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Aspertins A-D: Further Piperidine Alkaloids from *Andrachne aspera* (Euphorbiaceae)

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The aerial parts of *Andrachne aspera* belonging to the family Euphorbiaceae yielded four new piperidine alkaloids: aspertin-A (**1**), aspertin-B (**2**), aspertin-C (**3**) and aspertin-D (**4**). The structures of isolated alkaloids were elucidated by NMR, mass spectrometery and chemical means.

Key Words: *Andrachne aspera*, Euphorbiacae, Aerial parts, Piperidine alkaloids, Aspertins A-D, Spectroscopy.

Introduction

Andrachne aspera, a small perennial undershrub, is commonly found in the arid, stony and sandy regions of Pakistan[1]. Medicinally, this plant is used to improve eyesight and to treat eye sores[2−3]. A crude alkaloidal mixture has been reported to show various biological activities[4]. The biological significance of *A. aspera* motivated us to reinvestigate for further alkaloidal constituents fourteen years later[5−6]. The plant material was again collected from the same site (Karachi University campus). Unfortunately, it was not possible to obtain sufficient amounts of aspertins A-D for biological screening. The present communication deals with the isolation and structure elucidation of four new alkaloids (aspertins A-D) using NMR and mass spectrometric techniques.

Experimental

General experimental: The IR spectra were recorded on a JASCO A-302 spectrophotometer. HRMS spectra were scanned on a Finnigan MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. The NMR spectra were recorded on Bruker AM-300 and 500 spectrometers with TMS as an internal reference. The optical rotations were recorded on a Polartronic Universal Austratim standard K-157 digital polarimeter.

Plant material: The plant material (aerial parts) was collected from Karachi University campus during the summer (June, 1999) and identified by Prof. Khalida Khatoon, of the Department of Botany of the same university, where a voucher specimen is deposited in the herbarium.

Extraction and isolation: The fresh plant material (11.5 kg) was dried under shade for six days and then chopped and soaked in ethanol (17 L) at room temperature for a period of three days. After filtration and evaporation, the concentrated crude residue (158 g) was diluted with distilled water, acidified with 1% HCl and partitioned between $CHCl_3$ and H_2O. The aqueous phase was then basified with dilute NH_3 (pH 8-9), extracted with $CHCl_3$ until the Dragendorff's reagent gave a negative result. Evaporation of solvent *in vacuo* yielded a gummy brown residue (7.1 g). The crude material was then chromatographed on a silica gel column eluted with chloroform and methanol (3:1). Repeated column chromatography afforded a fraction showing two oval spots on TLC, which appeared on spraying with Dragendorff's reagent. This was re-chromatographed by low pressure liquid chromatography using a Lobar column (Lichroprep Si 60) using the same [chloroform and methanol (3:1)] mobile phase. As a result of this, four compounds (**1-4**) with minor R_f values were purified as gum.

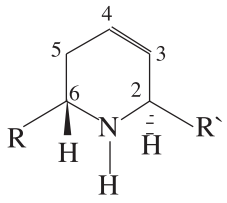
Aspertin-A (**1**): Yield: 11.3 mg, 0.00009%; $[\alpha]_D$: - 103^o (c 0.5, $CHCl_3$); IR ($CHCl_3$): 3315 (br., OH), 1630 (C=C) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.88 (1H, m, H-4), 5.67 (1H, dd, J=10.2, 1.2 Hz, H-3), 4.12 (1H, br.s, H-2), 3.89 (2H, br.s, H-2′ and H-2″), 2.89 (1H, br.s, H-6), 1.03 (3H, t, J=7.2 Hz, H-4′) and 0.92 (3H, t, J=7.2 Hz, H-5″); ^{13}C-NMR ($CDCl_3$,125 MHz): δ 55.1 (C-2), 136.5 (C-3), 128.6 (C-4), 34.0 (C-5), 46.8 (C-6), 39.1 (C-1′), 69.8 (C-2′), 30.8 (C-3′), 10.7 (C-4′), 40.1 (C-1″), 70.3 (C-2″), 39.8 (C-3″), 18.7 (C-4″) and 14.1 (C-5″); EI-MS: see Fig. 1; FD-MS: m/z 241; HRMS: m/z 241.20885 (m/z 241.204168 calcd. for $C_{14}H_{27}NO_2$), 212.15946 (m/z 212.165044 calcd. for $C_{12}H_{22}NO_2$), 182.15671 (m/z 182.154481 calcd. for $C_{11}H_{20}NO$), 168.13922 (m/z 168.138832 calcd. for $C_{10}H_{18}NO$).

Aspertin-B (**2**): Yield: 12.7 mg, 0.00011%; $[\alpha]_D$: -85^o (c 0.8, $CHCl_3$); IR ($CHCl_3$): 3100-3200 (br., OH), 1635 (C=C) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.55 (1H, m, H-4), 5.40 (1H, dd, J=10.3, 1.4 Hz, H-3), 4.10 (1H, br.s, H-2), 2.79 (1H, br.s, H-6), 3.81 (2H, br.s, H-2′ and H-2″), 0.90 (3H, t, J=7.4 Hz, H-4′) and 0.86 (3H, t, J=7.4 Hz, H-6″); ^{13}C-NMR ($CDCl_3$, 75 MHz) : δ 55.9 (C-2), 136.5 (C-3), 129.5 (C-4), 33.2 (C-5), 46.7 (C-6), 39.8 (C-1′), 70.5 (C-2′), 30.3 (C-3′), 10.4 (C-4′), 41.3 (C-1″), 68.9 (C-2″), 38.4 (C-3″), 20.1 (C-4″), 14.6 (C-5″) and 13.6 (C-6″); EI-MS: see Fig. 2; FD-MS: m/z 255; HRMS: m/z 255.22048 (m/z 255.219817 calcd. for $C_{15}H_{29}NO_2$), 212.16243 (m/z 212.165044 calcd. for $C_{12}H_{22}NO_2$); 182.15321 (m/z 182.154481 calcd. for $C_{11}H_{20}NO$), 168.13921 (m/z 168.138832 calcd. for $C_{10}H_{18}NO$).

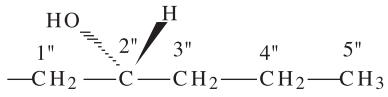
Aspertin-C (3): Yield: 9.9 mg, 0.00008%; $[\alpha]_D$: - 89^o (c 0.6, $CHCl_3$); IR ($CHCl_3$): 3190-3310 (br., OH) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.85 (2H, br.s, H-2′ and H-2″), 2.99 (2H, br.s, H-2, H-6) and 0.89 (6H, t, J=7.4 Hz, H-5′ and H-5″); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 52.1 (C-2), 34.4 (C-3), 18.7 (C-4), 34.4 (C-5), 52.6 (C-6), 40.0 (C-1′), 71.1 (C-2′), 40.0 (C-3′), 18.7 (C-4′), 13.9 (C-5′), 40.4 (C-1″), 71.0 (C-2″), 40.0 (C-3″), 18.7 (C-4″) and 13.9 (C-5″); EI-MS: see Fig. 3; FD-MS: m/z 257; HRMS: m/z 257.23359 (m/z 257.235466 calcd. for $C_{15}H_{31}NO_2$), 214.17952 (m/z 214.180694 calcd. for $C_{12}H_{24}NO_2$), 170.15430 (m/z 170.154481 calcd. for $C_{10}H_{20}NO$).

Aspertin-D (4): Yield: 11.3 mg, 0.00009%; $[\alpha]_D$: - 97^o (c 0.8, $CHCl_3$); IR ($CHCl_3$): 3210 (br., OH) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.85 (1H, m, H-2′), 4.10 (1H, m, H-2″), 3.02 (2H, br.s, H-2 and H-6) and 0.89 (6H, t, J=7.0 Hz, H-4′ and H-5″); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 53.0 (C-2), 33.3 (C-3), 18.9 (C-4), 34.2 (C-5), 52.4 (C-6), 38.4 (C-1′), 71.8 (C-2′), 30.4 (C-3′), 9.2 (C-4′), 39.6 (C-1″), 71.2 (C-2″), 40.2 (C-3″), 18.1 (C-4″) and 14.0 (C-5″); EI-MS: see Fig. 4; FD-MS: m/z 243; HRMS: m/z 243.22085 (m/z 243.219817 calcd. for $C_{14}H_{29}NO_2$), 200.17082 (m/z 200.165044 calcd. for $C_{11}H_{22}NO_2$), 170.15514 (m/z

170.154481 calcd. for $C_{10}H_{20}NO$), 156.13876 (m/z 156.138832 calcd. for $C_9H_{18}NO$).



(Aspertin-A) **1** R=



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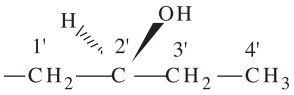
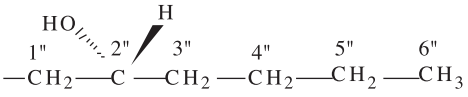
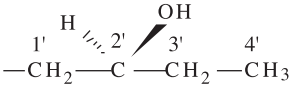
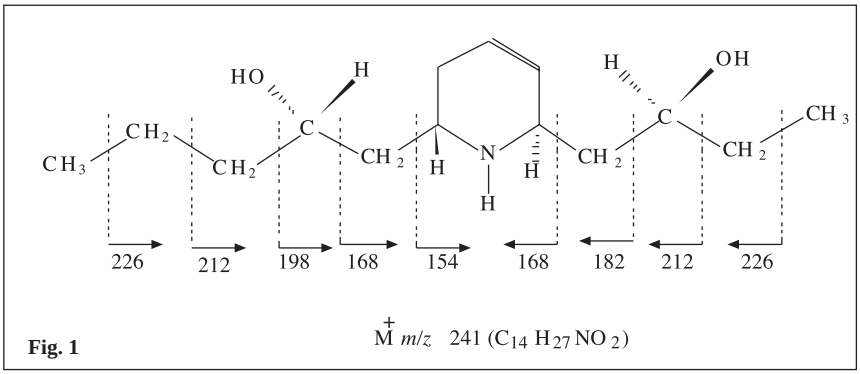


(Aspertin-B) **2** R=



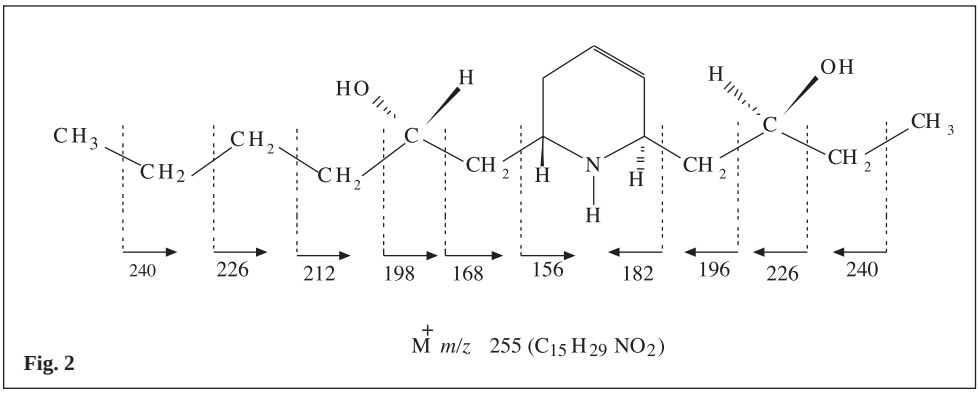
R`=





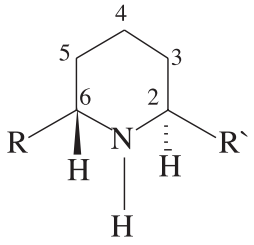
$\overset{+}{M}$ *m/z* 241 ($C_{14}H_{27}NO_2$)

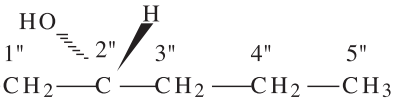
Fig. 1

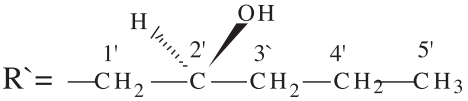


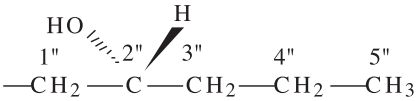
$\overset{+}{M}$ *m/z* 255 ($C_{15}H_{29}NO_2$)

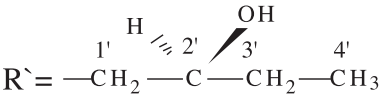
Fig. 2

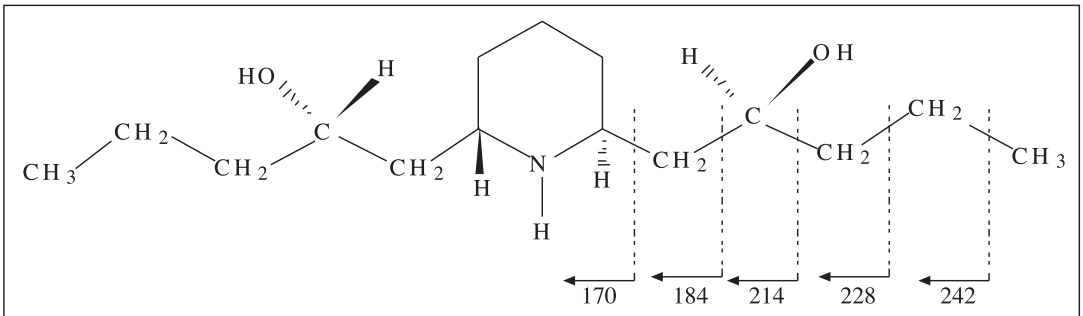


(Aspertin-C) **3** R= 

R`= 

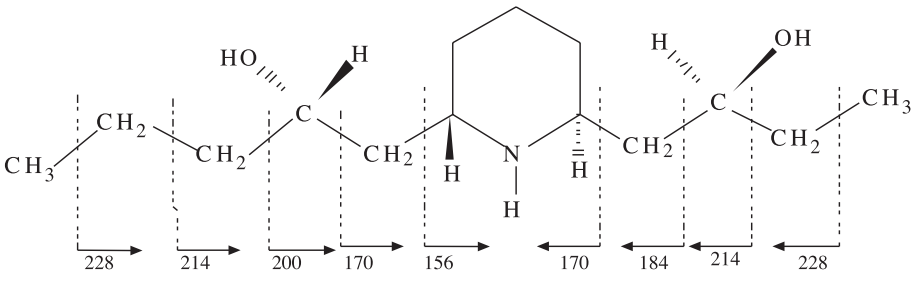
(Aspertin-D) **4** R= 

R`= 



M^{+} *m/z* 257 ($C_{15}H_{31}NO_2$)

Fig. 3



M^{+} *m/z* 243 ($C_{14}H_{29}NO_2$)

Fig. 4

Determination of absolute configuration by Horeau's method

The reaction vessel was charged with sample (2.0 mg each), 0.4 ml dry pyridine, and 2 M equivalent of (±) 2-phenylbutanoic anhydride and left to stand for 10 h at room temperature. The reaction mixture was then neutralized by 0.1 M NaOH and extracted with EtOAc. The aq. layer was then acidified with 1 M HCl and extracted with benzene (10 ml). The volume of benzene extract was adjusted to one ml. The optical rotation of 2-phenylbutanoic acid in solution was found to be positive, establishing the *S*-configuration of both hydroxyl groups at 2′ and 2″.

Results and Discussion

The ethanolic soluble part of *A. aspera* yielded four new *trans*-2,6-disubstituted-piperidine alkaloids (**1-4).** Their structures were elucidated by means of NMR and mass spectrometric methods. As all the isolated compounds have the same carbon skeleton, they are discussed here together.

Compounds **1** and **2** have the same structures except an additional methylene in the side chain of **2**. The IR spectra of both compounds showed the absorptions due to the presence of hydroxyl and olefinic functions [(**1**): 3315 (OH), 1630 (C=C); (**2**): 3100-3200 (OH), 1635(C=C) cm^{-1}]. The FDMS of **1** and **2** showed the molecular masses 241 and 255 a.m.u., and their corresponding formulae were determined through HRMS to be $C_{14}H_{27}NO_2$ and $C_{15}H_{29}NO_2$, respectively. Both formulae show the presence of two degrees of unsaturation in the molecules. Out of two, one degree of unsaturation should be due to the olefinic function, which was confirmed with the aid of IR spectra. Similarly, the presence of nitrogen in the molecules was counterchecked with the element detection method by fusing the samples with the sodium metal and also by spraying Dragendorff's reagent. The formula of **2** has an additional CH_2 in the molecule compared to **1**. The fragments appearing in the EI spectra of **1** and **2** are explained in Figs. 1 and 2, respectively.

The presence of a double bond in **1** and **2** was confirmed by the appearance of signals at δ 5.88 (m, H-4), 5.67 (dd, J=10.2, 1.2 Hz, H-3), 136.5 (C-3), 128.6 (C-4) and 5.55 (m, H-4), 5.40 (dd, J=10.3, 1.4 Hz, H-3), 136.5 (C-3), 129.5 (C-4), respectively, in the NMR spectra. The position of double bond was confirmed by decoupling and COSY experiments. The broad singlets at δ 3.89 (H-2′, H-2″) in **1** and 3.81 (H-2′, H-2″) in **2** were due to the carbinylic protons. Their corresponding carbons appeared at δ 69.8 (C-2′), 70.3 (C-2″) and 70.5 (C-2′), 68.9 (C-2″), respectively.

The two sharp triplets in **1** at δ 1.03 (H-4′) and 0.92 (H-5″), and in **2** at δ 0.90 (H-4′) and 0.86 (H-6″) were due to the methyls attached to the methylenes, and their corresponding carbon signals resonated at δ 10.7 (C-4′), 14.1(C-5″), 10.4 (C-4′) and 13.6 (C-6″), respectively

The signals due to H-2 and H-6 appeared at δ 4.12 (H-2), 2.89 (H-6) in **1**, and 4.10 (H-2), 2.79 (H-6) in **2** as very broad singlets. The broadness of these signals confirmed the diaxial *trans*-configuration of alkyl chain substitution at C-2 and C-6. The *trans*-configuration in sedenine had already been confirmed by x-ray crystallography[7].

Compounds **3** and **4** have almost identical chemical shifts in the NMR spectra and are given in the experimental section. They are different with respect to the carbon chain-lengths but have no double bond in the piperidine ring. The signals appearing at δ 18.7 and 18.9 in the carbon spectra of **3** and **4**, respectively, were assigned to C-4, and comparison of these values with the ^{13}C chemical shifts reported in the literature for C-4 *cis* and *trans*-2,6-disubstituted piperidine derivatives led to the assignment of *trans* configuration in **3** and **4**[8]. The lengths of the side chains in **3** and **4** were confirmed by spectrometry. The complete

fragmentation patterns of **3** and **4** are given in Figs. 3 and 4.

Application of Horeau's method[9] on **1-4** led to the isolation of (+) 2-phenylbutanoic acid. If we assume that the racemic 2-phenylbutanoic anhydride reacts with the OH groups at the $2'$ and $2''$ positions of alkyl side chains with equal optical yields due to similar environment, then it may be concluded that the absolute configuration at both these centers would be S.

The complete spectral data of **1-4** are given in the Experimental section and fragmentation patterns in Figs. 1-4. On the basis of their origin, the names of compounds **1-4** are given as aspertin-A(**1**), aspertin-B(**2**), aspertin-C(**3**) and aspertin-D(**4**). It should be clarified here that, due to insufficient amounts the bioactivity of **1-4** could not be determined.

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