Phytochemistry, Vol. 29, No. 11, pp. 3653–3655, 1990
Printed in Great Britain.

0031–9422/90 $3.00 + 0.00

SHORT REPORTS

DIBENZYL TRISULPHIDE AND *TRANS-N*-METHYL-4-METHOXYPROLINE FROM *PETIVERIA ALLIACEA*

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(*Received* 2 *January* 1990)

Key Word Index—*Petiveria alliacea*; Phytolaccaceae; stem; potassium nitrate; β-sitosterol; pinitol; allantoin; lignoceryl alcohol; lignoceric acid; lignoceryl lignocerate; α-friedelinol; dibenzyl trisulphide; *trans-N*-methyl-4-methoxyproline.

Abstract—A highly polar compound isolated from the stem of *Petiveria alliacea* was shown to be *trans-N*-methyl-4-methoxyproline on the basis of its microanalysis and spectral data. Dibenzyl trisulphide, previously unknown as a natural product, was isolated from the roots. Other compounds not yet reported as constituents of this plant are β-sitosterol, pinitol, allantoin, lignoceryl alcohol, lignoceric acid, lignoceryl lignocerate and α-friedelinol.

INTRODUCTION

Petiveria alliacea L. is a widely distributed shrub which is often found in vacant city lots from Florida, Mexico and the Antilles to Paraguay and Argentina. It grows in all the states of Brazil where it is known by many vernacular names among which the Portuguese 'erva-guiné' and 'guiné', 'pipi' and 'tipi', and the Tupi-Guaraní 'mucura-caá' are noteworthy. In the state of Minas Gerais an infusion of the roots of this plant is reputedly useful for the treatment of rheumatism [1], and elsewhere both the roots and the leaves are described as stimulants and abortefacients, but their long-term use in high doses is believed to cause brain damage [2].

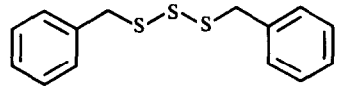
A TLC study on *P. alliacea* claimed the absence of triterpenes or alkaloids and the presence of a large number of 'coumarins' [3]. In a later publication, the isolation of benzyl hydroxyethyl trisulphide was reported [4]. The most recent study of the organic constituents of this plant showed the presence of the novel *cis*-3,5-diphenyl-1,2,4-trithiolan, '*trans*-stilbene', benzaldehyde, benzoic acid, and elemental sulphur [5]. The high nitrate content of *P. alliacea* is known to produce methaemoglobinaemia in cattle [6] and has been mentioned in a preliminary report as a risk factor in humans [1].

RESULTS AND DISCUSSION

In our hands, the petrol extract of all parts of the plant furnished a wax which upon hydrolysis and methylation gave the methyl esters of palmitic, linoleic, stearic and/or oleic and nonadecanoic acids. Only the stem afforded lignoceric acid. Potassium nitrate precipitated from the various ethanol extracts. β-Sitosterol was isolated from the roots and stem, allantoin was found in both leaves and stem, and the inflorescence afforded pinitol. The leaves also contained lignoceryl alcohol, lignoceryl lignocerate and α-friedelinol.

The roots yielded a thick oil with a garlicky odour which gave a relatively uninformative IR spectrum and a very simple ^{1}H NMR spectrum with a singlet at δ3.95 and another at δ7.25 with integrals in the ratio (2:5). The lower chemical shift was intermediate between those reported for benzyl groups bonded to two or three sulphur atoms [4], but the EI mass spectrum showed a molecular ion peak at m/z 278 and an $[M+2]^+$ peak with an intensity consistent with the molecular formula $C_{14}H_{14}S_3$. The compound was thus identified as dibenzyl trisulphide (**1**), which had been described previously as a synthetic product [7].

An additional stem constituent was the very hygroscopic proline derivative (**2**), isolated by chromatography



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on Florisil from a fraction containing mainly inorganic salts. It melted at 251–253° and showed $[\alpha]_D^{20}$ −32.9° (*c* 0.7; MeOH) which became increasingly negative towards the near UV $[\alpha]_{365}^{20}$ −112.6°. The only noteworthy peaks in its IR spectrum were a strong absorption attributable to water and N-H stretching, a large, broad signal centred at 1618 cm^{-1}, and several C-H bending absorptions. It is worth pointing out that proline, unlike primary amino acids, also absorbs strongly at 1618 cm^{-1}. The EI mass spectrum exhibited no conspicuous molecular ion, but FAB mass spectra gave strong peaks at *m/z* 160 and 182 which could be assigned to $[M+H]^+$ and $[M+Na]^+$, respectively, assuming the molecular formula to be $C_7H_{13}NO_3$. In retrospect, examination of the EI mass spectrum showed a weak molecular ion peak at *m/z* 159 (rel. int. 2.1 %). The elemental C–H analysis agreed with this result.

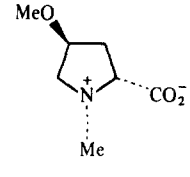
Intense *N*- and *O*-methyl singlets, the former unusually downfield (δ3.15), and six multiplets with rather large coupling constants (12.5 and 15.0 Hz) indicative of geminal methylene protons attached to a ring were seen in the ^1H NMR spectrum. Seven different carbon resonances, including the *N*- and *O*-methyl signals and a carbonyl peak, were identified in the ^{13}C spectrum. The structure was finally solved on the basis of homo- and heteronuclear 2D chemical shift-correlated NMR spectra. The CH-COSY diagram clearly showed the presence of two methylene groups with protons resonating at δ2.34 and 2.66, and at δ3.45 and 4.02, correlated with the carbon resonances at δ38.2 and 75.4, respectively. The assignments derived from the CH-COSY and CH-COLOC spectra are summarized in Table 1. Inspection of the HH-COSY diagram led directly to the HH relationships depicted in Scheme 1, while the CH-COSY spectrum showed that these HH relationships correspond to a CH-CH_2-CH-CH_2 moiety. The correlations observed in the CH-COLOC diagram between the *N*-Me protons (δ3.15) and the terminal atoms of the above four-carbon chain (δ75.4 and 77.8) indicated an *N*-methylpyrrolidine substructure. On the other hand, the lack of correlation between the carboxyl carbon nucleus and the methoxyl protons was proof that the compound is not an ester. Finally, a cross-signal corresponding to the pyrrolidine C-4 nucleus (δ67.7) and the methoxyl protons (δ3.40) indicated the location of this latter group. The compound is therefore an *N*-methyl-4-methoxyproline.

The NOE difference spectra clarified the relative configuration of this substance: apart from the expected reciprocal enhancement of the geminal proton signals, NOEs were clearly visible between the nuclei resonating at δ2.34 and 4.32, 2.66 and 4.58, 4.02 and 4.58, and 3.15 and 4.02 showing that the ammonium *N*-methyl group and the carboxylate function bear a *cis* relationship to each other. Consequently, the carboxylate and the methoxyl groups must be *trans*. The negative optical rotation, as in L-proline, *trans*-4-hydroxyproline [8] and *trans*-*N*-methyl-4-hydroxyproline [9] strongly suggests that the absolute configuration of this derivative should be 2*S*, 4*R*.

To the best of our knowledge, *trans*-*N*-methyl-4-methoxyproline has never been described before in spite of its trivial relationship to natural hydroxyproline and the well-known hygric acid. *trans*-4-Hydroxy-1-methyl-L-proline has been isolated on two occasions, from *Afrormosia elata* [9] and *Croton gubouga* [10]. Both *cis*- and

Table 1. Assignment of the CH-COSY and CH-COLOC spectra (ppm, 400/100.6 MHz, MeOH-d_4) of compound **2**

H		Carbon atoms separated by		
		one bond	two bonds	three bonds
CH-4	4.58	67.7		
CH-2	4.32	77.8	170.4	
CH_2-5	[3.45 AB 4.02]	75.4	67.7	
OMe	3.40	55.0	67.7	
NMe	3.15	49.1	77.8	75.4
CH_2-3	[2.34 AB 2.66]	38.2	77.8	



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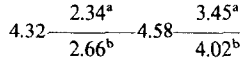

Scheme 1. HH Correlations in the HH-COSY spectrum (ppm, 400 MHz, MeOH-d_4) of *trans*-*N*-methyl-4-methoxyproline (**2**).

trans-4-methoxyprolines have been synthesized as intermediates in the search for new angiotensin converting enzyme inhibitors [11].

EXPERIMENTAL

General. The known compounds were identified by standard physical and spectrometric methods and by comparison with authentic samples.

Plant material. Whole plants of *Petiveria alliacea* were collected in the Botanical Garden of the Universidade Federal de Minas Gerais, Belo Horizonte, where voucher specimens are preserved. The air-dried plants were separated into roots, stems, leaves and inflorescences which were ground and extracted first with petrol and then with EtOH.

Dibenzyl trisulphide (**1**). From the petrol extract of the roots (720 g), isolated as a viscous, pungent-smelling oil (0.63 g) by CC on silica gel eluting with hexane–EtOAc. IR ν_{max}^{film} cm^{-1}: 3040, 3030, 2850, 1609, 1490, 1450, 760, 700; ^1H NMR ($CDCl_3$, 60 MHz) δ3.95 (2H, *s*, CH_2), 7.25 (5H, *s*, C_6H_5); EIMS (70 eV) *m/z* (rel. int.): 280 ($[M+2]^+$, 0.4), 278 ($[M]^+$, 3.0), 246 ($[M-S]^+$, 5), 213 (7), 123 (9), 91 (100), 77 (13), 65 (40), 51 (16).

trans-N-*Methyl*-4-*methoxyproline* (**2**). From the EtOH extract of the stems (3.87 kg), by CC on Florisil eluting with Me_2CO, needles (1.55 g), mp 251–253°; $[\alpha]_D^{20}$ −32.9°, $[\alpha]_{436}^{20}$ −69.3°, $[\alpha]_{365}^{20}$ −112.6° (0.7, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3370 (s, N-H stretch, H_2O), 1660 (m, amino acid I), 1618 (s), 1470, 1440 (w), 1400 (m), 1363, 1330 (w); ^1H NMR (MeOH-d_4, 200 MHz) δ:2.34 (1H, *dd*, J=15.0, 7.5 Hz, H-3a), 2.66 (1H, *ddd*, J=15.0, 10.5, 7.0 Hz, H-3b), 3.15 (3H, *s*, *N*-Me), 3.40 (3H, *s*, *O*-Me), 3.45 (1H, *dd*, J=12.5, 4.0 Hz, H-5a), 4.02 (1H, *dd*, J=12.5, 6.0 Hz, H-5b), 4.32 (1H, *dd*, J=10.5, 7.5 Hz, H-2), 4.58 (2H, *ddd*, J=7.0, 6.0, 4.0, H-4); ^{13}C NMR (MeOH-d_4, 50.3 MHz) δ:38.2 (*t*, C-3), 49.1 (*q*, *N*-Me) 55.0 (*q*, *O*-Me), 67.7 (*d*, C-4), 75.4 (*t*, C-5), 77.8 (*d*, C-2), 170.6 (*s*, CO_2^-). CH-COSY and CH-COLOC (400/100.6 MHz): Table 1; HH-COSY (400 MHz): Scheme 1. NOEDS (200 MHz) described in text. FABMS (thioglycerol) *m/z*: 182 ($[M+Na]^+$), 160 ($[M+H]^+$). (Found: C, 52.81; H, 8.32; N, 9.28. Calc. for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80).

Acknowledgements—We thank Prof. José Luis Pedersoli [Departamento de Botánica, ICB, UFMG] for identifying and collecting the plant material, and Prof. Jean Claude Tabet [Université de Paris VI] for the FABMS. This work was supported by the CNPq [Brazil], and the Ministerium für Wissenschaft und Forschung Nordrhein-Westfalen [F.R.G.].

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