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VIBURNINE, A MACROCYCLIC SPERMIDINE ALKALOID FROM VIBURNUM RHYTIDOPHYLLUM

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تم فصل والتعرف على مركب قلويدى جديد (فيبيورنين) من ازهار نبات الفيبيورنوم ريتيدوفيلوم المنزرع فى مصر هذا بالاضافة إلى خمس مركبات أخرى معروفة هى هنريوسيد ، سالسين ، أربيوتين ، كاتيشين وحمض الأورسيليك. وقد تم التعرف على المركبات المفصولة باستخدام طرق التحليل الطيفية المختلفة بالاضافة إلى مقارنة بعض هذه المركبات بعينات مرجعية.

A new macrocyclic spermidine alkaloid; viburnine was isolated from the flowers of Viburnum rhytidophyllum Hessel cultivated in Egypt together with five known compounds; henryoside, salicin, arbutin, catechin and ursolic acid. The identification of the isolated compounds was carried out by spectral analysis and / or comparison with authentic samples.

INTRODUCTION

The members of the genus *Viburnum* (F. *Caprifoliaceae*) are attractive ornamental shrubs growing in North and Central America, North Africa and East Asia. *V. rhytidophyllum* Hessel is an ornamental shrub cultivated in Egypt. The plants of the genus *Viburnum* have many folkloric medicinal uses as antispasmodic, anthelmintic for children, a remedy for tapeworm in animals, emmenagogue and as menorrhagia as well as in post partum haemorrhage[1-5].

A number of coumarins, phenolic glycosides, iridoides, triterpenes and other constituents were isolated from some plants belonging to the genus *Viburnum*[6-18]. However, there is no report traced on the presence of alkaloids in this genus.

We report herein detailed studies on the isolation and structural elucidation of a new alkaloid.

EXPERIMENTAL

General experimental procedure

Melting Points (uncorr.) were determined by Koffler hot stage microscope, type (ESP, Boetius M.) ^{1}H-NMR (90 MHz) and ^{13}C-NMR (22.4 MHz) spectra were recorded by Bruker instrument in $CDCl_3$ and CD_3OD using TMS as internal standard. IR spectra were determined using Unicam Sp-1025 infra red Spectrometer in KBr. UV spectra are determined in Unicam Sp-1750 ultra-violet Spectrometer. MS were recorded at 70 ev using direct inlet system with high resolution MS-50 Kratos A.E.I. Column chromatography was performed on Silica gel (70-230, E. Merck). while TLC using silica-gel G_{60} (E. Merck) and PC using whatman paper No.1 The following solvent systems were used

I- $CHCl_3$-MeOH	(90:10)
II- $CHCl_3$-MeOH-H_2O	(80:19:1)
III- $CHCl_3$-MeOH-H_2O	(70:27:3)
IV- Butanol-HOAc- H_2O	(4 :1 :2)

10% H_2SO_4 and Dragendorff's reagent are used for locating the spots over chromatoplates.

Plant material

The material used was obtained from cultivated plants collected from El-Orman Garden, Cairo. The identity of the plant was confirmed by Prof. Dr. N. El-Hadidi, Faculty of Science, Cairo University. A voucher sample is kept in the Faculty of Pharmacy, Assiut University.

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Extraction and isolation

Air-dried powdered flowers (1.5 kg) was defatted with petrol (60-80°, 3 liters x4) followed by exhaustive extraction with MeOH (3 liters x 4). The MeOH extract was evaporated under reduced pressure till nearly dry (35 g). 10 g of the dried crude MeOH extract were chromatographed over silica gel column (400 g, 7 x 120 cm). Gradient elution was performed with chloroform/ methanol collecting 50 ml fractions which were separately monitored by TLC. Fractions 20-30 eluted with $CHCl_3$ revealed a single spot (R_f 0.42, system I) and acquired positive colour with Dragedorff's; upon repeated purification afforded compound 1 (20 mg). Fractions 50-60 eluted with $CHCl_3$-MeOH (90:10) afforded a mixture of compounds which was separated to compounds 2 and 3 by preparative TLC; fractions 67-71 eluted with $CHCl_3$-MeOH (85:15) afforded componds 4 and 5 isolated by preparative TLC, while fractions 92-112 eluted with $CHCl_3$-MeOH (80:20) afforded compound 6 only.

Compound 1: Amorphous powder mp. 106-108°, $[\alpha]_D^{20}$ -2.6 (C=0.1, MeOH), UV λ_{max}^{MeOH} nm (log ϵ) 217(4.37), 222(4.28), 274(4.49).

IR $\nu_{max}^{CHCl_3}$ 3400-3240 (NH), 1655 (α,ß-unsaturated amide) and 1605, 1595 cm^{-1} (aromatic ring).

^{1}H-NMR ($CDCl_3$) δ 7.76 (d, J= 15.5 Hz, H-8"); 6.86 (d, J= 15.5 Hz, H-7"), 6.88-7.48 (m, aryl-H, 10 H), 2.73-3.48 (9 H, m), 2.12 (2H, br.s), 2.08 (3H, s, CH_3CON), 1.32-1.84 (8H, m); HRMS, m/z (% rel. int.) 447.2522 (Calc. for $C_{27}H_{33}N_3O_3$ 447.2521) (2), 375.2364 ($C_{24}H_{27}N_2O_2$) (3), 316.2028 ($C_{18}H_{26}N_3O_2$) (5), 290.187 ($C_{16}H_{24}N_3O_2$) (4), 287.1763 ($C_{17}H_{23}N_2O_2$) (2), 273.1603 ($C_{16}H_{21}N_2O_2$) (2), 260.1502 ($C_{15}H_{20}N_2O_2$) (3), 247.1654 ($C_{14}H_{19}N_2O_2$) (5), 231.1444 ($C_{14}H_{19}N_2O$) (10), 202.1211 ($C_{13}H_{16}NO$) (5), 200.1077 ($C_{13}H_{14}NO$), 186.0916 ($C_{12}H_{12}NO$) (4), 174.0915 ($C_{11}H_{12}NO$) (11) and 131.0491 (C_9H_7O), ^{13}C-NMR are cited in Table 1.

Table 1: ^{13}C-NMR Chemical Shifts for Viburnine (22.4 MHz, $CDCl_3$).

Carbon No.	ppm
C-2	47.6 [a]
C-3	26.1/26.4+
C-4	46.6 [a]
C-6	171.2
C-7	31.3
C-8	60.4/60.8+
C-10	48.2 [a]
C-11	27.6
C-12	28.1
C-13	47.2 [a]
C-1'	134.0
C-2', C-6'	128.1 [b]
C-3', C-5'	127.6 [c]
C-4'	129.5 [d]
C-1"	134.9
C-2", C-6"	128.2 [b]
C-3", C-5"	127.1 [c]
C-4"	129.8 [d]
C-7"	140.7
C-8"	121.2
C-9"	167.1
CH_3CO	170.1/23.4/170.7/23.3+

a-d: These assignments are interchangeable.
+ : Doubling of peaks (see discussion).

Ursolic acid (2): (500 mg) mp. 255°, IR $\nu_{max}^{CHCl_3}$, 3400, 2600-2300 cm^{-1} and further confirmation was done with an authentic sample.

Catechin (3): (200 mg), its physical and spectral data are similar with those reported[18].

Arbutin (4) (300 mg) mp. 200° and spectral data are identical to those reported[8,13].

Salicin (5): (1g) identical (^{1}H-NMR, mp and mmp) with an authentic sample.

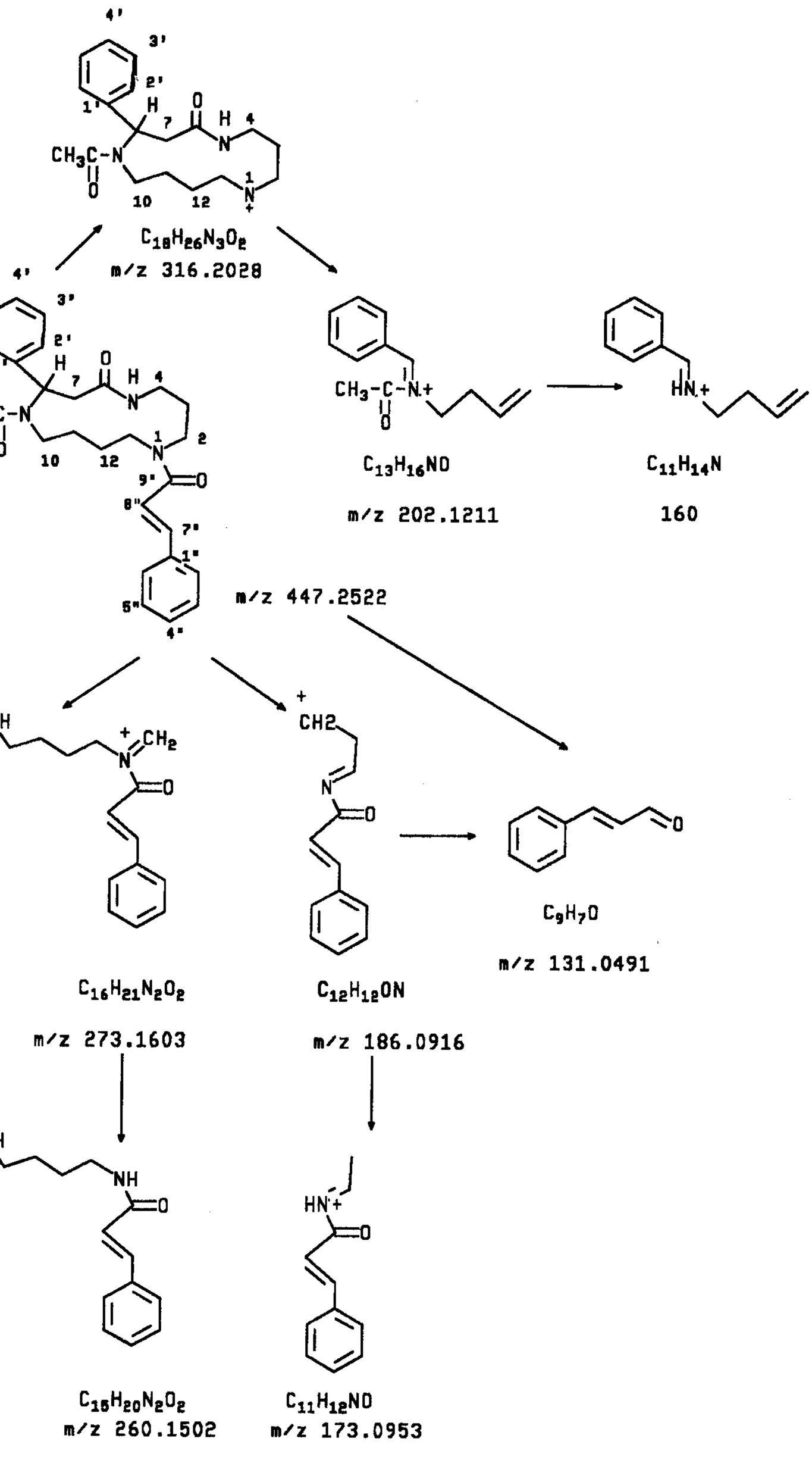

Fig. 1: Possible Fragmentation Pattern of Compound (1)

Henryoside (6), (20 mg), crystallised from EtOH, mp. 128-130°, $[\alpha]_D^{20}$-48° (C 0.4; MeOH). The spectral data are identical to those reported[9].

RESULTS AND DISCUSSION

The alkaloid under investigation is characterised by the presence of 13-membered ring, represents a new variant of the few known macrocyclic lactam alkaloids derived from spermidine. Detection of viburnine in *V. rhytidophyllum* confirms the fact that these alkaloids do not seem to be of specific occurrence in a particular plant family.

Viburnine: The molecular formula $C_{27}H_{33}N_3O_3$ was determined on the basis of high resolution mass spectrum. The UV spectrum displayed maxima at 217, 222 sh and 274 nm; these values are very close to those of celacinnine, a macrocyclic spermidine alkaloid isolated from *Maytenus serrata*[19].

The IR spectrum showed broad band at 3340-3240 (NH), 1655 (α,ß-unsaturated amide) 1605 and 1590 cm^{-1} (aromatic rings).

The ^{1}H-NMR spectrum of [1] showed few distinctive signals, it showed a pair of doublet (J= 15.5 Hz) at δ 7.76 and 6.86 assigned to the olefinic protons of the trans cinnamic acid residue in addition to signals for two monosubstituted aromatic rings between δ 7.48~6.88. These signals together with ultra-violet absorption bands at λ_{max}^{MeOH} 274 nm suggests the presence of a trans-cinnamyl group[19,20]. The multiplet between δ 1.32~1.84 (8H,m) was assigned to the three methylene groups CH_2-3, CH_2-12 and CH_2-13, singlet at δ 2.08 (3H,s, CH_3-CO), broad singlet at 2.12 (2H, brs, CH_2-7) and multiplet between 2.73-3.48 (9H,m) for methylene groups adjacent to nitrogen (CH_2-2,4,10 and CH_2-13) and CH-8 adjacent to nitrogen.

The macrocyclic structure of (1) was ultimately assigned to viburnine on the basis of high resolution mass spectral data and by comparing the ^{13}C-NMR spectral data (Table 1) with those of other spermidine alkaloids[19,20].

The HRMS showed peak at m/z 146 (C_9H_8NO) and a base peak at m/z 131 (C_9H_7O) characteristic for the cinnamyl moiety[21] and subsequent cleavage of N-C bond respectively. Peak at m/z 375 (M^+-C_3H_6NO) attributed to a double cleavage in which the ring amide and either the C(7)-C(8) bond or C(4) methylene groups are lost, provide additional support for the proposed structure. A significant peaks at m/z 316, ($C_{18}H_{26}N_3O_2$), (M^+-C_9H_7O) formed by the cleavage of N(1)-C(9") and m/z 202 ($C_{13}H_{16}NO$) formed by the cleavage of the liable bond C(7)-C(8) followed by the N(1)-C(13) bond and hydrogen transfer. This fragment gave m/z 160 ($C_{11}H_{14}N$) through the loss of acetyl group and this confirms structure [1] of viburnine as a celacinnine skeleton but not dihydroperiphylline skeleton[21-27]. The principal fragmentation of the alkaloid is shown in Fig. 1.

The complete set of ^{13}C-NMR data is compiled in Table 1. ^{1}H and ^{13}C-NMR spectra showed doubling of some peaks which may be due to slowly interconverting conformers of the N-acetate; a similar phenomenon has been reported by other authors in amides[28-32]. However, the observed doubling of the signals could also be due to the existence of conformers in solution as a result of the flexibility of the macrocyclic molecule.

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