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ALKALOIDS FROM STEM BARK AND LEAVES OF *PESCHIERA BUCHTIENI*

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Key Word Index—*Peschiera buchtieni*; Apocynaceae; leaves; stem bark; indole alkaloids; NMR.

Abstract—During an investigation of the stem bark and leaves of *Peschiera buchtieni*, 34 alkaloids were isolated. Eight new alkaloids were obtained from the stem bark, *N*-methyl-pericyclivine, 18,19(*R*)-dihydroxycoronaridine, chloromethylene affinisinium, demethylaccedinisine, 18-hydroxyaffinisine, buchtienine, 3-hydroxytetrahydroolivacine and demethylceridimine. The known alkaloids from the stem bark were coronaridine, voaphylline, coronaridine hydroxyindolenine, heyneanine, eglandine, eglandulosine, 19-epi-heyneanine, voachalotine, ochropamine, olivacine, ibogamine, affinisine, *N*-oxyaffinisine, vallesamine, (*E*)-isositsirikine, voaphylline hydroxyindolenine, normacusine B, janetine, 3,14-dihydroolivacine, 3′(*R*/*S*)-hydroxy-*N*-demethyl-tabernamine, 4′,17β-dihydrotchibangensine, ceridimine and *N*-demethyltabernamine. Apodine, voacristine, voacristine hydroxyindolenine and olivacine were identified in leaves.

INTRODUCTION

As part of a survey of medicinal plants from tropical regions of Bolivia, we have examined the chemical composition of *Peschiera buchtieni* (Syn. *Tabernaemontana buchtieni* Mgf.) [1]. This tree is rather common in the Chapare rain forest and is used locally as a treatment for leishmaniasis [2, 3].

RESULTS AND DISCUSSION

Alkaloids were displaced from their salts by means of aqueous ammonia solution and extracted with ethyl acetate. They were then separated from neutral compounds by extraction with aqueous H_2SO_4. Neutralization of the water phase and extraction with $CHCl_3$ yielded the crude alkaloid mixture (AM) with the following yields: 31.5 g kg^{-1} for stem bark and 2.6 g kg^{-1} for leaves. The pure alkaloids were separated from the AM by means of column chromatography, centrifugal TLC, prep. TLC and recrystallization. Products from the stem bark are numbered in order of increasing polarity. They are, coronaridine (**1**, 5.4% AM), voaphylline (**2**, 0.16% AM), coronaridine hydroxyindolenine (**3**, 0.11% AM), heyneanine (**4**, 16.6% AM, recrystallized from Et_2O), eglandine (**5**, 0.52% AM), eglandulosine (**6**, 0.05% AM), 19-epi-heyneanine (**7**, 0.004% AM), *N*-methylpericyclivine (**8**, 0.025% AM), voachalotine (**9**, 0.11% AM), ochropamine (**10**, 0.025% AM), olivacine (**11**, 6.5% AM), ibogamine (**12**, 0.03% AM), 18, 19(*R*)-dihydroxycoronaridine (**13**, 0.03% AM), affinisine (**14**, 2.5% AM), *N*-oxyaffinisine (**15**, 0.07% AM), vallesamine (**16**, 0.24% AM), chloromethylene-affinisinium (**17**, 1.04% AM), (*E*)-isositsirikine (**18**, 0.13% AM), voaphylline hydroxyindolenine (**19**, 0.013% AM), normacusine B (**20**, 0.017% AM), janetine (**21**, 1.15% AM), demethylaccedinisine (**22**, 0.032% AM), 18-hydroxyaffinisine (**23**, 0.021% AM), 3.14-dihydroolivacine (**24**, 0.025% AM), 3′(*R*/*S*)-hydroxy-*N*-demethyltabernamine (**25**, 0.06% AM), buchtienine (**26**, 0.36% AM), 4′,17β-dihydrotchibangensine (**27**, 0.021% AM), 3-hydroxytetrahydroolivacine (**28**, 0.03% AM), ceridimine (**29**, 0.034% AM), demethylceridimine (**30**, 1.29% AM) and *N*-demethyltabernamine (**31**, 0.034% AM). From the leaves were isolated in order of increasing polarity, apodine (**32**, 0.22% AM), voacristine (**33**, 2% AM), voacristine hydroxyindolenine (**34**, 0.2% AM) and olivacine (**11**, 1% AM). More polar fractions have not been investigated.

The known compounds, **1**, **2**, **4**–**7**, **10**, **12**, **14**–**16**, **18**, **20**, **27**, **32** and **33**, were identified by comparison of their spectroscopic data with those published in the literature and by co-TLC with authentic samples. Compounds **9**, **25**, **29**, **31** and **34** have only been identified by comparison with published data. Compounds **3**, **11**, **19**, **21** and **24** were also identified by comparison with authentic samples, but their isolation provided the opportunity to complete or modify previously reported spectral data (see Experimental). The ^{1}H NMR spectrum of coronaridine hydroxyindolenine (**3**) [4, 5], olivacine (**11**) [6, 7] and 3,14-dihydroolivacine (**24**) [8] have not been previously

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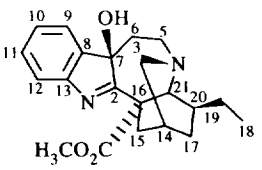
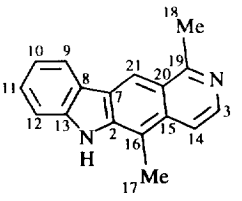
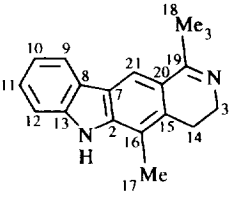


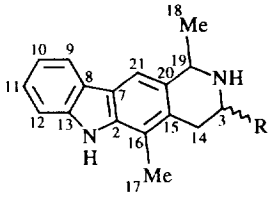
Coronaridine hydroxyindolenine (**3**)



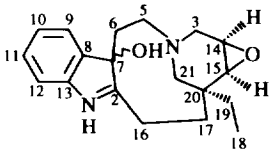
Olivacine (**11**)



3,14-dihydro-olivacine (**24**)



R = H Janetine (**21**)
R = OH 3-hydroxy-tetrahydro-olivacine (**28**)



Voaphylline hydroxyindolenine (**19**)

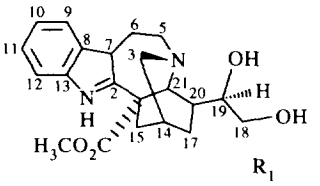
reported. Careful inspection of COSY [9] data from voaphylline hydroxyindolenine (**19**) allowed reassignment of ^{1}H NMR resonances [10], i.e. the permutation of signals of H-10 with H-11 and of H-14 with H-15. The combination of COSY, HMBC [11] and HMQC [12] data allowed the complete and unambiguous assignment of all signals in the ^{1}H and ^{13}C NMR spectra of janetine (**21**) [13]. Compounds **8**, **13**, **17**, **22**, **23**, **26**, **28** and **30** are new; their structures were elucidated as follows.

The UV spectrum of *N*-methylpericyclivine (**8**) showed maxima characteristic of an indole chromophore. Its mass spectrum exhibited a fragmentation pattern similar to that observed for pericyclivine, but the $[M]^+$ was heavier by 14 mu. Peaks at *m/z* 182 and 183 were characteristic of a *N*-methyltetrahydro-*β*-carboline ring system. The presence of a NMe group was confirmed in the ^{1}H NMR spectrum by the presence of a singlet signal at δ3.4 (three protons). The aliphatic part of the ^{1}H NMR spectrum was interpreted using data for 10-methoxypericyclivine [14]. Shielding of the methyl ester protons (*s* at δ3.1) indicated a 16(*S*)-configuration. Deshielding of H-3 (δ5.12) may be caused by salification of the basic nitrogen atom. No ^{13}C NMR data are available owing to the paucity of material.

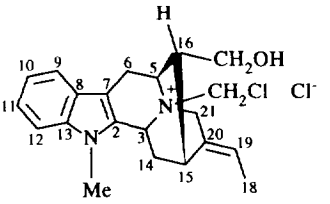
The mass spectral fragmentation pattern of **13** was related to that of heyneanine, but the $[M]^+$ was heavier by 16 mu. In the ^{1}H NMR spectrum, there was no signal corresponding to a methyl group at position 18. Supplementary data from ^{13}C NMR and COSY spectra led to its identification as 18,19-dihydroxycoronaridine. The C-19 configuration was suggested by chemical shift comparison of H-19 in **13** with those of 19(*R*)- and 19(*S*)-heyneanine [15, 16]. Substitution of one of the H-18 protons by a hydroxyl group caused a deshielding of H-19 (*ca* 0.3 ppm). The δ value of this proton at 4.19 ppm in **13** is only compatible with a 19(*R*) absolute configuration.

The ^{1}H NMR spectrum of **17**, as well as its mass spectral fragmentation pattern strongly suggested an affinisine structure. However, two supplementary proton signals at δ5.1 and at δ5.85 (*d*, J = 10.3 Hz, AB system) have only been interpreted after a full NMR study. Combination of COSY, HMBC and HMQC data, analysed by the LSD program [17], led to the structure of an affinisne–CH_2Cl_2 adduct. Compound **17** has been synthesized by refluxing affinisine in CH_2Cl_2 in the presence of silica. Alkaloid **17** is thus an extraction artefact. Reaction of CH_2Cl_2 with tertiary amines has already been reported [18].

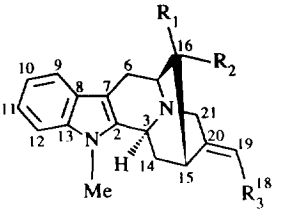
Compound **22** was a perivinol–affinisine dimer alkaloid. Analysis of COSY data allowed the assignment of the two moieties. Branching at position 10′ of the affin-



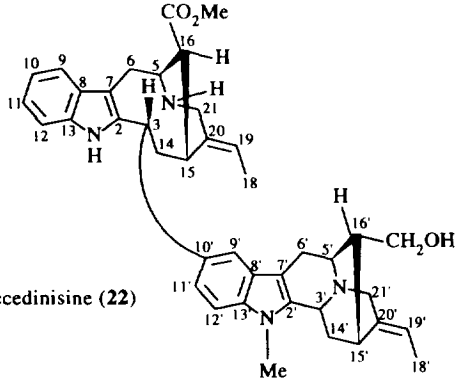
18,19(*R*)-Dihydroxy-coronaridine (**13**)



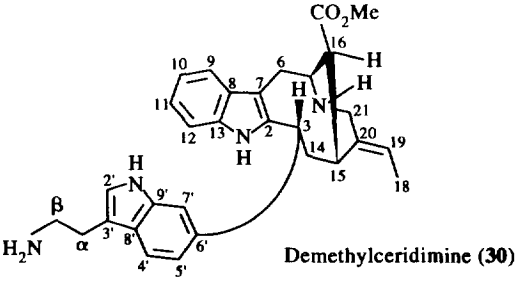
Chloromethylene-affinisinium (**17**)



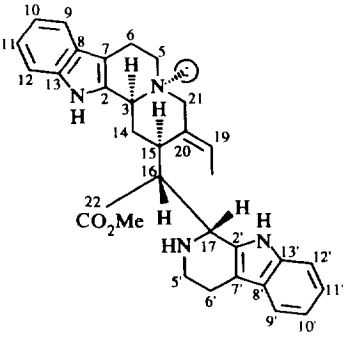
R_1	R_2	R_3	
CO_2Me	H	Me	*N*-methyl-pericycline (**8**)
H	CH_2OH	CH_2OH	18-Hydroxy-affinisine (**23**)



Demethyl-accedinisine (**22**)



Demethylceridimine (**30**)



Buchtienine (**26**)

isine part was suggested by comparison with literature data concerning the aromatic proton patterns [19]. This alkaloid was simultaneously isolated from *P. van heurkii*. Its structure was confirmed by methylation, which yielded accedinisine [20].

Compound **23** contained an intact indole unit as revealed from its UV spectrum. The mass spectrum ($[M]^+$ at *m/z* 324) and analysis of ^{1}H NMR and COSY data were in agreement with a structure similar to affinisine, but hydroxylated at position 18. This was supported by coupling of the olefinic proton H-19 with a methylene group resonating at δ4.0 and δ4.2. The 16 (*S*)-configuration was deduced by chemical shifts comparison with 16(*S*)- and 16(*R*)-affinisine [21].

The quasidimeric structure of buchtienine (**26**) was suggested from its $[M]^+$ at *m/z* 494, as well as by the intense fragments ions at *m/z* 169 and 170 typical of a tetrahydro-β-carboline. Analysis of UV and NMR specta (^{1}H, ^{13}C, COSY, relayed COSY, HMBC, HMQC) indicated that **26** possesses a 4′,17β-dihydrotchibangensine skeleton [22]. An additional CO_2Me group was deduced from the IR spectrum (ν C=O at 1730 cm^{-1}), ^{13}C NMR (C=O at δ174.3 and Me at δ51.3) and ^{1}H NMR (*s* at δ3.3). Its connection at C-16 is in agreement with the presence of only one H-16 at δ2.8. Moreover, application of standard ^{13}C chemical shift increments to C-14 (− 2 ppm), C-15 (+ 2 ppm) and C-17

(+2 ppm) in 4',17β-dihydrotchibangensine [22] for the substitution of a hydrogen atom by an ester group is in good agreement with the ^{13}C NMR buchtienine data. COSY spectra revealed the nature of the connection between the two parts of the molecule by observation of H-16–H-17 and H-17–H-6' couplings. The H-3–N-4 ring junction was shown to be *cis*, because the H-3 ^{1}H NMR signal is a broad singlet at δ4.3. By comparison with 16(*R*)- and 16(*S*)-isositsirikine data [23], the chemical shift of this proton in **26** favours a 16(*R*) absolute configuration, assuming the biogenetic hypothesis of a common 15α-H configuration. The chemical shift value of H-19 at δ5.5 is in agreement with an *E*-configuration of the C-18, C-19 double bond [24].

The chromophore of **28** was identical to that of janetine (**21**), as shown by its complex UV spectrum and the aromatic part of the ^{1}H NMR spectrum. The hypothesis of an hydroxylated janetine structure was inferred from the $[M]^+$ at *m/z* 266 in the mass spectrum, 16 mu more than janetine. The hydroxylation site was proposed on the basis of the chemical shift value for H-3 (*t* at δ4.9), which is compatible with the presence of hemiaminal group. The small amount of isolated product did not permit confirmation of this hypothesis by ^{13}C NMR.

Analysis of ^{1}H NMR and COSY data of **30** suggested a perivinol-type alkaloid. However, ^{13}C and HMQC data revealed the presence of two supplementary methylene groups and of enough aromatic carbon atoms to account for a supplementary tryptamine unit, as in ceridimine (**31**) [25]. The $[M]^+$ at *m/z* 482 and the absence of a NMe signal in the ^{1}H NMR spectrum lead to the demethylceridimine structure for **30**. Moreover, the ^{13}C chemical shifts of ceridimine and **30** differ mainly by an upfield shift of 10 ppm in the latter compound, for C-5 and C-21. HMQC and HMBC spectra have been used vigorously in order to complete the assignment of quaternary carbons. The superimposition of proton resonances in the aromatic region nevertheless leads to ambiguities, alleviated by chemical shift considerations. The branching point between the two moieties was confirmed as C-3–C-6' by observation of H-3–C-6' long-range coupling in the HMBC spectrum.

Natives of the Bolivian Chapare tropical region also use *P. buchtieni* and *P. van heurkii* to treat cutaneous leishmanianis. Preliminary antileishmania screening show that only *P. van heurkii* exhibited significant activity against parasites [20] in relation to the presence of *bis*indole alkaloids of the conodurine-type. *P. buchtieni* possesses a great variety of indole alkaloids belonging to the three biogenetic types I–III. The striking feature of this species is the presence of quasidimers. Compound **30** is the second example [25] of such an alkaloid containing a tryptamine unit with a free side-chain and linked by its aromatic part to a vobasine skeleton. Alkaloids with a dihydrotchibangensine structure, e.g. **26** and **27**, are common in the Loganiaceae (*Strychnos*), are present in the Rubiaceae (*Cinchona*) and occur sometimes in the Apocynaceae (*Dyera* [26], *Ervatamia* [16]). Buchtienine **26** is a novel example within this group. The presence of a carbomethoxy group at position C-16 suggests a biogenetic pathway from geissoschizine. Thus, this compound could be a biogenetic precursor of tchibangensine derivatives.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR were recorded at 300 and 75 MHz, respectively. Chemical shifts are reported in δ from TMS. 1D (^{1}H, ^{13}C) and 2D (COSY, relayed COSY, HMQC, HMBC) expts were performed using standard Bruker microprograms.

Plant material. Peschiera buchtieni was collected in June 1989 in the Sacta Valley in the Chapare region of Bolivia. A voucher specimen is kept at the National Herbarium of La Paz (Bolivia) under number Moretti-1453.

Extraction. Dried powdered leaves (2.8 kg) were wetted with 6 M aq. NH_3, macerated overnight in EtOAc, then lixiviated. The lixiviate was extracted with 0.33 M H_2SO_4. The aq. layer was basified with 12 M aq. NH_3 and extracted with $CHCl_3$. The $CHCl_3$ layers were dried (Na_2SO_4) and evapd *in vacuo* to give 5.7 g of crude alkaloid mixt. (AM, 2.6 g kg^{-1}). Stem bark (1 kg) extraction was performed following an identical process, yielding 31.5 g of AM.

Isolation. AM from leaves was purified by flash-CC. Elution was performed initially with CH_2Cl_2 and then with CH_2Cl_2–MeOH mixts of increasing polarity. Frs (20 ml) were collected, analysed by TLC and combined according to their composition. Further purifications were achieved by prep. TLC and CTLC. Apodine (**32**) was isolated from initial frs, eluted by CH_2Cl_2. The other isolated products, **33**, **34**, **11**, were contained in frs eluted CH_2Cl_2–MeOH (99:1). Other CC frs were not investigated owing to their great complexity.

AM from stem bark was purified by CC. Elution (150 ml frs) was performed initially with CH_2Cl_2 (5.2 l), then with CH_2Cl_2–MeOH mixts (99.5:0.5, 1.8 l), (99:1, 1.35 l), (98.5:1.5, 1.5 l), (49:1, 1.5 l), (19:1, 1.2 l), (9:1, 1.5 l), (4:1, 2.55 l) and MeOH (1.35 l). Alkaloid **1** was in frs 11–35, **2** in frs 36–47, **3** in frs 48–56, **4**–**6** in frs 57–64, **7** and **8** in frs 66–67, **9** and **10** in frs 68–77, **11**–**13** in frs 78–90, **14**–**19** in frs 91–107, **20**–**23** in frs 108–109, **24**–**26** in fr. 110, **27**–**28** in fr. 114, **29**–**30** in fr. 115, and **31** in fr. 116. Affinisine (**14**) salt was present in the final MeOH frs.

Coronaridine hydroxyindolenine (**3**). Ceric-spray: green grey. $[\alpha]_D$ −7° (EtOH; *c* 1). UV λ_{max}^{MeOH} nm: 223, 286, 293. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1740. MS *m/z* (rel. int.): 354 $[M]^+$ (100), 337 (95), 325 (9), 295 (15), 230 (10), 188 (15), 160 (15), 136 (18), 122 (25). ^{1}H NMR ($CDCl_3$): δ7.45 (*d*, *J* = 7 Hz, H-9), 7.2–7.35 (*m*, H-10, H-11, H-12), 3.7 (*s*, CO_2Me), 3.55 (*d*, *J* = 3 Hz, H-21), 3.5 (*ddd*, *J* = 4, 12, 15 Hz, H-6'), 3.0 (*dd*, *J* = 15, 4 Hz, H-6), 2.75 (*d*, *J* = 15 Hz, H-3), 2.5 (*ddd*, *J* = 15, 4, 2 Hz, H-3'), 2.05 (*bd*, *J* = 15 Hz, H-5), 1.95 (*m*, H-14), 1.9 (*m*, H-5'), 1.75 (*m*, H-15'), 1.5–1.4 (*m*, H-19, H-19'), 1.4 (*m*, H-20), 1.1 (*m*, H-15), 0.9 (*t*, *J* = 7.5 Hz, H-18).

Olivacine (**11**). Ceric-spray: yellow-green. ^{1}H NMR (CD_3OD): δ8.7 (*s*, H-21), 8.22 (*d*, *J* = 7.6 Hz, H-9), 8.17 (*d*, *J* = 6.3 Hz, H-3), 7.8 (*d*, *J* = 6.3 Hz, H-14), 7.52 (*m*,

H-11, H-12), 7.3 (*ddd*, J = 3.2, 5.1, 7.6 Hz, H-10), 3.2 (*s*, H-17), 2.9 (*s*, H-18).

3,14-Dihydroolivacine (**24**). Ceric-spray: green. UV λ_{max}^{MeOH} nm: 203, 235, 274, 282, 298, 310, 370. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2950, 1750, 750. MS *m/z* (rel. int.): 248 $[M]^+$ (100), 247 (70), 233 (30), 218 (5), 204 (10). ^1H NMR ($CDCl_3$): δ8.25 (*bs*, NH), 8.0 (*d*, J = 8 Hz, H-9), 8.1 (*s*, H-21), 7.58 (*t*, J = 7 Hz, H-11), 7.75 (*dd*, J = 7, 8 Hz, H-10), 7.23 (*d*, J = 7 Hz, H-12), 3.7 (*t*, J = 7 Hz, H-3, H-3'), 2.8 (*t*, J = 7 Hz, H-14, H-14'), 2.55 (*s*, H-18), 2.4 (*s*, H-17).

Voaphylline hydroxyindolenine (**19**). Ceric-spray: pinkish. UV λ_{max}^{MeOH} nm: 208, 221, 267, 286. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 3200, 2950, 1470, 760. MS *m/z* (rel. int.): 312 $[M]^+$ (100), 295 (40), 265 (30), 222 (20), 186 (25), 172 (50), 156 (70), 146 (20), 130 (20), 124 (40), 77 (30). ^1H NMR ($CDCl_3$): δ7.45 (*d*, J = 7 Hz, H-9), 7.33 (*t*, J = 7 Hz, H-10), 7.3 (*d*, J = 7 Hz, H-12), 7.2 (*t*, J = 7 Hz, H-11), 4.0 (*dd*, J = 12, 14 Hz, H-16), 3.25 (*d*, J = 13 Hz, H-3), 3.1 (*d*, J = 4 Hz, H-14), 3.0 (*d*, J = 4 Hz, H-15), 2.58 (*d*, J = 13 Hz, H-3'), 2.52 (*d*, J = 13 Hz, H-21), 2.45 (*m*, H-17), 2.42 (*m*, H-6), 2.4 (*m*, H-16'), 2.22 (*m*, H-5), 2.19 (*m*, H-17'), 2.05 (*dt*, J = 6, 11, 11 Hz, H-5'), 1.95 (*d*, J = 12 Hz, H-21'), 1.65 (*m*, H-6'), 1.3 (*m*, H-19), 0.95 (*t*, J = 7 Hz, H-18).

Janetine (**21**). Ceric-spray: green. $[\alpha]_D$ + 8° (EtOH; *c* 0.95). UV λ_{max}^{MeOH} nm: 217, 232, 240, 250, 262, 287 (sh), 298, 328, 341. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1720, 1610, 1580, 1450, 1250, 750. MS *m/z* (rel. int.): 250 $[M]^+$ (16), 236 (18), 235 (100), 233 (8). ^1H NMR ($CDCl_3$): δ8.0 (*d*, J = 7.7 Hz, H-9), 7.85 (*s*, NH), 7.7 (*s*, H-21), 7.45 (*bd*, J = 7.1 Hz, H-12), 7.2 (*ddd*, J = 1.3, 6.7, 7.9, H-11), 7.3 (*dt*, J = 8, 1.1 Hz, H-10), 4.32 (*q*, J = 7.2 Hz, H-19), 3.4 (*dt*, J = 12.6, 5 Hz, H-3), 2.8–3 (*m*, H-14–H-14'), 3.13 (*ddd*, J = 5.3, 8.1, 12.6 Hz, H-3'), 2.42 (*s*, H-17), 1.6 (*d*, J = 7.2 Hz, H-18). ^{13}C NMR ($CDCl_3$): δ139.8 (C-13), 137.9 (C-2), 132.4 (C-20), 130.7 (C-15), 125.3 (C-11), 123.9 (C-8), 120.9 (C-7), 120.0 (C-9), 119.1 (C-10), 117.1 (C-16), 114.7 (C-21), 110.5 (C-12), 52.4 (C-19), 41.9 (C-3), 28.0 (C-14), 23.5 (C-18), 12.9 (C-17).

N-Methylpericyclivine (**8**). Ceric-spray: blue grey. $[\alpha]_D$ + 7.5° ($CHCl_3$; *c* 0.29). UV λ_{max}^{MeOH} nm: 222, 280, 293. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740. MS *m/z* (rel. int.): 336 $[M]^+$ (100), 321 (20), 305 (5), 277 (40), 263 (20), 196 (15), 183 (90), 182 (95), 167 (40), 154 (20). ^1H NMR ($CDCl_3$): δ7.4 (*d*, J = 7.6 Hz, H-9), 7.3–7.05 (*m*, H-10, H-11, H-12), 5.55 (*q*, J = 6.1 Hz, H-19), 5.12 (*bd*, J = 10 Hz, H-3), 4.25–4.1 (*m*, H-5, H-21, H-21'), 3.75 (*dd*, J = 18, 5 Hz, H-6'), 3.45 (*dd*, J = 18, 2 Hz, H-6), 3.4 (*s*, N-Me), 3.18 (*m*, H-15), 3.1 (*s*, CO_2Me), 2.95 (*dd*, J = 3, 10 Hz, H-16), 2.75 (*dd*, J = 12, 3 Hz, H-14'), 2.15 (*t*, J = 12 Hz, H-14), 1.7 (*d*, J = 7 Hz, H-18).

18,19-(R)-*Dihydroxycoronaridine* (**13**). Ceric-spray: blue grey. $[\alpha]_D$ − 6° (MeOH; *c* 0.3). UV λ_{max}^{MeOH} nm: 213, 228 (sh), 275 (sh), 285, 294. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1735. MS *m/z* (rel. int.): 370 $[M]^+$ (20), 352 (10), 339 (100), 323 (25), 214 (10), 180 (5), 168 (10), 154 (15). ^1H NMR ($CDCl_3$): δ7.85 (*bs*, NH), 7.7 (*d*, J = 7.7 Hz, H-9), 7.27 (*d*, J = 7.8 Hz, H-12), 7.18 (*dt*, J = 1.1, 7.5 Hz, H-11), 7.1 (*dt*, J = 1.1, 7.3 Hz, H-10), 4.19 (*ddd*, J = 6.9, 4.7, 2 Hz, H-19), 3.9 (*s*, H-21), 3.72 (*s*, CO_2Me), 3.63 (*dd*, J = 11, 7.3 Hz, H-18'), 3.5 ((*m*, H-5', H-6', H-18), 3.1–3.25 (*m*, H-5, H-6), 3.0 (*m*, H-3'), 2.83 (*bd*, J = 9.1 Hz, H-3), 2.0–2.1 (*m*, H-14, H-17), 1.9 (*ddt*, 12, 6.3, 2 Hz, H-15'), 1.65 (*dd*, J = 16.6, 10 Hz, H-20), 1.55 (*m*, J = H-15). ^{13}C NMR ($CDCl_3$): δ122.4 (C-11), 119.5 (C-10), 118.5 (C-9), 110.5 (C-12), 75.8 (C-19), 64.9 (C-18), 59.2 (C-21), 53.0 (CO_2Me), 52.2 (C-5), 51.1 (C-3), 36.8 (C-17), 36.3 (C-20), 26.6 (C-14), 23.7 (C-15), 21.5 (C-6).

Chloromethylene-affinisinium (**17**). Ceric-spray: light purple. $[\alpha]_D$ + 4° (MeOH; *c* 0.4). UV λ_{max}^{MeOH} nm: 221, 282, 293. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350; MS *m/z* (rel. int.): 322 $[M - Cl]^+$ (40), 321 (50), 320 (100), 308 (35), 183 (60), 182 (40), 170 (60). ^1H NMR ($CDCl_3$): δ7.4 (*d*, J = 7.8 Hz, H-9), 7.3 (*m*, H-11, H-12), 7.15 (*t*, J = 7.2 Hz, H-10), 6.5 (*d*, J = 10 Hz, H-3), 5.85 (*d*, J = 10.3 Hz, CH_2Cl), 5.55 (*q*, J = 6.7 Hz, H-19), 5.1 (*d*, J = 10.2 Hz, CH_2Cl), 5.0 (*bd*, J = 15.5 Hz, H-21'), 4.45 (*d*, J = 15.2 Hz, H-21), 4.05 (*m*, H-5), 3.72 (*s*, NMe), 3.5–3.65 (*m*, H-17, H-17'), 3.25 (*bs*, H-6, H-6'), 2.95 (*bs*, H-15), 2.7 (*t*, J = 11.4 Hz, H-14'), 2.28 (*q*, J = 7.3 Hz, H-16), 2.0 (*dd*, J = 12.4, 4.6 Hz, H-14), 1.65 (*d*, J = 6.8, H-18). ^{13}C NMR ($CDCl_3$): δ137.7 (C-13), 131.0 (C-2), 125.6 (C-20), 125.3 (C-8), 122.8 (C-11), 121.7 (C-19), 120.0 (C-10), 118.5 (C-9), 109.3 (C-12), 100.8 (C-7), 64.7 (CH_2Cl), 62.9 (C-5), 62.6 (C-17), 59.7 (C-21), 58.6 (C-3), 42.7 (C-16), 31.3 (C-14), 29.8 (N-Me), 26.3 (C-15), 24.0 (C-6), 12.7 (C-18).

Demethylaccedinisine (**22**). Ceric-spray: grey. $[\alpha]_D$ − 30° (MeOH; *c* 0.3). UV λ_{max}^{MeOH} nm: 232, 286, 293. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2940, 1730, 1620. ^1H NMR (CD_3OD): δ7.6 (*m*, H-9), 7.25 (*s*, H-9'), 7.2 (*d*, J = 8 Hz, 1H), 7.0–7.1 (*m*, 5H), 5.5 (*q*, J = 7 Hz, H-19), 5.4 (*q*, J = 7 Hz, H-19'), 4.65 (*dd*, J = 3, 13 Hz, H-3), 4.35 (*bt*, J = 8 Hz, H-5), 4.2 (*d*, J = 8 Hz, H-3'), 4.1 (*d*, J = 15 Hz, H-21), 3.8 (*m*, H-15), 3.7 (*m*, H-6), 3.6 (*m*, H-6, N'-Me, 2 H-21'), 3.5 (*m*, 2 H-17'), 3.45 (*d*, J = 15 Hz, H-21), 3.1 (*dd*, J = 6, 14 Hz, H-6'), 2.8 (*m*, H-5', H-15'), 2.7 (*m*, H-14, H-16, H-6'), 2.5 (*s*, CO_2Me), 2.2 (*t*, J = 14 Hz, H-14'), 2.1 (*m*, H-14), 1.85 (*q*, J = 7 Hz, H-16'), 1.7 (*d*, J = 7 Hz, H-18), 1.6 (*d*, J = 7 Hz, H-18').

18-*Hydroxyaffinisine* (**23**). Ceric-spray: green-grey. UV λ_{max}^{MeOH} nm: 223, 227, 285, 293. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2900, 1480, 1100, 750. MS *m/z* (rel. int.): 324 $[M]^+$ (20), 323 (22), 322 (25). 309 (5). 307 (7), 293 (8), 214 (18), 183 (28), 182 (15). ^1H NMR ($CDCl_3$): δ7.45 (*d*, J = 7 Hz, H-9), 7.3 (*d*, J = 7 Hz, H-12), 7.2 (*t*, J = 7 Hz, H-11), 7.1 (*t*, J = 7 Hz, H-10), 5.7 (*bt*, J = 7 Hz, H-19), 4.25 (*m*, H-18), 4.2 (*d*, J = 8 Hz, H-3), 4.0 (*dd*, J = 7, 12 Hz, H-18'), 3.7 (*m*, H-21'), 3.65 (*s*, N-Me), 3.6 (*m*, H-17', H-21), 3.45 (*t*, J = 10.5 Hz, H-17), 3.08 (*dd*, J = 14, 6 Hz, H-6'), 3.05 (*bs*, H-15), 2.72 (*t*, J = 6 Hz, H-5), 2.6 (*d*, J = 14 Hz, H-6), 1.9 (*ddd*, J = 1.5, 12, 10 Hz, H-14'), 1.85 (*m*, H-16), 1.7 (*dt*, J = 12, 3 Hz, H-14).

Buchtienine (**26**). Ceric-spray: yellow. UV λ_{max}^{MeOH} nm: 224, 275, 282, 290. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400–3100, 1730, 1630. MS *m/z* (rel. int.): 494 $[M]^+$ (4), 464 (4), 449 (3), 434 (3), 406 (5), 364 (7), 324 (35), 323 (30), 295 (5), 265 (6), 252 (30), 251 (35), 249 (40), 247 (35), 235 (10), 223 (5), 185 (20), 170 (60), 169 (100), 156 (20), 154 (20). ^1H NMR ($CDCl_3$): δ8.7 (*bs*, NH), 7.7 (*bs*, NH), 7.6 (*d*, J = 7.5 Hz, H-9), 7.45 (*d*, J = 8 Hz, H-9'), 7.15–7.25 (*m*, H-10, H-11, H-12), 7.1 (*dt*, J = 7, 1.4 Hz, H-10'), 7.05 (*dt*, J = 7, 1.4 Hz, H-11'),

7.0 (*bd*, $J = 8$ Hz, H-12'), 5.5 (*q*, $J = 6.8$ Hz, H-19), 4.6 (*d*, $J = 1.6$, H-17), 4.3 (*bs*, H-3), 3.75 (*bd*, $J = 12$ Hz, H-21a), 3.6 (*m*, H-15), 3.5 (*m*, H-5'a), 3.35 (*m*, H-5a), 3.3 (*s*, OMe), 3.1 (*m*, H-6a), 3.1 (*m*, H-5b), 2.93 (*d*, $J = 12$ Hz, H-21b), 2.87 (*m*, H-5'b), 2.8 (*dd*, $J = 2.5$, 11.6 Hz, H-16), 2.7 (*m*, H-6'), 2.62 (*d*, $J = 10.1$ Hz, H-6b), 2.55 (*bd*, $J = 14$ Hz, H-14a), 2.1 (*dt*, $J = 6.2$, 14 Hz, H-14b), 1.6 (*dd*, $J = 1$, 6.8 Hz, H-18). ^{13}C NMR ($CDCl_3$): δ174.3 (C-22), 135.6 (C-19, C-13'), 135.3 (C-20), 134.6 (C-2), 132.1 (C-2'), 128.1 (C-8), 127.1 (C-8'), 122.3 (C-19), 121.7 (C-11), 121.5 (C-11'), 119.9 (C-10), 119.1 (C-10'), 118.5 (C-9), 117.9 (C-9'), 111.1 and 111.0 (C-12 and C-12'), 52.7 (C-3), 52.3 (C-17), 52.0 (C-21), 51.4 (C-5), 51.3 (Me), 49.1 (C-16), 44.0 (C-5'), 31.2 (C-15), 28.7 (C-14), 22.5 (C-6'), 17.4 (C-6), 13.0 (C-18).

3-Hydroxytetrahydroolivacine (**28**). Ceric-spray: green. $[\alpha]_D$ + 8° (EtOH; *c* 0.3). UV λ_{max}^{MeOH} nm: 220, 236, 242, 251, 261, 287, 297, 329, 346. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 1620, 1580. MS *m/z* (rel. int.): 266 $[M]^+$ (15), 251 (100), 233 (25). ^{1}H NMR ($CDCl_3$): δ8.05 (*d*, $J = 7.5$ Hz, H-9), 7.95 (*bs*, NH), 7.8 (*s*, H-21), 7.45 (*m*, H-11, H-12), 7.2 (*dt*, $J = 2$, 7.5 Hz, H-10), 4.9 (*t*, $J = 2$ Hz, H-3), 4.29 (*q*, $J = 7.2$ Hz, H-19), 3.4 (*dd*, $J = 13$, 2 Hz, H-14), 3.1 (*dd*, $J = 13$, 2 Hz, H-14'), 2.65 (*s*, H-17), 1.65 (*d*, $J = 7.2$ Hz, H-18).

Demethylceridimine (**30**). Ceric-spray: purple-blue. $[\alpha]_D$ − 167° (EtOH; *c* 1.3). UV λ_{max}^{MeOH} nm: 225, 285, 293. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400–3100, 1730. MS *m/z* (rel. int.): 482 $[M]^+$ (30), 452 (5), 436 (5), 316 (20), 287 (8), 271 (20), 269 (25), 256 (15), 248 (15), 186 (30), 166 (40), 159 (35), 156 (25), 130 (20), 122 (15). ^{1}H NMR ($CDCl_3$): δ8.2 (*bs*, NH), 7.5 (*m*, H-4', H-9), 7.18 (*m*, H-7'), 7.08 (*m*, H-5', H-11), 7.0 (*m*, H-2', H-10, H-12), 5.25 (*q*, $J = 7$ Hz, H-19), 5.05 (*dd*, $J = 13$, 2.4 Hz, H-3), 4.15 (*bt*, $J = 9$ Hz, H-5), 4.1 (*d*, $J = 15$ Hz, H-21'), 3.85 (*bt*, $J = 9$ Hz, H-15), 3.7 (*dd*, $J = 10$, 14 Hz, H-6'), 3.3 (*m*, H-6, H-β'), 3.1 (*d*, $J = 15$ Hz, H-21), 3.1 (*m*, H-β, H-α'), 3.0 (*m*, H-α), 2.9 (*q*, $J = 15$ Hz, H-14'), 2.55 (*t*, $J = 3$ Hz, H-16), 2.45 (*s*, CO_2Me), 2.0 (*ddd*, $J = 15$, 3, 7 Hz, H-14), 1.6 (*bd*, $J = 7$ Hz, H-18). ^{13}C NMR ($CDCl_3$): δ171.3 (C-22), 139.7 (C-20), 139.3 (C-6'), 135.9 (C-9'), 135.8 (C-13), 135.6 (C-2), 129.2 (C-8), 127.7 (C-8'), 121.4 (C-11 and C-2'), 118.9 (C-5'), 118.3 (C-10), 118.2 (C-4'), 117.6 (C-19), 117.4 (C-9), 110.6 (C-7'), 109.8 (C-12), 109.1 (C-7), 108.0 (C-3'), 53.1 (C-5), 50.0 (CO_2Me), 49.9 (C-16), 41.3 (C-β), 35.2 (C-3), 34.9 (C-14), 33.8 (C-15), 26.8 (C-α), 24.6 (C-6), 12.0 (C-18).

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