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TETRAMETHYLPUTRESCINE FROM YOUNG PLANTS OF *RUELLIA ROSEA*

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Abstract—Tetramethylputrescine was isolated from the roots and aerial parts of *Ruellia rosea*. The compound was characterized by MS, ^{13}C-NMR-spectroscopy and comparison with a synthetic specimen.

INTRODUCTION

The plant family Acanthaceae contains quinazoline and imidazolylquinoline alkaloids like peganine and macrorungine respectively [1]. The genus *Ruellia* comprises about 200 species. Searching for alkaloids we investigated young plants of 10 *Ruellia* species which were cultivated in a greenhouse.

RESULTS AND DISCUSSION

In the roots and upper parts of 4- to 6-month-old plants of *Ruellia rosea* Hook. a substance was found giving a strong positive reaction with Dragendorff's reagent. After removing non-basic compounds the substance was extracted with a mixture of chloroform–methanol after alkalinization. The alkaloid-like compound was steam volatile and could be identified as tetramethylputrescine. The fragmentation pattern was in accordance with the data published by Veith *et al.* [2]. The ^{13}C-NMR spectra of the hydrochlorides of both bases putrescine and tetramethylputrescine were obtained using a pulsed Fourier transform system with proton noise-decoupling and off-resonance decoupling. The resonances were assigned with the aid of their multiplicity in the off-resonance spectra. The use of substituent chemical shift principles for NH_3^+ and NH_2 substituents on an alkane chain to give calculated values of chemical shifts for all carbon atoms of both compounds. We have used the CMR chemical shifts of *n*-butane (C_1: 13·0 and C_2: 24·8 ppm) [3] and the substituent chemical shifts from Table 1. Table 2 gives a comparison of observed and calculated chemical shifts for the carbons of the hydrochlorides of both bases. In general the calculated chemical shifts are in close agreement with the corresponding observed values.

The IR spectrum of the hydrochloride in nujol revealed strong absorption bands between 1250–1040 cm^{-1} indicating a tertiary amine and an absorption in the region 3000–2000 cm^{-1} (amino groups). The hydrochloride of the natural product was identical in every respect (UV, IR, MS, TLC)

Table 1 Substituent effects of CMR chemical shifts (ppm)

	C_1	C_2	C_3
$\delta R\text{-}\overset{+}{N}H_3 - \delta R\text{-}H$*	26	7 5	−4 6
$\delta R\text{-}NH_2 - \delta R\text{-}H$*	29	11 4	−4 6
$\delta R\text{-}N(Me)_2 - \delta R\text{-}NH_2$†	18	−6 5	1 0

* Values from [3] † Values were estimated from differences of corresponding chemical shifts of 1-dimethylamino-3-aminopropane [3] and 1 3-diaminopropane (calculated from chemical shifts of propane [3] using values of substituent effects for the NH_2 group)

Table 2. Comparison of observed and calculated (in brackets) CMR chemical shifts (ppm downfield from TMS)

$$R_2\overset{+}{N}H-\underset{1}{CH_2}-\underset{2}{CH_2}-CH_2-CH_2-H\overset{+}{N}R_2$$

	C_1	C_2	R
R = H	39·8 (39·0)	24·7 (27·7)	–
R = Me	57·6 (57·0)	22·0 (22·2)	43·6 (42·4*)

* Calculated from chemical shift of methyl group of 1-dimethylamino-3-aminopropane (45·4 ppm) [3] using difference of substituent effects for the NH_3^+ and NH_2 groups (see Table 1).

with a synthetic specimen prepared according to Keil [4].

Tetramethylputrescine has been found only sporadically in the plant kingdom, mainly in the Solanaceae. It was first isolated by Willstätter and Heubner in 1907 [5] from *Hyoscyamus muticus* and later on only from the upper parts of the same plant by Potjewijd [6]. Furthermore it is present in the roots of *Hyoscyamus reticulatus* [7] and in *Hyoscyamus niger* [8]. Recently the methylated diamine was detected as a constituent of the roots of *Duboisia leichhardtii* [9]. Interestingly this compound was metabolized after administration in detached shoots of this particular plant. Tetramethylputrescine is also a major alkaloid of the root bark of *Duboisia myoporoides* [10]. It has been argued that this putrescine derivative might be involved in the biosynthesis of tropane alkaloids in *Duboisia* [10]. Outside the Solanaceae this substance was isolated from the above-ground parts of *Oldenlandia affinis* (Rubiaceae) at a level of 0·1% [11]. We have detected tetramethylputrescine in the Acanthaceae. Greenhouse cultivated plants of *Ruellia rosea* contain in the roots and in the above-ground parts 0·05–0·1% of this putrescine derivative.

EXPERIMENTAL

Plant material. Seeds of *Ruellia rosea* Hook. were obtained from the Botanical Garden, Amsterdam, and cultivated in a greenhouse. After 4 to 6 months the plants were harvested and the roots and upper-parts separately dried. A voucher specimen is deposited at the Institute of Plant Biochemistry, Halle/Saale.

Isolation and identification. Dried plant material (200 g) was milled and made alkaline with NaOH (10%) and extracted for 12 hr with a mixture (1·3 l.) of $CHCl_3$–MeOH (3:1) in a Soxhlet. Concentrated extract from the latter was treated with HCl and again evaporated *in vacuo*. Residue dissolved in H_2O was exhaustively extracted with Et_2O. The aq soln was made alkaline with NaOH and extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated *in vacuo* and the constituents separated by preparative TLC (Si gel PF_{254}). The following solvent systems were used: (i) $CHCl_3$–MeOH–diethylamine (16:3:1), R_f: 0·5; (ii) $CHCl_3$–MeOH–NH_4OH (25%) (100:5:10), R_f: 0·5. After elution with $CHCl_3$ and evaporation, the oily residue was precipitated with HCl–Et_2O and recrystallized from EtOH–Et_2O mp 280° (120 mg). UV (EtOH) of the oily base λ_{max}nm 217, IR of the hydrochloride ν_{max}^{Nujol} 3015, 1469, 1408, 1380, 1330, 1278, 1252, 1167, 1120, 1047, 1008, 955 cm^{-1}. High resolution MS *m/e*: M^+, (measured 144·1623 and calculated as 144·1626 for $C_8H_{20}N_2$); $M^+ - 15$ (measured 129·1396 and calculated 129·1392 for $C_7H_{17}N_2$); $M^+ - N(Me)_2$ (measured 100·1125 and calculated 100·1126 for $C_6H_{14}N$); 129 – H_2N–Me (measured 98·0972 and calculated 98·0970 for $C_6H_{12}N$); $M^+ - 50$ (measured 84·0809 and calculated 84·0813 for $C_5H_{10}N$); $M^+ - 73$ (measured 71·0733 and calculated 71·0735 for C_4H_9N); $H_3C-\overset{+}{N}H{=}CH-Me$ (base peak) measured 58·0640 calculated 58·0657 for C_3H_8N); 45; 42; 30. The synthesis of tetramethylputrescine was achieved according to the method of Keil [4]. The natural abundance ^{13}C NMR spectra were determined on a Fourier transform spectrometer operating at 25·15 MHz. The spectral width was 100 ppm using 8192 data points and 450–36·500 accumulations. The pulse length was 10 μsec (*ca* 45°) at a pulse interval of 2 sec. The samples were dissolved in D_2O, and the solvent deuterium provided the lock signal. Dioxane was used as an internal standard. Chemical shifts, positive when towards low field, are referred to tetramethylsilane by taking this to be −67·39 ppm from dioxane. The shifts are estimated to be accurate to 0·1 ppm.

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