Phytochemistry, 1970, Vol. 9, pp. 655 to 657. Pergamon Press. Printed in England

SHORT COMMUNICATION

THE ISOLATION AND SYNTHESIS OF DOLICHOTHELINE[1]

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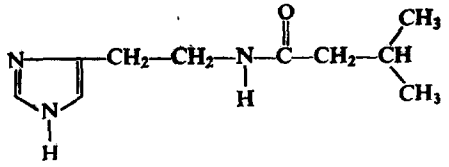
(*Received* 14 *July* 1969)

Abstract—An imidazole alkaloid named *dolichotheline* has been isolated from *Dolichothele sphaerica* (Dietrich) Britton and Rose, and characterized as 4(5)-[2-*N*-isovalerylaminoethyl]imidazole (*N*-isovalerylhistamine) (I). Its structure has been confirmed by synthesis and a biogenetic pathway is proposed.



(I)

INTRODUCTION

WITH the exception of a single published account of an unknown alkaloid,[2] the genus *Dolichothele* (family Cactaceae) has been void of phytochemical investigation. Preliminary TLC analysis of a crude extract from *Dolichothele sphaerica*, a small cactus indigenous to southern Texas and northern Mexico, revealed a relatively high concentration of material staining yellow–gold with tetrazotized benzidine reagent.[3] The following report relates to the isolation, characterization and synthesis of this alkaloid which we have named dolichotheline.

RESULTS AND DISCUSSION

The isolated crystalline material obtained from the condensed non-phenolic fraction melted at 130–131°. The new compound is soluble in water and ethanol, moderately soluble in chloroform, and insoluble in ether. With a modified Pauly's reagent, dolichotheline gave the characteristic red coloration of nonsubstituted ring-nitrogen imidazoles.[4–6] From analytical data and molecular weight determination by mass spectroscopy, the molecular formula, $C_{10}H_{17}N_3O$ was assigned to the compound. Dolichotheline forms a picrate ($C_{16}H_{20}N_6O_8$), m.p. 150–152° and an acetate ($C_{12}H_{19}N_3O_2$), m.p. 76–78°.

The presence of a monosubstituted amide function was indicated by a strong peak at 1640 cm^{-1} in the i.r. spectrum. The u.v. spectrum, however, proved to be of little value as no characteristic absorption maxima were observed. This is in agreement with the general behavior of imidazoles not possessing a carbonyl function in conjugation with the imidazole

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ring.[7] The NMR spectrum integrated for seventeen protons. Signals for two aromatic protons appeared as singlets at 7·52δ and 6·78δ and were assigned to the 2 and 4(5) positions respectively of the imidazole nucleus.[8] A broad band at 7·93δ, which exchanged with D_2O, represented the imino hydrogen while the methylene group adjacent to the imidazole ring showed a triplet centered at 2·77δ ($J = 7$ cps). Signals at 0·88δ ($J = 5$ cps) and 0·95δ ($J = 3$ cps) appeared as a doublet of doublets and represent the protons of the 2 methyl groups of the isovaleryl radical. A multiplet at 1·97δ representing three protons was assigned to the methylene and methine group of the isovaleryl moiety. The remaining three protons, the amido hydrogen and the adjacent methylene group, appeared as a multiplet of 3·40δ.

Hydrolysis of dolichotheline with 10% HCl yielded an acid and an amine. The i.r. spectrum of the sodium salt of the acid was identical with that of sodium isovalerate. The amine, isolated as the dihydrochloride, was identical to histamine dihydrochloride with respect to the i.r. spectrum, m.p. and elemental composition.

Additional evidence for the identity of the alkaloid was provided by high resolution mass spectral data. Dolichotheline showed a strong molecular ion peak at *m/e* 195, corresponding to I and confirmed the empirical formula $C_{10}H_{17}N_3O$, with an accurate mass of 195·1382 (calc. 195·1372). The isovaleryl side-chain led to simple acyl cleavage ($M-C_4H_9$) and a McLafferty rearrangement ($M-C_3H_6$).[9] Further, there was a strong peak at *m/e* 85 corresponding to the isovaleryl portion and one at *m/e* 111 corresponding to the amine. The fragmentation pattern of the isolated amine was identical with that reported for histamine[10] and confirmed the empirical formula $C_5H_9N_3$ with an accurate mass of 111·0792 (calc. 111·0796).

N-Isovalerylhistamine was synthesized by refluxing histamine with isovaleric anhydride.[11] The resulting crystalline base proved to be identical to natural dolichotheline with respect to m.p., R_f value on TLC, and i.r. spectrum. The m.p. and i.r. spectra of its picrate and acetate derivatives were also identical to those of natural dolichotheline.

In the biogenesis of dolichotheline the two amino acids histidine and leucine could play major roles. Histidine would be decarboxylated to histamine[12] and this reacts with isovaleryl CoA to yield dolichotheline. The isovaleryl radical could arise from α-keto-isocaproic acid which could be formed by oxidative deamination of leucine[13] or by oxidative decarboxylation of α-hydroxy-β-carboxyisocaproic acid.[14]

Dolichotheline establishes the existence of an imidazole nucleus in cactus alkaloids in addition to the known β-phenethylamine and tetrahydroisoquinoline structures. Although the imidazole nucleus occurs quite widely in nature, few of these known alkaloids (*N*-cinnamoylhistamine,[15] *N*-acetylhistamine,[16] *N*-α-(4-oxodecanoyl)-histamine[17] and casimiroedine[18]) occur in amide linkage with histamine.

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EXPERIMENTAL

Instrumentation

Melting points (corrected) were determined on a Fisher–Johns melting-point apparatus. Ultra-violet spectra were measured on a Beckman DK-2A ratio recording spectrophotometer. Infra-red spectra were recorded on a Perkin–Elmer 337 grating i.r. spectrophotometer employing the solid KBr pellet technique. NMR spectra were taken on a Varian A-60 instrument in deuterated MeOH with tetramethylsilane as the internal standard. The high resolution mass spectra were obtained through the courtesy of the Hoffmann-LaRoche Inc., on a CEC-21-110 spectrometer at 70 eV, using a photoplate. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Source of Plant Material

Living plants of *Dolichothele sphaerica* were obtained from the University of Michigan Botanical Gardens for the initial TLC screen. Subsequent larger quantities of the cactus were purchased from the El Paso Cactus Gardens. Sample specimens were potted and serve as reference plants.

Extraction and Isolation Procedures

The cactus plants were sliced, dried, ground (1 kg) and freed from lipids by exhaustive Soxhlet extraction with 30–60° light petroleum for 36 hr. The marc, after air-drying and basification, was extracted with $CHCl_3$ for 72 hr. The $CHCl_3$ extract was evaporated *in vacuo* to a syrupy residue and 1 l. 1 N HCl added. After filtration, the acid extract was extracted with $CHCl_3$ until the organic layer was colorless. The aqueous solution was then made basic to a pH 9·5 (NH_4OH) and extracted several times with equal portions of $CHCl_3$. The basified aqueous extract was further submitted to extraction with hot $CHCl_3$ in a liquid–liquid extractor for 24 hr. The combined $CHCl_3$ extracts were evaporated to dryness *in vacuo* and passed through an ion-exchange column to separate non-phenolic from phenolic fractions.[19] The addition of benzene:acetone (1:1) to the condensed non-phenolic extract caused the crystallization of dolichotheline, m.p. 130–131° (yield 0·7%). The compound was found to be homogeneous by TLC (R_f 0·24 in 2-butanone:*N*,*N*-dimethylformamide:NH_4OH, 13:1·9:0·1). (Found: C, 61·57; H, 8·73; N, 21·55. $C_{10}H_{17}N_3O$ required: C, 61·51; H, 8·77; N, 21·52%.)

Preparation of Derivatives

Acetate. Dolichotheline was dissolved in acetic anhydride and pyridine was added. The acetate derivative was recrystallized from ether and the product had a m.p. 76–78°. (Found: C, 60·65; H, 8·09; N, 17·70. $C_{12}H_{19}N_3O_2$ required: C, 60·74; H, 8·07; N, 17·71%.)

Picrate. Picrate derivative, m.p. 150–152°. (Found: C, 44·69; H, 4·68; N, 19·55. $C_{16}H_{20}N_6O_8$ required: C, 45·28; H, 4·75; N, 19·80%.)

Hydrolysis. Dolichotheline (300 mg) was refluxed for 24 hr in 10% HCl (10 ml). The hydrolysate was distilled at 40° under vacuum and 5 ml collected. The distillate was neutralized (1 N NaOH) and evaporated to dryness. The residue, a mixture of NaCl and the sodium salt of the acid, was used directly for the i.r. spectrum of the acid. An identical procedure was used to prepare sodium isovalerate.

The remaining hydrolysate was adjusted to pH 9·5 (7·5 N NaOH) and then freeze-dried. The solid was Soxhlet extracted with $CHCl_3$ for 24 hr, the extract evaporated and the residue dissolved in MeOH. Dry HCl gas was bubbled through the solution and the addition of Et_2O gave crystalline amine dihydrochloride (yield 110 mg, m.p. 227–231°). (Found: C, 32·63; H, 6·01; N, 22·74; Cl, 38·55. Calc. for $C_5H_{11}N_3Cl_2$: C, 32·62; H, 6·02; N, 22·83; Cl, 38·53%.)

Synthesis of N-*isovalerylhistamine*

Isovaleric anhydride (1 g, from isovaleric acid with acetic anhydride[20]) and histamine (380 mg) were refluxed for 1 hr. Several drops of water were added and the mixture condensed on a steam bath to a thick, oily residue. The addition of acetone caused the crystallization of *N*-isovalerylhistamine which was recrystallized from EtOH–Et_2O (yield 323 mg, m.p. 130°). Picrate and acetate derivatives were prepared as described above.

Acknowledgements—The authors wish to thank Dr. Fortüne Kohen, College of Pharmacy, The University of Michigan, for the NMR determinations, and Dr. W. Benz, Hoffmann-La Roche Inc., Nutley, N.J., for the high resolution mass spectral data.

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