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A NEW ALKALOID FROM SOUTH AFRICAN *CONIUM* SPECIES

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Key Word Index—*Conium maculatum*; Umbelliferae; piperidine alkaloids; 2-propyl-5-hydroxy-*N*-methyl piperidine; *N*-methylpseudoconhydrine; relative configuration; conformation.

Abstract—Recent screening of South African *Conium* species for alkaloids as part of taxonomic studies has yielded γ-coniceine, coniine, methylconiine, conhydrine and a new alkaloid *N*-methylpseudoconhydrine. The relative stereochemistry of *N*-methylpseudoconhydrine was ascertained by ^{1}H NMR decoupling experiments. This latter alkaloid was found in significant amounts in the leaf and stem of some plants investigated and was the major alkaloid along with conhydrine in the leaf and stem of one group of high altitude plants. These plants also contained significant amounts of volatile oil, the major monoterpene being myrcene.

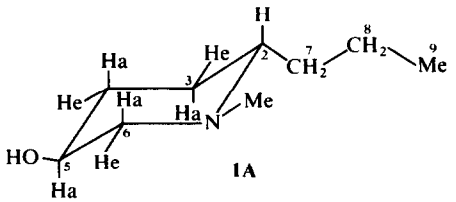
INTRODUCTION

The recent collections of South African *Conium* species* have yielded material with considerable taxonomic variation and indeed more than one species may occur (Hilliard, O. and Burtt, B. L., unpublished results). These plants contained comparatively high levels of volatile oil, the major constituent of which has been identified (GLC, MS) as the monoterpene myrcene (Rowan, M. and Roberts, M. F., unpublished results). The fruits of these plants have clearly defined schizogenous canals especially those outside the rib bundles. In European species these canals are less well defined. Plants were initially separated according to whether they were yellow or white flowered and according to habitat. Levels of volatile oil were in general higher in the plants growing at the higher altitudes and these plants had low alkaloid content averaging at 0.04% (<10% that recorded for European *Conium* [1]. Other samples had an alkaloid content similar to the European *Conium*.

RESULTS AND DISCUSSION

All plants contained alkaloids and from all samples γ-coniceine, coniine and methylconiine were isolated along with an unknown alkaloid (**1A**) which attained significant levels in some plants. Plants collected at a higher altitude than 3000 m (H and B 8859) contained conhydrine and in these particular plants the unknown alkaloid was the major constituent of the leaf and stem. In other plants this alkaloid was a minor constituent of the leaf and stem. In all plants coniine and methylconiine were the major constituents of the fruits where the highest levels of alkaloid occurred. The known alkaloids were identified by TLC and GLC with reference to authentic samples. The unknown alkaloid (**1A**), identified as *N*-methylpseudoconhydrine, was readily detected by PC and TLC. However, despite this, it has not been previously detected in *Conium maculatum* from Europe and the U.S.A. The new alkaloid was isolated using PC, identified and its stereochemistry determined from IR, NMR and MS data. X-ray analysis of pseudoconhydrine hydrobromide has shown that the substituents in the ring are *trans* [2] and it was anticipated that the naturally occurring *N*-methyl derivative would be similar. This is substantially confirmed by the data given in Table 1 and the Experimental.

The MS data showed the expected fragmentation pattern with the base peak at *m/e* 114 equivalent to the piperidine ring less the three carbon side chain on the carbon α to the nitrogen. It also indicated additional substitution in this ring compared with pseudoconhydrine of Me which the ^{1}H NMR data showed to be attached to the nitrogen.



1A

* *Conium* in S. Africa has frequently been given the name *C. chaerophylloides*. However, in view of the taxonomic variations in our collected material, the precise application of this name is uncertain.

Table 1. ^{1}H NMR parameters of *N*-methylpseudoconhydrine in $CDCl_3$ at 300 MHz

δ	Proton	Multiplicity	*J* Hz (coupled proton)
3.70	H-5	*sept.*	9(6a), 4.5(6e), 9(4a), 4.5(4e)
2.94	H-6e	*dq*	11(6a), 4.5(5), 2(4e)
2.22	*N*-Me	*s*	
2.16	OH	*s*	
1.94	H-4e	*m*	10(4a), 4.5(5), 2(6e), ?(3a, e)
1.92	H-6a	*dd*	11(6e). 9(5)
1.4, 1.1	5H	*m* ca.	1.2 H-4a, ca. 1.5 H_2-8
0.85	H_3-9	*t*	7(8-H_2)

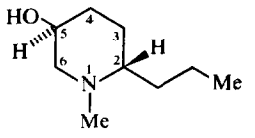
s = singlet, *dd* = doublet of doublets, *dq* = double quartet, *m* = multiplet, *sept.* = septuplet.

Partial analysis of the 300 MH_2 ^{1}H NMR spectrum with double irradiation showed clearly that the piperidine ring existed as a single chair conformation with the 5-hydroxyl in an equatorial orientation. Consequently the 2-propyl group was also equatorial and the 2 and 5 substituents have necessarily to be *trans* to each other as in **1A**. The alternative *cis* configuration, **2**, would have comprised two interconvertible axial–equatorial conformers **2A** and **2B**, but the observed coupling constants were not compatible with equilibration between **2A** and **2B**. If the *cis* 4-alkylcyclohexanols are considered, since they constitute excellent models for the corresponding piperidines [3], the conformer with an axial hydroxyl group greatly predominates. Its proportion increases with the size of the alkyl group from *ca* 90% for an equatorial methyl group to virtually 100% for an equatorial *t*-butyl group. Hence in the present case the *cis* isomer would exist essentially as conformer **2B** with an axial 5-hydroxyl group in contradiction of the experimental findings for the isolated alkaloid (Table 1).

Thus H-5 was observed as a septuplet at δ 3.70: the two *trans* diaxial couplings of 9Hz to H-6a and H-4a which were revealed by decoupling experiments showed conclusively that it had an axial orientation. Its identity was further confirmed by a downfield shift >1 ppm on acylation. The couplings to the other C-6 and C-5 protons (Table 1) were likewise compatible only with a chair conformation of the piperidine: in particular there was a significant long range 1,4-interaction of 2 Hz between the equatorial H-4 and H-6 in accord with their perfect in-plane W arrangement. The data on acetylation and benzoylation further substantiated these assignments. The IR spectra of the parent compound in $CHCl_3$ always had a sharp peak at 3600 cm^{-1} for free OH, but a broad hydroxyl band at *ca* 3300 cm^{-1} diminished with increasing dilution and showed that only intermolecular hydrogen bonding occurs. *N*-Methylpseudoconhydrine was therefore unequivocally assigned structure **1A**.

Biosynthetic investigations of *Conium maculatum* have shown that the major biosynthetic route is γ-coniceine → coniine → methylconiine although plants vary considerably in their ability to reduce γ-coniceine and methylate the product coniine [4–6]. Under certain environmental conditions γ-coniceine may form conhydrinone and conhydrine or pseudoconhydrine [7, 8]. The occurrence therefore of *N*-methylpseudoconhydrine (**1**) in the S. African material was of interest.

Enzymic studies (Roberts, M. F., unpublished results) have shown that plants do not appear to contain the



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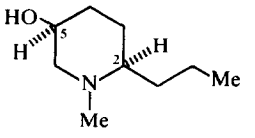
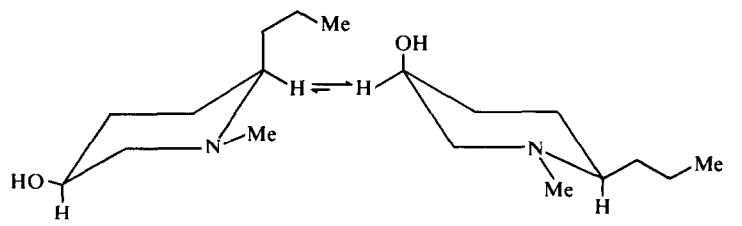


2



2A 2B

hydroxylated alkaloids but have an active coniine: *S*-adenosylmethionine methyltransferase will methylate pseudoconhydrine to *N*-methylpseudoconhydrine. The limiting step in the formation of the hydroxylated alkaloids therefore is the allylic oxidation of γ-coniceine. It was also observed that conhydrine is not methylated by the cell free preparations from *Conium* sp. and this may relate to the fact that the stereochemistry of this molecule almost certainly would mean that intramolecular hydrogen bonding of the C-1′-OH to the nitrogen lone pair [9] exists in this compound.

EXPERIMENTAL

Plant material. The following *Conium* samples from South Africa were extracted for alkaloids: A, yellow flowered: Hilliard and Burtt 9426 (Underberg, Natal) H & B 9463 (Harrismith OFS) H & B 11063 (Stutterheim E. Cape). B, white flowered: (1) High level 3000 m damp grass below cliffs H & B 8859 (Sani Pass, Natal), (2) 1800–2300 m; streams in open grassland H & B 9151 (Oshoek, S. Transvaal); H & B 10525 (Underberg, S. Natal); (3) 1200–1700 m, partial shade of forest streams H & B 11190 (Benvie Karkloof Range, Natal) H & B 11211 (Ixopo, Natal). Plants were divided into root, stem, leaf, flowers and fruits, the component parts extracted with methanol and the alkaloids isolated as previously described [6]. γ-Coniceine, coniine, methylconiine and conhydrine were isolated and identified by reference to authentic samples.

N-*Methylpseudoconhydrine* gave a crystalline hydrochloride, colourless hexagonal rods, mp 157° $[\alpha]_D^{25}$ + 25° (CH_3OH). The free base, a liquid, gave the following accurate Masses: M^+ 157.0962, calc. 157.0964 for $C_9H_{19}QN$; 114.099, calc. 114.0919 for $C_6H_{12}NO$; 96.0817, calc. 96.0813 for $C_6H_{10}N$. MS (EI, 70eV, 90°) *m/e* 157 M^+ (33%, rel int.), 114 (100), 96 (99). IR ($CHCl_3$) cm^{-1} 3600(S), 2900(S), 1600, 1460(S), 1380, 1090, 1050(S), 1000(S), 960(S), 880(S), ^1H NMR, 300 MHz ($CDCl_3$) see Table 1. Elemental analysis: $C_9H_{19}ON$ (Found: C 55.926; H, 10.680; N, 7.133 requires N, 55.900; H, 9.913; N 7.247).

N-*Methylpseudoconhydrine acetate.* MS(Cl) *m/e* 199 M^+. (EI 70 eV 90°) *m/e* 156 (33%, rel int.) 96 (100). ^1H NMR 90 MHz ($CDCl_3$, TMS int. std.) δ 0.88 ppm (H_3-9 *t*; *J*, 7, H_2-8) 2.25 (N-Me *s*); δ 3.0 [H-6e *dq.* 11 (6a), 4.5 (5) 2, (4e)]; δ 2.82 (H-5 sept. 9 (6a), 4.5 (6e), 9 (4a), 4.5 (4e)).

N-*Methylpseudoconhydrine benzoate.* $C_{16}H_{23}NO_2$ requires 261.1731, Mass measured 261.1729. MS, (EI), *m/e* 162 M^+ + 1 (<0.1% rel. int.) 218, (26%), 105 (100), 96 (35).

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