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7,8-DIHYDROAJUGASTERONE C, NORHYGRINE AND OTHER CONSTITUENTS OF *NIEREMBERGIA HIPPOMANICA*

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Key Word Index—*Nierembergia hippomanica*; Solanaceae; (20*R*,22*R*)-2β,3β,11α,14α,20,22-hexahydroxy-5β-cholestan-6-one (7,8-dihydroajugasterone C, 7,8-dihydro-11α-hydroxyponasterone A); ecdysterone; norhygrine.

Abstract—The new ecdysteroid-like polyhydroxyketosterol (20*R*,22*R*)-2β,3β,11α,14α,20,22-hexahydroxy-5β-cholestan-6-one (7,8-dihydroajugasterone C or 7,8-dihydro-11α-hydroxyponasterone A) and the new pyrrolidine alkaloid, norhygrine, were identified as constituents of whole plants of *Nierembergia hippomanica*, together with the known compounds, ecdysterone, quercetin 3-*O*-β-D-galactopyranoside, D-mannitol, choline and tigloidine.

INTRODUCTION

Nierembergia hippomanica is a plant native to Argentina, well known for its toxicity to cattle. We have previously reported on the structural elucidation of some of its components, the toxicosis by ingestion of this plant [1] and its lethal constituent identified as pyrrol-3-carbamidine [2, 3]. Furthermore, this species also contains the flavonoids, pinocembrin 7-*O*-β-neohesperidoside [4], pinocembrin 7-*O*-β-(2‴-*O*-acetyl)neohesperidoside [5], pinocembrin 7-*O*-β-(3‴-*O*-acetyl)neohesperidoside and pinocembrin 7-*O*-β-(6‴-*O*-acetyl)neohesperidoside [6], the pentacyclic triterpenoids ursolic and oleanolic acid, α- and β-amyrins and uvaol [7], the coumarin scopoletin [7] and the alkaloids, β-phenethylamine, tyramine, *N*-methyltyramine, hordenine and hygrine [7]. This species was shown to be devoid of antimicrobial activity [8].

In continuation of our investigation, we now describe the isolation and identification of ecdysterone **1**, the new (20*R*,22*R*)-2β,3β,11α,14α,20,22-hexahydroxy-5β-cholestan-6-one **2** (7,8-dihydroajugasterone C or 7,8-dihydro-11α-hydroxyponasterone A), the new norhygrine **3**, quercetin 3-*O*-β-D-galactopyranoside, D-mannitol, tigloidine and choline from a methanolic extract of *N. hippomanica*.

RESULTS AND DISCUSSION

The precipitate obtained from a methanolic extract of whole plants of *N. hippomanica* was separated by column chromatography. The ethyl acetate extract of one methanolic fraction (see Experimental) contained the acylated flavonoids [4–6], along with the two terpenoids **1** and **2** (TLC, silica gel: R_f 0.24, **1**; 0.37, **2**; $CHCl_3$–Me_2CO, 35:65; anisaldehyde-sulphuric acid or vanillin-sulphuric acid; green spots). These terpenoids were freed from the flavonoids by column chromatography on alumina. Flavonoids were retained, while compounds **1** and **2** were eluted and further purified by CC.

The UV spectrum of **1** showed a maximum at 242 nm, suggesting an α,β-unsaturated carbonyl group. UV and IR spectra corresponded to a 7-en-6-one of ecdysteroids [9]. On the basis of some test reactions, the spectroscopic data and comparison with an authentic sample, compound **1** was identified as (20*R*,22*R*)-2β,3β,14α,20,22,25-hexahydroxy-5β-cholest-7-en-6-one (ecdysterone, 20-hydroxyecdysone). The ^1H and ^{13}C NMR assignments were in agreement with those previously reported [10].

Compound **2**, present in small quantities, showed properties similar to those of **1**, suggesting the occurrence of a polyhydroxylated steroid. However, no strong UV absorption was observed and the IR spectrum showed the signal of an isolated carbonyl group at 1695 cm^{-1}. The ^1H NMR spectrum of **2** showed a doublet at δ0.82 (6H, J = 6 Hz) of an isopropyl group and three singlets of angular methyls. It is known that the presence of a hydroxyl at C-20 shifts downfield the resonances of Me-21 and Me-18 of an ecdysteroid. Accordingly, the singlets at δ1.53 and 1.23 were assigned to Me-21 and Me-18, respectively. The isopropyl group was assigned to Me-26 and Me-27. The remaining singlet was assigned to Me-19 (δ1.40), even when this methyl appeared downfield from that of an ecdysteroid, indicating a close hydroxylation

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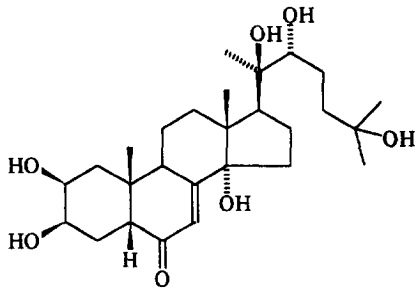

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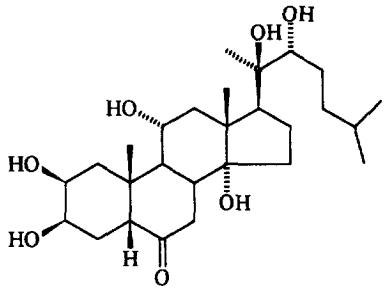


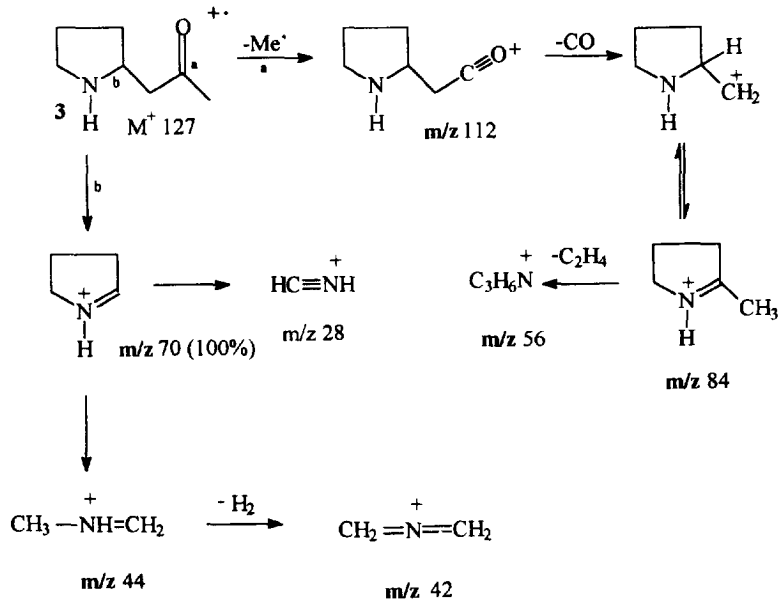
2

[11, 12]. The signal at δ4.68 was attributed to a methine proton bonded to a 11-hydroxyl group. Then, the ^{1}H NMR spectrum of **2** was quite similar to that of ajugasterone C [13], except for the signals of the ring-B protons owing to the absence of the 7-double bond. The α-equatorial orientation of HO-11 was determined on the basis of coupling constants of H-9 and H-12 axial. The α-axial H-9 showed two large coupling constants (J_{ax-ax}) resulting from the axial-axial coupling with the H-8 and H-11 (β)-axial. Likewise, the large coupling constants of H-12 axial were due to its coupling with the geminal proton and with the vicinal β-axial H-11, in agreement with the spectral data of ajugasterone C [13] and other 11-hydroxyecdysteroids. Chemical shifts and coupling constants of the ring-A protons were also similar to those of ajugasterone C [13], suggesting the same syn-orientation for HO-2 (β), HO-3 (β) and H-5 (β). The relative stereochemistry was further confirmed by ^{13}C NMR. The $[M]^+$ of **2** was *m/z* 482 (FAB-mass spectrum) and the EI-mass spectrum showed the highest peak at *m/z* 467, along with *m/z* 464, both due to the respective loss of Me· and water from the $[M]^+$. Successive dehydrations were observed from these fragments. After 101 mu, a new series of water losses with intense peaks at *m/z* 363, 345, 327 and 309 was observed. The ion at *m/z* 363 might account for the steroidal nucleus, as in ecdysterone; however, this mass was not in agreement with the lack of UV absorption, which indicated saturation of the Δ^7-double bond. Hence, in this case the steroidal nucleus corresponded to *m/z* 381 (< 4%), which after the loss of water led to the same dehydration sequence. The extra hydroxyl group must be located close to Me-19, according to ^{1}H NMR analysis. Moreover, odd fragments suggested the presence of a 20,22-diol in the side-chain. Finally, fragments *m/z* 145, 127, 109, 101 of low intensity and an important *m/z* 83 appeared in the region of low masses. The ion *m/z* 145 was assigned to the C-17/C-20 fission in a side-chain with two hydroxyls, *m/z* 127 and 109 to two successive losses of water, and *m/z* 101 to the C-20/C-22 fission giving rise to *m/z* 83 after subsequent dehydration. Thus, the side-chain with only two hydroxy groups, e.g. HO-20 and HO-22, was fully determined.

The ^{1}H NMR spectrum of the acetyl derivative of **2** showed the presence of four acetyl groups and four signals of methines α to acetyls, from which a multiplet at δ5.40 was coincident with that of the H-11 of the tetraacetyl derivative of ajugasterone C [11], with an α-oriented hydroxyl. The other signals of tetraacetylated **2** were also in agreement with the tetraacetate of ajugasterone C, except for the shielding of Me-19 and deshielding of Me-26 and Me-27 in **2**, owing to the saturation of the 7-double bond and the concomitant change in the conformation of ring B. A DEPT ^{13}C NMR spectrum indicated that compound **2** contained five methyl groups, eight methylenes, nine methines and four quaternary carbons, together with one isolated carbonyl group. Because of the lack of literature ^{13}C NMR data on 7,8-dihydroecdysteroids ^{13}C signal assignments were performed by comparison with the spectra of ajugasterone C [13] and ecdysterone [10], as well as by calculated chemical shifts. Comparison of the C-20 and C-22 chemical shifts (δ76.7 and 77.0, respectively) with reported values for four possible 20,22-dihydroxy sterol diastereomers [14, 15], confirmed the 20*R*,22*R* stereochemistry of **2**. Taken together, the above data indicated that compound **2** was (20*R*,22*R*)-2β,3β,11α,14α,20,22-hexahydroxy-5β-cholestan-6-one (7,8-dihydroajugasterone C, 7,8-dihydro-11α-hydroxyponasterone A). To our knowledge, this is the first report on the occurrence of 7,8-dihydroajugasterone C.

The methanolic extract of *N. hippomanica* was subjected to an alkaloid analysis at different pH values. Column chromatography of the ether-soluble alkaloids obtained at pH 10 yielded four main fractions. The first oily fraction was analysed by GC-mass spectrometry at low temperature giving two peaks of t_R 7.31 (**3**) and 7.77 (hygrine) [7]. The mass spectra of both compounds were quite similar and only differed in 14 mu. Compound **3** ($[M]^+$ *m/z* 127) showed a base peak at *m/z* 70 (Scheme 1), while hygrine ($[M]^+$ *m/z* 141) at *m/z* 84 $[M - 57]^+$. The first important loss of both was $[M - Me]^+$ (*m/z* 112 for **3** and *m/z* 126 for hygrine), followed by $[M - Me - CO]^+$ in both cases. The even base peak, *m/z* 70, for **3** indicated a 2-alkylpyrrolidine nucleus [16] by analogy to that of *N*-methyl-2-alkylpyrrolidine. Thus, this closely related substance was norhygrine **3**, the *N*-demethylated analogue of hygrine, which has not been previously reported.

In addition to the compounds **1**–**3**, the already known quercetin 3-*O*-β-D-galactopyranoside, D-mannitol,



Scheme 1. Mass spectral fragmentation of norhygrine (3).

choline and tigloidine were also isolated and identified (see Experimental). The occurrence of, quercetin 3-*O*-β-D-galactopyranoside and ornithine derivatives, such as tropane- and hygrine-alkaloids, is in agreement with the characteristic constituents of the Solanaceae [17–20]. However, the medicinally useful alkaloid, tigloidine, unlike the 3α-tigloyl derivative, is of limited distribution in this family [19]. Its occurrence suggests that *N. hippomanica* contains the stereospecific tropinonreductase system for pseudotropine (tropan-3β-ol) derivatives [18]. Norhygrine **3** is reported here for the first time, but other noralkaloids occur in this family, probably due to the fact that ornithine derivatives are formed in the roots and sometimes, as in this species, they are transported to the buds where secondary transformations, such as *N*-demethylation take place [18].

The occurrence of ecdysterone (**1**) and, in particular, the novel 7,8-dihydroajugasterone C (**2**) is of considerable biogenetic interest. Only two other ecdysteroids lacking the 7,8-double bond are known – 7,8-dihydroecdysone (cheilanthone A) and 25-deoxy-7,8-dihydroecdysone (cheilanthone B); these were isolated, together with ecdysone, from the fern *Cheilanthes tenuifolia* [21]. Cheilanthones A and B were biologically inactive when tested up to 0.6 μg per animal in isolated abdomens of *Calliphora stygia*. Unlike ecdysone, cheilanthone A when injected into larvae did not accelerate puparium formation [22]. Thus, even when the molecule possesses the 2β,3β,14α-trihydroxy-6-one system the unsaturated carbonyl is the structural requirement for a moulting hormone. Accordingly, compound **2** is probably inactive. Moreover, in the biosynthesis of 7,8-dihydroajugasterone C (**2**) in *N. hippomanica* the introduction of the 7-double bond may take place at a stage after hydroxylation. Ecdysteroids are not only steroidal moulting hormones [23] of insects and crustaceans, they also induce protein synthesis in other phyla, such as mammals. Phytoecdysteroids [24, 25] are probably protective agents against phytophagous insects. They could also play a role in synchronizing the photoperiodic responses of plants and insects [25].

EXPERIMENTAL

General. UV were recorded in MeOH, IR in Nujol. ^{1}H (200 MHz) and ^{13}C NMR (50 MHz) spectra were recorded at 303 K using TMS as int. standard. Multiplicities of ^{13}C NMR were assigned by the Distortionless Enhancement by Polarization Transfer (DEPT) expts. EI-MS and GC-MS were obtained at 70 eV. Mass scanning was performed in the range 30–800 for each peak sample. FABMS with a matrix of glycerol were performed in a high-resolution mass spectrometer. TLC was carried out on Merck precoated silica gel F_{254} plates.

Plant material. Whole plants of *N. hippomanica* Miers. were collected in Chacharramendi, La Pampa Province, Argentina, and identified by Ing. Guillermo Covas (INTA). Voucher specimens are deposited in the Herbarium of INTA (La Pampa) under Nr. 1324. Whole plants of the same species were also collected in La Calera, Córdoba Province, Argentina, and in the Ancasti mountains at 1000 m high (Estación Experimental INTA Sumalao, Valle Viejo, Catamarca Province, Argentina). The chemical components **1–7** were found in plants from the three different sources.

Fractionation. Dried ground whole plants were successively extracted with petrol and MeOH. At room temp., K_2SO_4 (0.02% dry wt) separated out from the MeOH extract. Upon concn of the MeOH filtrate to one-tenth of its vol., an organic ppt. (1.85% dry wt) was obtained. Ursolic and oleanolic acid, α- and β-amyrins,

uvaol, scopoletin [7], pinocembrin 7-*O*-β-neohesperidoside [4], quercetin 3-*O*-galactopyranoside (0.06% dry wt) and D-mannitol (0.22% dry wt) were isolated from this ppt by CC on silica gel. A portion of the remaining MeOH filtrate was concd, suspended in H_2O and successively extracted with $CHCl_3$, EtOAc and *n*-BuOH. The EtOAc extract (0.7% dry wt) contained the three previously reported [5, 6] monoacetylated derivatives of pinocembrin 7-*O*-β-neohesperidoside and traces of other minor flavonoids, along with the two terpenoids **1** (ecdysterone; 0.028% dry wt) and **2** (the novel 7,8-dihydroajugasterone C; 0.014% dry wt). Alkaloids were analysed in another portion of the MeOH filtrate, yielding the novel norhygrine **3** ($5.2.10^{-4}$% dry wt), hygrine ($4.7.10^{-4}$% dry wt) and tigloidine (0.006% dry wt). Choline was also identified (reineckate at pH 12; 0.1% dry wt).

Quercetin 3-O-β-D-*galactopyranoside* (*hyperin*; *hyperoside*). Obtained from fr. 9 from the CC of the organic ppt from the MeOH extract. Further purification by CC on silica gel and CC on Sephadex LH-20. TLC: Silica gel; R_f 0.53 (quercetin 3-*O*-β-D-galactopyranoside) and 0.28 (quercetin 3-*O*-diglycoside) (EtOAc–Me_2CO–HOAc, 100:20:1). Polyamide; R_f 0.10 (quercetin 3-*O*-β-D-galactopyranoside) and 0.30 (quercetin 3-*O*-diglycoside) (H_2O–*n*-BuOH–Me_2CO–HOAc, 16:2:1:1). 2D chromatography, Whatman No. 1 (quercetin 3-*O*-β-D-galactopyranoside), R_f 0.64 (*t*-BuOH–HOAc–H_2O, 3:1:1); R_f 0.43 (15% HOAc). Spots were visualized by NH_3 vapours, bright yellow fluorescence at 366 nm, 2% $FeCl_3$ in ethanol: green (phenolic hydroxyls); 10% $SbCl_3$ in chloroform: yellow fluorescence at 366 nm (flavonol). UV λ_{max} 256, 266sh, 295, 356 nm; + $AlCl_3$: 275, 300sh, 330, 428 nm; + $AlCl_3$/HCl: 273, 298sh, 353, 395 nm; + NaOMe: 272, 330, 385 nm; + NaOAc: 272, 321, 383 nm; + NaOAc/H_3BO_3: 263, 370 nm. ^{1}H NMR in agreement with that previously reported [26]. Upon acid hydrolysis (1N HCl, reflux for 4 hr) D-galactose (TLC, cellulose: R_f 0.51; *n*-BuOH–pyridine–H_2O, 6:4:3, two developments; visualized with $AgNO_3$–NH_4OH and *p*-anisidine) and quercetin (TLC, silica gel: R_f 0.30; $CHCl_3$–EtOH, 19:1) were obtained. UV, EI-MS [27] and ^{1}H NMR [26] spectra were in agreement with those previously reported.

D-*Mannitol*. Evapn of the column fr. yielded a syrup that was recrystallized from EtOH as needles, mp 166–168° (EtOH). UV: no absorption. IR (cm^{-1}) 3330 (ν_{O-H}), 1420 and 1380 (δ_{O-H}), 1080 and 1010 (str. C–O), 960, 930, 880, 865, 785, 700 (str. C–O and C–C). ^{1}H NMR (DMSO-d_6): δ3.60 (*m*, 4H, H-1 and H-6), 4.15 (*d*, *J* = 3 Hz, 2H, H-3 and H-4), 4.40 (*m*, 2H, H-2 and H-5). EI-MS *m/z* (rel. int): 182 ($[M]^+$, 1), 164 ($[M-18]^+$, 2), 151 ($[M-31]^+$, 2), 146 ($[M-18-18]^+$, 3), 133 ($[151-18]^+$, 11), 121 ($[M-61]^+$, 25), 115 ($[133-18]^+$, 5.5), 103 ($[121-18]^+$, 21), 91 ($[182-91]^+$, 8), 85 ($[103-91]^+$, 21), 73 ($[91-18]^+$, 100), 61 (42), 57 (74), 43 (54).

Ecdysteroids. Compounds **1** and **2** were sepd from acylated flavonoids by CC on neutral alumina with MeOH as solvent and subsequently purified on silica gel with EtOAc–MeOH (49:1; 19:1; 9:1). TLC (silica gel; $CHCl_3$–Me_2CO, 7:13; anisaldehyde–sulphuric acid or vanillin–sulphuric acid; green spots): R_f 0.24 (**1**) and 0.37 (**2**).

Ecdysterone (20-*Hydroxyecdysone*) **1**. $[\alpha]_D^{22}$ + 62.1° ($CHCl_3$; *c* 0.03). UV λ_{max} (nm): 242 (MeOH); 240, 300 nm after treatment with MeOH–HCl at 70° for 15 min. IR (cm^{-1}) 3560 (ν_{OH}), 2860 (ν_{CH}), 1660 and 1640 ($\nu_{C=O}$ and $\nu_{C=C}$ of the 7-en-6-one system), 1460, 1445 and 1380 (asym. and sym. fl. of the methyl), 1320, 1270, 1230 and 1215 (δ_{OH}), 1075, 1060, 1030 (str. C–O), 885 and 682 (C=C). ^{1}H and ^{13}C NMR in agreement with those previously reported [10, 14]. FAB-MS: *m/z* 481 $[MH]^+$. EI-MS *m/z* (rel. int.): 462 ($[M-18]^+$, 2), 444 ($[M-2\times18]^+$, 8), 429 ($[M-2\times18-15]^+$, 10), 426 ($[M-3\times18]^+$, 37), 411 ($[M-3\times18-15]^+$, 5), 408 ($[M-4\times18]^+$, 17), 393 ($[M-4\times18-15]^+$, 10), 363 ($[M-117]^+$, side-chain from C-20/22 fission, 28), 345 ($[363-18]^+$, 79), 344 ($[363-18-1]^+$, 73), 327 ($[345-18]^+$, 67), 309 ($[365-3\times18]^+$, 17), 301 ($[M$ – C-20 to C-27 – $18]^+$, 20), 300 ($[M$ – C-20 to C-27 – 18 – $1]^+$, 39), 285 ($[300-15]^+$, 25), 173 ([C-17 to C-27-H]$^+$, 31), 161 ([C-20 to C-22]$^+$, 10), 143 ($[161-18]^+$, 31), 125 ($[161-2\times18]^+$, 18), 117 ([C-22 to C-27]$^+$, 5), 99 ($[117-18]^+$, 100), 81 ($[99-18]^+$, 69), 71 (23), 69 (28), 59 (30), 57 (18), 56 (16), 55 (29), 43 (94).

Ecdysterone 2,3,22-*triacetate*. ^{1}H NMR ($CDCl_3$): δ0.85 (*s*, 3H, Me-18), 1.03 (*s*, 3H, Me-19), 1.20 and 1.21 (each *s*, 6H, Me-26 and 27), 1.24 (*s*, 3H, Me-21), 1.20–2.20 (*m*, 16H, methylenes), 1.97 (*s*, 3H, CH_3CO), 2.08 (*s*, 6H, 2 CH_3CO), 2.34 (*m*, 1H, H-5), 2.60 (*m*, 1H, H-17), 3.12 (*m*, 1H, H-9), 4.80 (*dd*, *J* = 4 and 8 Hz, 1H, H-22), 5.25 (*m*, 2H, H-2 and H-3), 5.80 (*d*, *J* = 2 Hz, 1H, H-7). FAB-MS: *m/z* 607 $[MH]^+$. EI-MS *m/z* (rel. int.): 528 ($[M-60-18]^+$, 5), 510 ($[M-60-2\times18]^+$, 16), 495 ($[M-60-2\times18-15]^+$, 8), 492 ($[M-60-3\times18]^+$, 9), 477 ($[M-60-3\times18-15]^+$, 3), 450 ($[M-60-2\times18]^+$, 2), 437 ([510–C-24 to C-27]$^+$, 5), 385 ($[M$ – C-20 to C-27 – $18]^+$, 10), 334 ([437–1–60–42]$^+$, 11), 185 ([C-20 to C-27 – 18]$^+$, 8), 173 (11), 167 ($[185-18]^+$, 7), 125 ($[185-60]^+$, 15), 123 ([C-22 to C-27–2×18]$^+$, 11), 99 ([C-22 to C-27–60]$^+$, 14), 81 ($[99-18]^+$, 30), 79 (11), 69 (42), 67 (12), 59 (15), 55 (25), 43 ($[CH_3CO]^+$, 100).

7,8-*Dihydroajugasterone C* (7,8-*Dihydro*-11α-*hydroxyponasterone A*) **2**. Amorphous powder. UV: No absorption. $[\alpha]_D^{22}$ + 43.2° ($CHCl_3$; *c* 0.01). IR cm^{-1}: 3350 (ν_{OH}), 2870 (ν_{C-C}), 1695 ($\nu_{C=O}$), 1445 and 1375 (sym and asym fl. Me), 1265, 1230, 1215 and 1200 (δ_{OH}), 1095 (str. CO), 1010 (str. C–O), 860 (str. C–C). ^{1}H NMR (pyridine-d_5): δ0.82 (6H, *d*, *J* = 6 Hz, Me-26 and Me-27), 1.23 (3H, *s*, Me-18), 1.40 (3H, *s*, Me-19), 1.53 (3H, *s*, Me-21), 1.58 (1H, *m*, H-25), 1.68 (1H, *m*, H-23b), 1.71 (1H, *m*, H-24b), 1.80 (1H, *m*, H-4*ax*), 1.85 (1H, *m*, H-7*eq*), 1.95 (1H, *m*, H-15a), 2.00 (1H, *m*, H-1*ax*), 2.10 (2H, *m*, H-16a and H-15b), 2.20 (1H, *dt*, *J* = 4 and 14 Hz, H-4*eq*), 2.23 (1H, *dd*, *J* = 4 and 14 Hz, H-7*ax*), 2.30 (1H, *m*, H-8), 2.50 (1H, *m*, H-16b), 2.70 (1H, *dd*, *J* = 12 and 6 Hz, H-12*eq*), 3.00 (1H, *t*, *J* = 8 Hz, H-17α), 3.20 (1H, *m*, H-5β), 3.42 (1H, *t*, *J* = 12 Hz, H-12*ax*), 3.45 (1H, *dd*, *J* = 16 and 4 Hz, H-1*eq*), 3.80 (1H, *dd*, *J* = 5 and 10 Hz, H-22), 3.90 (1H, *t*, *J* = 9 Hz, H-9), 4.30 (1H, *m*, H-3*eq*), 4.60 (1H, *m*, H-2

ax), 4.68 (1H, *m*, H-11*ax*). Signals of H-23a (*δ ca* 1.40) and H-24a (*δ ca* 1.50) were overlapped with those of the methyl groups. ^{13}C NMR (pyridine-d_5): *δ* 16.4 (C-19), 21.5 (C-21), 22.0 (C-27), 22.6 (C-26), 26.9 (C-18), 28.7 (C-25), 31.8 (C-23), 32.3$_a$ (C-4), 32.9$_a$ (C-16), 36.4 (C-24), 37.7$_b$ (C-8), 37.9$_b$ (C-15), 40.6 (C-7), 41.2 (C-10), 43.5$_c$ (C-12), 43.9$_c$ (C-1), 51.4 (C-9), 53.3 (C-17), 55.4 (C-5), 60.1 (C-13), 65.2 (C-2), 67.5$_d$ (C-11), 67.8$_d$ (C-3), 76.7$_e$ (C-20), 77.0$_e$ (C-22), 93.8 (C-14), 214.5 (C-6). (a–e, signals may be interchangeable). FAB-MS: *m/z* 483 $[MH]^+$. EI-MS *m/z* (rel. int.): 467 ($[M-15]^+$, 2), 464 ($[M-18]^+$, 5), 428 ($[M-3\times18]^+$, 2), 413 ($[428-15]^+$, 2). 381 ([M – C-22 to C-27]$^+$, 4), 363 ($[381-18]^+$, 84), 345 ($[363-18]^+$, 41), 327 ($[345-18]^+$, 28), 309 ($[327-18]^+$, 16), 285 (8), 267 (10), 189 (12), 157 ([C-17 to C-27-H]$^+$, 11), 145 ([C-20 to C-27]$^+$, 41), 127 ($[145-18]^+$, 9), 109 ($[127-18]^+$, 33), 101 ([C-22 to C-27]$^+$, 2), 83 ($[101-18]^+$, 75), 71 (36), 67 (33), 55 (100), 43 (94).

7,8-Dihydroajugasterone C 2,3,11,22 tetraccetate. ^1H NMR ($CDCl_3$): *δ*0.83 (3H, *s*, Me-18), 0.90 (3H, *s*, Me-19), 1.05 (6H, *d*, *J* = 6 Hz, Me-26 and 27), 1.21 (3H, *s*, Me-21), 1.90, 1.98, 2.02 and 2.08 (each *s*, 12H, 4 CH_3CO), 1.20-2.00 (*m*, other methylenes and methines), 2.20 (1H, *t*, *J* = 14 Hz, H-9), 1.85 (2H, *d*, *J* = 14 Hz, H-7), 2.34 (1H, *dd*, *J* = 7 and 10 Hz, H-5), 4.80 (1H, *dd*, *J* = 4 and 9 Hz, H-22), 5.15 (1H, *m*, H-2), 5.30 (1H, *m*, H-3), 5.40 (1H, *m*, H-11).

Alkaloids. A portion of the MeOH filtrate was concd and poured into a soln of 0.1N HCl (1l). After standing overnight at room temp., a ppt was filtered off and the acid aq. soln successively extracted with Et_2O and $CHCl_3$. The pH of the aq. layer was adjusted to pH 10 (NH_4OH) and the free bases extracted with Et_2O and $CHCl_3$. The same procedure was performed at pH 12 (K_2CO_3). Quaternary alkaloids were analysed in the remaining aq. layer as Reineckates (Reinecke salt: $[Cr(SCN)_4(NH_3)_2^-NH_4^+]$) obtained by precipitation with NH_4 Reineckate at different pH values. At pH 12, choline was obtained in large quantities. At pH 8, small quantities of other quaternary alkaloids (positive Ehrlich test) were obtained but not analysed. No ppt occurred at pH 5 or pH 1.

Ether-soluble alkaloids obtained at pH 10 (0.07% dry wt). The Et_2O-sol. alkaloids showed two positive Dragendorff spots on TLC (silica gel, R_f 0.30 and 0.76; $CHCl_3$ satd with ammonia–MeOH, 3:2). A quantitative variation was observed depending on the origin of plant material, viz., both spots were found 1:1 in MeOH extracts from La Pampa plants, while those from Córdoba and Catamarca contained mainly the higher R_f alkaloid. The R_f 0.30 alkaloid was unstable. Alkaloids were separated by CC on neutral alumina using cyclohexane–$CHCl_3$, $CHCl_3$, gradients of $CHCl_3$–MeOH and MeOH–H_2O as eluents. Four main frs were obtained. Fr. 1 was analysed by GC and GC–MS. The novel norhygrine **3** (R_t 7.31 min) and hygrine (R_t 7.77 min) were obtained. Fr. 2: *β*-phenethylamine and hordenine [7]. Fr. 3: *N*-methyltyramine [7]. Fr. 4: tyramine [7].

Norhygrine **3.** GC conditions Apiezon column, *N*-FID at 50° (isothermal), R_t rel. to hygrine: 0.94. EI-MS *m/z* (rel. int.): 127 ($[M]^+$, 5], 112 ($[M-15]^+$, 19), 84 ($[M-43]^+$, 15), 70 ($[M-57]^+$, pyrrolidinium ion, 100), 56 ($[CH_2=N^+(H)-CH=CH_2]^+$, 11), 44 ($[CH_3N^+(H)=CH_2]^+$, 40), 42 ($[CH_2=N^+=CH_2]^+$, 44), 31 (43).

Hygrine. EI-MS *m/z* (rel. int.): 141 ($[M]^+$, 13), 126 ($[M-15]^+$, 33), 98 ($[M-43]^+$, 15), 84 ($[M-57]^+$, pyrrolidinium ion, 100), 70 ($[CH_2=N^+(Me)-CH=CH_2]^+$, 9), 56 ($[C_3H_6N]^+$, 12), 44 (34), 42 ($[HC\equiv N^+-CH_3]^+$, 89), 31 (20) [28].

Chloroform-soluble alkaloids obtained at pH 10 (0.02% *dry wt*) . CC on neutral alumina using $CHCl_3$ and gradients of $CHCl_3$–MeOH as eluents yielded the phenethylamine hordenine [7] and the tropane alkaloid [29], tigloidine.

Tigloidine (3β-*tigloyloxytropine*). R_t 10.38 min (GC, OV-101, 50–250°, 10° min^{-1}). TLC. silica gel; R_f 0.75 (Me_2CO–H_2O–NH_4OH, 90:7:3), coincident with that of a tigloidine picrate standard. Standards of 3α-tigloyloxytropine picrate: R_f 0.42, tropine: R_f 0.36, hordenine: R_f 0.85. TLC (silica gel, EtOAc–MeOH–NH_4OH, 15:5:1): tigloidine R_t 0.80; 3α-tigloyoxytropine: R_f 0.45; tropine: R_f 0.38. HPTLC plates were also used. MS *m/z* (rel. int.): 223 ($[M]^+$, 18), 140 ($[M-83]^+$ (acyl), 7), 124 ($[M-99]^+$ (acyloxy), 100), 110 (*N*-methylhydroxypyridinium ion, 12), 96 ($[C_6H_{10}N]^+$, 12), 95 ($[C_6H_9N]^+$, 13), 94 ($[C_6H_8N]^+$, 30), 83 ($[C_5H_8N]^+$, 29), 82 ($[C_5H_8N]^+$, 44), 67 (15), 55 (17), 42 ($[HC\equiv N^+-CH_3]^+$, 25).

Choline. Obtained from the Reineckate at pH 12 (0.1% dry wt) after treatment with Amberlite IRA-400 resin (HO^- form) eluting with EtOH. Further separation on a neutral alumina column with $CHCl_3$–MeOH–HOAc (90:10:1) yielded choline. TLC (cellulose, EtOH–NH_4OH, 4:1) R_f 0.60, coincident with a standard of choline chloride. TLC (cellulose, EtOAc–*n*-BuOH–HOAc–H_2O, 8:2:3:2): R_f 0.53. ^1H NMR (D_2O): *δ*3.18 (*s*, 9H, Me_3N^+–), 3.50 (*m*, 2H, $-CH_2-N^+Me_3$), 4.03 (*m*, 2H, $-CH_2OH$). Choline picrate: a soln of picric acid in EtOH was added to an EtOH soln of choline, mp 240–242° (EtOH; yellow needles) [30]. Mmp with a standard was not depressed.

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