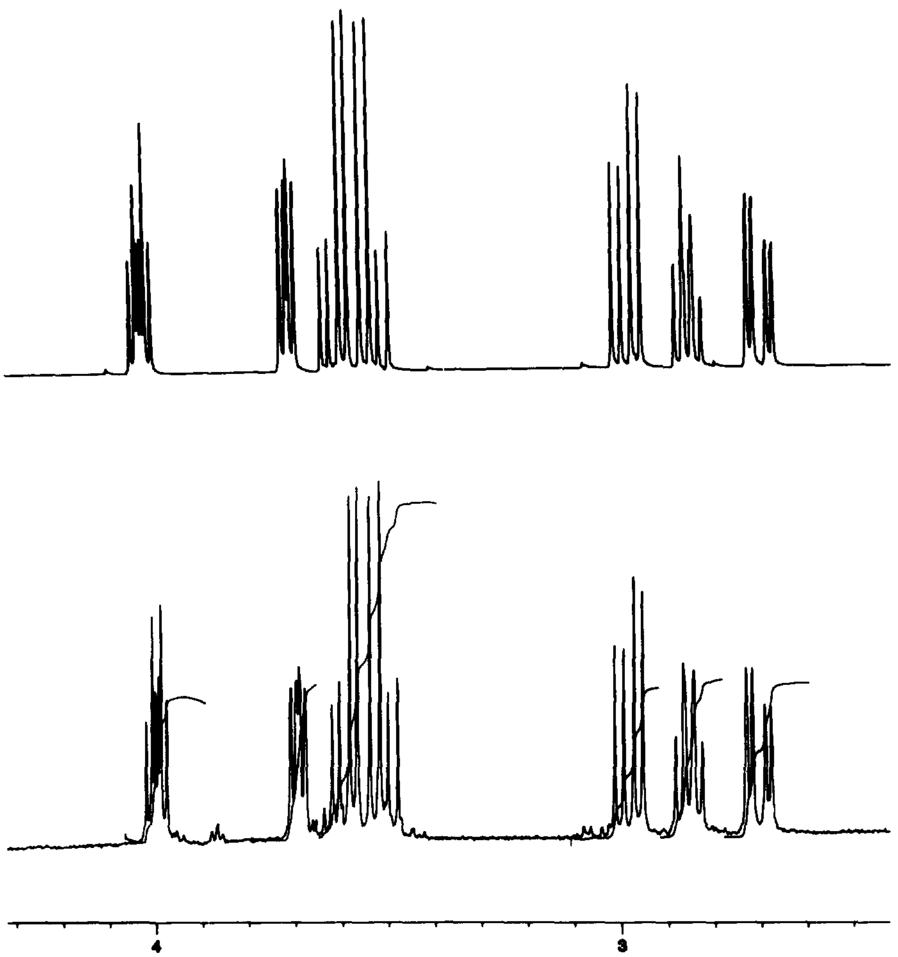

Fig. 1. The 300 MHz ^{1}H NMR spectrum of 2-hydroxymethyl-3,4-dihydroxypyrrolidine. Lower trace: actual spectrum (HOD resonance not shown). Upper trace: simulated spectrum.

(200 mg/ml). The extracts, after filtration and concn, were subject to 2D PC on Whatman No. 1 paper using the ascending method. Solvents used were: (A) *n*-BuOH–HOAc–H_2O (12 : 3 : 5) followed by (B) PhOH–H_2O (4 : 1, w/v) in the presence of NH_3. Papers were developed with ninhydrin reagent (0.2 % w/v in 95 % aq. Me_2CO) and aq. sodium nitroprusside (10 %), acetaldehyde (10 %), Na_2CO_3 (5 %) (1 : 1 : 2). R_f values of **1** were 0.38 (A) and 0.89 (B). The extracts were also analysed by high voltage ionophoresis on Whatman No. 1 paper (70 V/cm for 30 min) in buffer solns of pH 1.9, 3.6 [2], 8.0 (0.05 M borate) and 9.2 (0.06 M borate). The ionic mobilities of **1** relative to arginine were 0 89 (pH 1.9), 1.2 (pH 3.6), 0.8 (pH 8.0) and 0.5 (pH 9.2).

Isolation of **1**. Finely ground ripe fruits (25 g) of *A. boutiqueanus* Touss (Sophoreae) were extracted with aq. 75 % EtOH. After filtration the extract was applied to a column (30 × 2 cm) of cation exchange resin (Dowex 50 × 8) in the H^+ form. The resin was washed with H_2O and the neutral and acidic amino acids were displaced with 2.5 M C_5H_5N. The 'unknown', together with arginine, was displaced with 2 M NH_4OH soln. After concn and removal of NH_3 under red. pres., the 'unknown' was separated from arginine on a column (30 × 1 cm) of cation exchange resin (Amberlite CG120) in the NH_4^+ form by elution with 0.2–1 M NH_4OH soln. The fractions containing the 'unknown' were concd under red. pres. and passed through a column (20 × 1 cm) containing anion exchange resin (Amberlite CG 400) in the OH^- form. The resin was washed with H_2O, the eluate was reduced in vol. and the 'unknown' was crystallized from aq. EtOH to give a hygroscopic solid, yield 40 mg.

Identification of **1**. Chemical ionization MS (NH_3) *m/z* (rel. int.): 151 $[M+NH_4]^+$ (28), 134 $[M+H]^+$ (100); (CH_4): 174 $[M+C_3H_5]^+$ (2), 162 $[M+C_2H_5]^+$ (4), 134 $[M+1]^+$ (100). Electron impact MS *m/z* (rel. int.): 158 (31, impurity), 134.0809 ($C_5H_{12}NO_3$) $[M+1]^+$ (10), 133 $[M]^+$ (3), 116 (8), 102 (100), 84 (17), 55 (53), 30 (60). ^{1}H NMR: (see Fig. 1 and structure **1**): δ4.01 (H-4), 3.70 (H-3), 3.60 (H-6), 3.53 (H-6'), 2.99 (H-5), 2.87 (H-2), 2.72 (H-5'); $J_{3,4} = 3.7$ Hz, $J_{4,5} = 5.8$ Hz, $J_{4,5'} = 3.8$ Hz, $J_{2,3} = 5.3$ Hz, $J_{6,6'} = -11.6$ Hz, $J_{2,6} = 4.9$ Hz, $J_{2,6'} = 6.4$ Hz, $J_{5,5'} = -12.1$ Hz. ^{13}C NMR: δ (multiplicity in SFORD spectrum): 79.9 (*d*, CH–O), 78.4 (*d*, CH–O), 66.4 (*d*, CH–N), 62.9 (*t*, CH_2–O), 51.6 (*t*, CH_2–N).

Acknowledgements—We thank W. A. Thomas, I. W. A. Whitcombe and A. J. Moore of Roche Products Ltd. for the

NMR and CIMS spectra, Jardin Botanique de Belgique for gifts of *Angylocalyx* spp., and the Science and Engineering Research Council and Natural Environmental Research Council for financial support.

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Phytochemistry, Vol 24, No 7, pp 1622–1624, 1985
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0031–9422/85 $3.00 + 0.00

USPALLATINE, A PYRROLIZIDINE ALKALOID FROM *SENECIO USPALLATENSIS*

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(*Received* 18 *September* 1984)

Key Word Index—*Senecio uspallatensis*; Compositae; pyrrolizidine alkaloids; uspallatine.

Abstract—A new pyrrolizidine alkaloid uspallatine was isolated from roots of *Senecio uspallatensis* together with the previously known retrorsine. The structure of the new alkaloid was established by spectroscopical data and chemical transformation.

INTRODUCTION

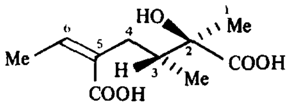
As part of our chemical systematic study of the genus *Senecio* (Senecioneae: Compositae) which grows in the western region of Argentina, we have investigated the roots and aerial parts of *S. uspallatensis* with regard to its alkaloid content. *Senecio uspallatensis*, commonly known as 'chachacoma', is a common plant in the mediun Andes cordillera and is particularly frequent in Paramillos de Uspallata. Its infusion is drunk by the villagers instead of mate [1]. From this species a new macrocyclic pyrrolizidine diester alkaloid, which was named uspallatine, was isolated together with the previously known retrorsine. Uspallatine is a diester of a new amino alcohol, 6α,7β-dihydroxy-1-hydroxymethyl-1,2-dehydro-8α-pyrrolizidine (**1**), hereafter called uspallatinecine.

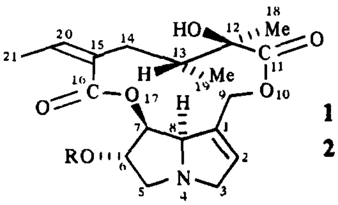
Up to now three pyrrolizidine triols have been reported. Eight esters of crotanecine (unsaturated triol pyrrolizidine necine) have been characterized in *Crotalaria* sp. [2, 3]. Croalbinecine (saturated pyrrolizidinetriol necine) was isolated as the base constituent of croalbidine from *C. albida* [4]. Rosmarinecine, a stereoisomer of croalbinecine, has been isolated from *S. angulatus* as a constituent of rosmarinecine and angularine [4]. Thus, this is the first report of the occurrence of unsaturated necine triols in *Senecio*.

RESULTS AND DISCUSSION

The empirical formula of uspallatine (**1**), is $C_{18}H_{25}NO_6$ (from the high resolution mass spectrum). The peaks at *m/z* 307, 154, 152, 137, 136, 135, 95 and 94 indicated that it is an alkaloid of the pyrrolizidine type with an unsaturated triol base, esterified by a C-10 dicarboxylic acid forming a macro ring. Several of these peaks are prominent also in the spectra of anacrotine and madurensine [2].

Important information regarding its structure was obtained from NMR analysis. The ^1H NMR data are given in Table 1 and that for ^{13}C NMR in Table 2. The ^1H NMR showed signals appropriate to senecic acid or its diastereoisomers (*cis*-grouping CH_3–CH=C [3], CH_3–C–OH, CH_3–CH) and also exhibited the character-





1 R = H
2 R = CO – Me
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