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Biologically active indole and bisindole alkaloids from *Tabernaemontana divaricata*

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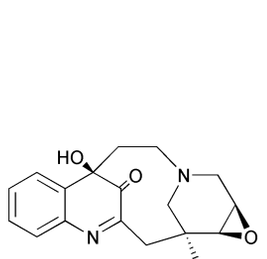
The ethanol extract of the leaves of *Tabernaemontana divaricata* (double flower variety) provided a total of 23 alkaloids, including the new aspidosperma alkaloids, taberhanine, voafinine, *N*-methylvoafinine, voafinidine, voalenine and the new bisindole alkaloid, conophyllinine in addition to the previously known, biologically active bisindole, conophylline and its congener, conofoline. The structures of the new alkaloids were established by spectroscopic methods. The preparation and characterization of the corresponding quinones of the biologically active bisindoles are also described in relation to a structure–activity study of these compounds with respect to their action in stimulating insulin expression.

Plants of the genus *Tabernaemontana* (Apocynaceae) have a widespread distribution and are known to provide alkaloids of intriguing molecular structure as well as novel biological activity.[1–3] We have reported many examples of new alkaloids from various Malaysian representatives of this genus that are distinguished by their structural novelty, as well as useful bioactivity.[4–15] For instance, we have previously reported the structure of the novel tetracyclic indole voaharine **1**, as well as the novel bisindoles, conophylline **2**, conophyllidine **3**, and conofoline **4** from the leaf extract of *T. divaricata* (L.) R. Br. ex Roem. & Schult.[4–6] The bisindole conophylline **2** which is found in both the single-flower as well as the double-flower variety of *T. divaricata* has been recently found to exhibit important biological activity.[16–19] It was shown to be a potent inhibitor of *ras* functions and, very recently, it has also been found to induce morphological change as well as insulin production in pancreatic acinar carcinoma AR42J cells.[20] In view of the promising biological significance of conophylline with respect to its potential in pancreatic β-cell regeneration therapy, we undertook a detailed investigation of the alkaloidal constituents of *T. divaricata* (double-flower variety) with a view to addressing several issues. Firstly, a full study may uncover useful new analogues (hence allowing the preparation of semisynthetic derivatives) for projected structure–activity studies, with respect to the activity of conophylline and its congeners in stimulating insulin expression.[20] Secondly, it is of interest to uncover the existence of the monomeric moieties constituting the bisindole compounds, which would furnish useful information regarding their biogenetic origin. Thirdly, the present large scale effort would complete the study of the full alkaloidal composition of this important plant.[21]



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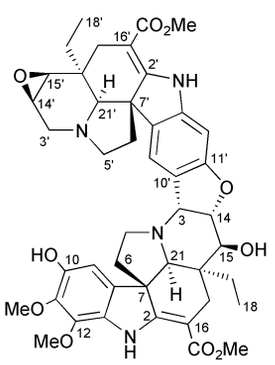

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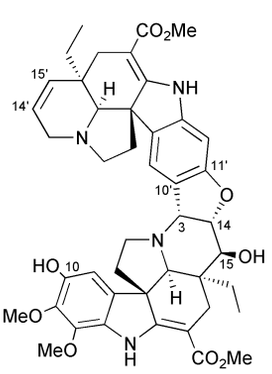

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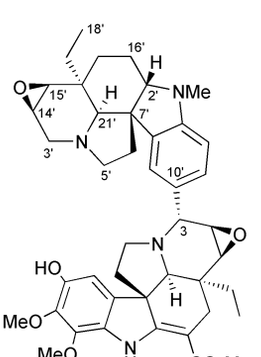

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Results and discussion

One pertinent question that has arisen with respect to the alkaloidal composition of this plant is that, although a common, highly oxygenated tabersonine-like unit occurs in all three bisindoles, conophylline **2**, conophyllidine **3** and conofoline **4**, this unusual unit has not been previously detected and has eluded repeated attempts to demonstrate its independent existence.[3–6] We have finally succeeded in isolating small amounts of this elusive compound for which we give the trivial name taberhanine. Taberhanine **5** was obtained as a light yellow oil. The UV spectrum showed absorption maxima at 202, 238, 307 and 340 nm, consistent with a β-anilinoacrylate chromophore, and resembling that of conophylline **2**. The IR spectrum showed bands at 3383 and 1674 cm^{-1} due to *N*H/OH and conjugated carbonyl functions respectively. The APIMS spectrum showed an MH^+ ion at *m*/*z* 429 and HREIMS measurements yielded the molecular formula $C_{23}H_{28}N_2O_6$, corresponding to a DBE value of 11. The ^{1}H NMR spectrum of **5** showed the presence of three methoxy groups at δ 3.91, 3.93 and 3.78 (two aromatic methoxy and one carbomethoxy respectively), an indolic *N*H (δ 8.82), an OH function (δ 5.45), one isolated aromatic H (δ 6.61) and an ethyl side chain {δ 0.74, t, *J* 7.5 Hz, H(18); 0.93, dq, *J* 15, 7.5 Hz; 1.00, dq, *J* 15, 7.5 Hz, H(19)}. The presence of only one aromatic hydrogen indicated a highly substituted indole ring, while the two low field aromatic methoxy carbon

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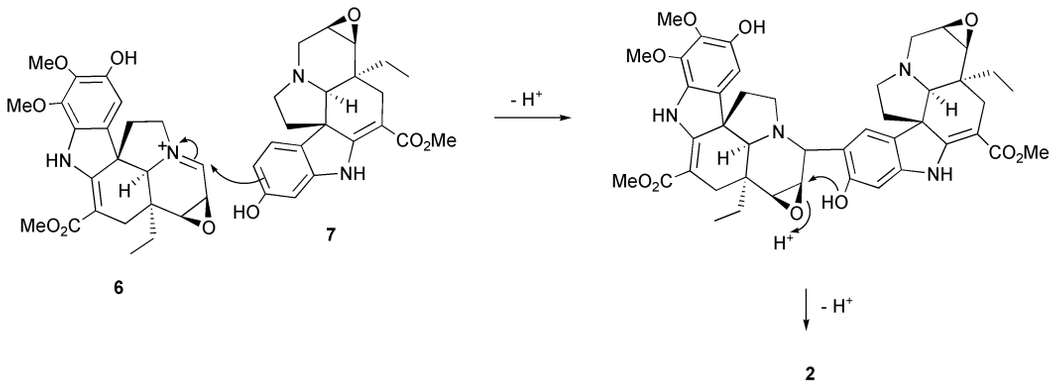
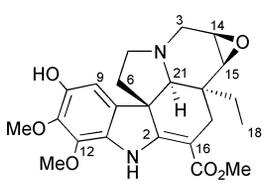


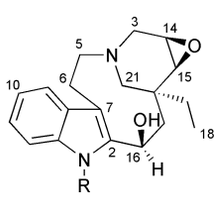
Scheme 1

resonances at δ 60.5 and 61.1, suggested that they are in an *ortho* arrangement.[4,22] The observed NOE interaction between the indolic *N*H and the aromatic methoxy at δ 3.93 (δ_C 60.5) allowed the placement of this methoxy substituent at C(12). The other methoxy substituent was therefore at C(11) and the hydroxy function at C(10). The observed NOE interaction between the lone hydrogen at C(9) with H(21) as well as the phenolic OH at C(10), provided further confirmation of the ring substitution pattern. Such a substitution pattern is reminiscent of that found in the highly oxygenated unit common in the bisindoles **2**–**4**, and examination of the H and C resonances of taberhanine confirmed a basic similarity, with pronounced departure observed only for the piperidine ring D resonances.[4,5] The ^{13}C NMR spectrum of taberhanine showed a total of 22 peaks indicating overlap of two carbon signals. HMQC experiments indicated that the signal at δ 51.0 was due to overlap of the C(5) and methyl ester signal. The presence of an epoxide function is indicated by the characteristic H(14) and H(15) signals at δ 3.26 and 3.07, respectively, and the corresponding C(14) and C(15) resonances at δ 52.1 and 56.3, respectively. This is also consistent with the *N*CH_2CHCH fragment revealed by the COSY spectrum. The stereochemistry of the epoxide function is deduced to be β from the observed carbon resonances at position 14, 15, and 21,[23] as well as from the observed NOE interaction between H(15) and H(18). The remaining H and C signals can be readily assigned with the application of standard 2-D techniques and reveal taberhanine to be the highly oxygenated tabersonine-β-epoxide, **5**. One notable feature of the ^{1}H NMR spectrum of conophylline **2**, is the significantly shielded signal due to H(9) at δ 5.55, which is a consequence of the structure of the dimer, which incorporates a central dihydrofuran ring with *C*(3α)-substitution. Such an arrangement produces a structure which results in placement of the aromatic H(9) directly within the anisotropic influence of the second tabersonine-epoxide unit, resulting in the observed upfield shift. In taberhanine **5**, this is no longer the case, as duly reflected in the observed H(9) resonance at δ 6.61. With the present isolation of taberhanine **5** in the same plant, a possible biogenetic origin for conophylline **2** is conceivable, as arising from electrophilic attack of the iminium ion derived from taberhanine (**6**) on the activated position 10′ of the hypothetical 11′-hydroxypachysiphine acceptor unit (**7**), followed by intramolecular nucleophilic attack by the 11′-OH function on the epoxide unit of the taberhanine moiety from the more accessible α-face of the less hindered C(14), as shown in Scheme 1.

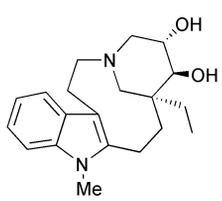


5

Four other new alkaloids of the aspidosperma type were also obtained in this study, *viz.*, voafinine **8**, *N*(1)-methylvoafinine **9**, voafinidine **10**, and voalenine **11**. Voafinine **8** showed a molecular ion at *m*/*z* 312 in EIMS consistent with the formula $C_{19}H_{24}N_2O_2$. The ^{1}H and ^{13}C NMR spectral data indicated that it shares a similar carbon skeleton with voaphylline which, together with *N*(1)-methylvoaphylline, constitute the major alkaloids present in the leaves.[6] The main difference is the presence of an OH group, which is indicated by the signals at δ 6.40 and 5.21 in the ^{1}H NMR spectrum, corresponding to OH and the oxymethine, respectively. The corresponding oxymethine carbon resonance is observed at δ 68.9. The location of the hydroxy function is deduced to be at C(16) from the observed long range heteronuclear correlation from H(17) to C(19) and C(21), which is consistent with the location of the OH function on C(16). Additional confirmation is provided by the observed NOE interaction between 16-OH and H(6β). *N*(1)-Methylvoafinine **9** is readily shown to be the *N*(1)-methyl substituted analogue of voafinine **8** from the MS (M^+ 326, $C_{20}H_{26}N_2O_2$), and the ^{1}H and ^{13}C NMR spectral data (*N*Me instead of *N*H at δ_H 3.68, δ_C 30.3). In addition, the observed NOE interactions between *N*Me and H(16), as well as between 16-OH and H(6β), confirm both the location as well as the β-stereochemistry of the 16-OH function. The stereochemistry of the epoxide unit in both **8** and **9** is confirmed as β from the observed NOE between H(15) and H(18).



8 R = H
9 R = Me



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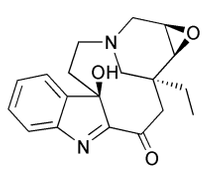
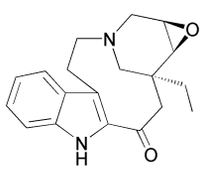


11

Voafinidine **10** showed an M^+ at *m*/*z* 328 ($C_{20}H_{28}N_2O_2$) in EIMS. The ^{13}C NMR spectrum showed a total of 19 carbon resonances, indicating overlap involving one of the peaks {C(5) and C(21) from the HMQC spectrum}. The ^{13}C NMR spectrum of voafinidine was essentially similar to that of *N*-methylvoaphylline[6] except for departure of the piperidine ring resonances, indicating modifications in the piperidine ring.

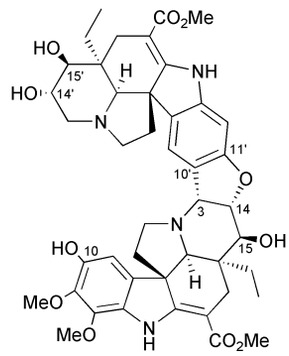
The piperidine ring incorporates two oxymethines from the resonances at δ 70.7 and 80.8, with the corresponding H shifts at δ 3.20 and 3.52, respectively, suggesting the presence of a 1,2-diol function. This was further confirmed by the *N*CH_2CH(O)-CH(O) fragment from the COSY spectrum, corresponding to the C(3)–C(14)–C(15) unit. The observed $J_{14–15}$ value of 9 Hz indicated the *trans*-diaxial disposition of H(14) and H(15), in turn suggesting that the hydroxy groups are both equatorial in the chair-like piperidine ring. On steric grounds, it might be inferred that the stereochemistry of the 14- and 15-substituents are 14α and 15β, assuming that the *trans* diol arises from nucleophilic attack by water on a 14,15-β-epoxide function of the putative precursor, *N*(1)-methylvoaphylline, which would be anticipated to occur on the less-hindered C(14). In the event, this was shown to be the case from NOESY as well as NOE difference experiments. Irradiation of H(14β) resulted in NOE enhancement of H(17β), while irradiation of H(18) resulted in enhancement of H(15α), confirming the stereochemistry of the 15-OH function as β.

Voalenine **11** showed an M^+ at *m*/*z* 326 consistent with the formula $C_{19}H_{22}N_2O_3$. The NMR spectral data again pointed to a voaphylline-like carbon skeleton with an intact C(20) ethyl side chain, and a 14,15-β-epoxide function. The notable absence of the indolic NH, coupled with the characteristic downfield C(2) shift of δ 177.3, and the hydroxy peak at δ 4.43 (exchanges with D_2O), indicated that voalenine is a 7-hydroxy-indolenine derivative. The molecular formula indicated that voalenine has incorporated an additional oxygen compared to the other voaphylline derivatives, and the observed carbon resonance at δ 197.5 indicated a conjugated ketone, immediately suggesting position 16 as the site of oxygenation. This is entirely consistent with the COSY spectrum which revealed the presence of two isolated methylenes, in addition to an ethylene, and *N*CH_2CHCH fragments. Additional confirmation is provided by the HMBC spectrum which showed three-bond correlations from H(17) to C(19). Voalenine **11** is therefore the 7-hydroxyindolenine of 16-oxovoaphylline **12**, which was, however, not obtained despite repeated attempts.



12

A new bisindole, conophyllinine **13**, was also obtained from this study, albeit in a minute amount. The UV and IR spectrum were similar to that of conophylline **2**, showing the presence of a β-anilinoacrylate chromophore, and *N*H/OH conjugated carbonyl functions, respectively. The APIMS spectrum showed an MH^+ ion at *m*/*z* 813. HRFABMS gave the formula $C_{44}H_{52}N_4O_{11}$, differing from conophylline by the addition of a molecule of water. The NMR spectral data indicated that **13** is an analogue of conophylline **2**. The ^{13}C NMR spectrum revealed that the highly-oxygenated tabersonine-like unit, is intact, as in conophylline, and that changes have occurred in the second monomer unit. The similarity of the piperidine ring D H(3), H(14) and H(15) resonances of **13** in the ^1H NMR spectrum with that of conophylline **2**, as well as conophyllidine **3**, indicated a similar mode of connection of the monomeric entities, *i.e. via* formation of a central dihydrofuran ring. Examination of the carbon resonances of the other monomeric unit revealed an essential similarity with that in conophylline, except for the presence of a 1,2-diol function at positions 14′ and 15′, in place of a 14′,15′-β-epoxide function. This is reflected in changes in both the ^1H and ^{13}C resonances of the piperidine ring of the second monomeric unit when compared with conophylline **2**. The most significant change involves carbons 14′ and 15′, which are now oxymethines with δ_C at 69.9 and 81.3, respectively, while the corresponding H(14′) and H(15′) resonances are observed at δ 3.92 and 3.55, respectively. The observed $J_{14'–15'}$ value of 9 Hz suggested a *trans*-diaxial arrangement. This was further confirmed by the observation that irradiation of H(15′) had no effect on H(14′) and *vice versa*. Instead, irradiation of H(15′) caused NOE enhancement of H(18′), H(19′) and H(21′), indicating that the stereochemistry of the OH substituent at C(15′) is β {H(14′β) and H(15′α)}.

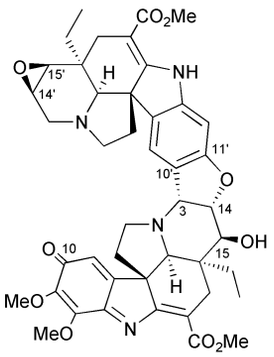


13

In addition to the alkaloids already mentioned, a further 13 known alkaloids were obtained, including the recently reported novel quinolinic alkaloid, voastrictine, from *T. corymbosa*.[10]

The structure of conophylline **2** suggests that the highly oxygenated tabersonine-like unit with a 10-hydroxy function should undergo ready oxidation to a quinone derivative, resulting in extended conjugation. Cyclic voltammetry of conophylline **2**, on a platinum electrode in acetonitrile–dichloromethane, in the presence of 0.1 M Et_4NClO_4 as supporting electrolyte, exhibited a reversible wave at 0.60 V (*versus* Ag/AgCl). The relatively low oxidation potential compared to other indole alkaloids and derivatives, as well as the reversible nature of the process, confirmed the initial supposition that conophylline should undergo facile oxidation.[24] Coulometry indicated that the oxidation is a two-electron process, and controlled potential electrolysis (Pt gauze anode, Pt cathode) proceeded smoothly until consumption of 2 F mol^{-1}, when all the starting material was consumed.[24] Work up of the electrolysed solution, which turned progressively orange as the oxidation progressed, furnished the quinone derivative in quantitative yield. Conophylline quinone **14** is a reddish-orange solid. APIMS and FABMS showed an MH^+ ion at *m*/*z* 793 consistent with the formula $C_{44}H_{48}N_4O_{10}$, corresponding to the loss of 2H compared to conophylline **2**. The UV spectrum (EtOH) showed absorption maxima at 202, 248, 329, and 393 nm. The ^1H NMR spectrum was essentially similar to that of **2**, except for changes consistent with the transformation to a quinone structure in the oxygenated tabersonine-like unit, such as the absence of peaks due to the indolic *N*H and the phenolic 10-OH, and the changes of the proximate H(9) and 11-OMe signals, which have been shifted slightly upfield and downfield, respectively.

Likewise, the notable changes in the ^{13}C NMR spectrum of **14** compared to **2**, involved changes at C(10), with δ at 182.8, consistent with a conjugated ketone function, and the imine C(13) at δ 166.4. Conophylline can also be readily converted to its quinone derivative by MnO_2 oxidation in CH_2Cl_2. Conophylline **2** can be readily regenerated from the quinone **14** by treatment with ascorbic acid or $NaBH_4$. The corresponding quinones of the other bisindoles, *viz.*, conophyllidine **3** and conofoline **4**, *i.e.* **15** and **16** respectively, were similarly prepared by MnO_2 oxidation (see experimental section) for structure–activity studies in connection with the action of these compounds (conophylline and its congeners) in stimulating insulin expression.[20]



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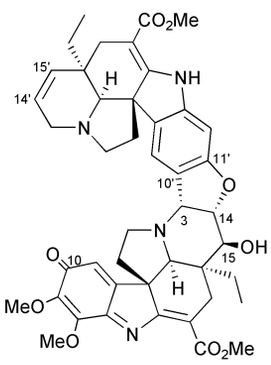

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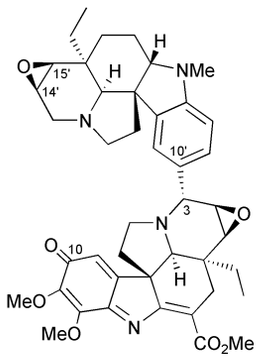

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Experimental

Melting points were determined on a Leitz Wetzler melting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-3101PC spectrophotometer. IR spectra were recorded on a Perkin-Elmer 1600 Series FTIR spectrophotometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter and the $[\alpha]_D$-values are given in 10^{-1} deg cm^2 g^{-1}. APIMS were obtained on a Perkin Elmer API 100 instrument. HREIMS and HRFABMS were obtained on a JEOL GCmate mass spectrometer courtesy of Professor K. Umezawa, Department of Applied Chemistry, Keio University, Yokohama, Japan. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JMN-LA400 spectrometer at 400 and 100 MHz respectively. Coupling constants (*J*) are reported in Hz. Assignments are confirmed by COSY, HMQC, HMBC and NOE experiments. All solvents were of analytical grade and were distilled before use. Acetonitrile and dichloromethane were distilled from calcium hydride. All electrochemical experiments (cyclic voltammetry, coulometry, preparative electrolysis) were performed on a BAS 100B electrochemical analysis system using a 100 mL cylindrical glass cell (BAS MR-1195) fitted with a Teflon cell top. The electrodes used for cyclic voltammetry were a platinum wire electrode (1.6 mm diameter), with platinum as the counter electrode, and Ag/AgCl/NaCl (3 M) as the reference electrode. Preparative electrolyses were performed with a platinum gauze electrode (diameter 4 cm, height 5 cm). The progress of electrolysis was also monitored by TLC as well as cyclic voltammetry.[24]

Isolation of alkaloids

Extraction of the ground leaf material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid, as has been described in detail elsewhere.[25] The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH, followed by rechromatography of appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal TLC were Et_2O–hexane (1 : 4; 3 : 1), Et_2O–hexane (2 : 1, NH_3-saturated), $CHCl_3$–hexane (10 : 1), $CHCl_3$, NH_3-saturated EtOAc–hexane (1 : 1, NH_3-saturated; 3 : 1, NH_3-saturated), EtOAc, Et_2O–MeOH (20 : 1), and $CHCl_3$–MeOH (50 : 1). The yields (g kg^{-1}) of the alkaloids were as follows: **1** (0.0003), **2** (0.116), **4** (0.105), **5** (0.0014), **8** (0.010), **9** (0.008), **10** (0.002), **11** (0.041), **13** (0.003), (−)-mehranine (0.064), pachysiphine (0.009), voastrictine (0.015), peduncularidine (0.005), voaphylline (0.26), *N*(1)-methylvoaphylline (0.12), 16(*R*)-19,20-*E*-isositsirikine (0.004), 16(*R*)-19,20-*E*-isositsirikine oxindole (0.0007), ibogaine (0.002), ibogamine (0.003), voacangine (0.061), voacristine (0.014), apparicine (0.22), and 16-hydroxy-16,22-dihydroapparicine (0.0008).

Taberhanine **5**, was obtained as a light yellowish oil, $[\alpha]_D$ −185 (*c* 0.08, $CHCl_3$), ν_{max} (film)/cm^{-1} 3383, 1674 and 1605; λ_{max} (EtOH)/nm 202 (log ε/dm^3 mol^{-1} cm^{-1} 4.13), 238 (3.94), 307 (3.96) and 340 (3.93); δ_H (400 MHz; $CDCl_3$; Me_4Si) 0.74 (3H, t, *J* 7.5, H-18), 0.93 (1H, dq, *J* 15 and 7.5, H-19), 1.00 (1H, dq, *J* 15 and 7.5, H-19), 1.71 (1H, dd, *J* 11.5 and 4.5, H-6), 2.06 (1H, td, *J* 11.5 and 6.5, H-6), 2.38 (1H, d, *J* 1.5, H-21), 2.51 (1H, d, *J* 15.5, H-17), 2.66 (1H, ddd, *J* 11.5, 8 and 4.5, H-5), 2.68 (1H, dd, *J* 15.5 and 1.5, H-17), 2.85 (1H, d, *J* 13, H-3), 3.00 (1H, dd, *J* 8 and 6.5, H-5), 3.07 (1H, d, *J* 4, H-15), 3.26 (1H, br d, *J* 4, H-14), 3.56 (1H, dd, *J* 13 and 1, H-3), 3.78 (3H, s, CO_2Me), 3.91 (3H, s, 11-OMe), 3.93 (3H, s, 12-OMe), 5.45 (1H, br s, 10-OH), 6.61 (1H, s, H-9) and 8.82 (1H, br s, *N*H); δ_C (100 MHz; $CDCl_3$; Me_4Si) 7.2 (C-18), 23.4 (C-17), 26.5 (C-19), 37.2 (C-20), 44.0 (C-6), 49.6 (C-3), 51.0 (C-5, $CO_2\mathit{Me}$), 52.1 (C-14), 55.2 (C-7), 56.3 (C-15), 60.5 (12-OMe), 61.1 (11-OMe), 71.0 (C-21), 91.1 (C-16), 104.3 (C-9), 129.3 (C-13), 133.6 (C-8), 137.1 (C-12), 139.3 (C-11), 143.7 (C-10), 165.2 (C-2) and 168.7 ($\mathit{C}O_2$Me); *m*/*z* (EI) 428.1954 (M$^+$, 58%. $C_{23}H_{28}N_2O_6$ requires 428.1947), 427 (9), 413 (16), 305 (37), 290 (68), 214 (19), 151 (85), 138 (79), 108 (57) and 69 (100).

Voafinine **8**, was obtained as a light yellowish oil, $[\alpha]_D$ −13.5 (*c* 0.021 $CHCl_3$), ν_{max} (film)/cm^{-1} 3272; λ_{max} (EtOH)/nm 229 (log ε/dm^3 mol^{-1} cm^{-1} 4.08), 285 (3.57) and 292 (3.52); δ_H (400 MHz; $CDCl_3$; Me_4Si) 0.72 (3H, t, *J* 7.5, H-18), 1.23 (2H, m, 2 × H-19), 1.47 (1H, dd, *J* 12 and 1, H-21), 1.96 (1H, dd, *J* 15 and 2, H-17), 2.35 (1H, td, *J* 13 and 3, H-5), 2.48 (1H, ddd, *J* 15, 5.5 and 1, H-17), 2.50 (1H, dt, *J* 12 and 1, H-21), 2.64 (1H, br d, *J* 13, H-3), 2.66 (1H, ddd, *J* 13, 5 and 3, H-5), 2.86 (1H, dt, *J* 14 and 3, H-6), 2.91 (1H, dd, *J* 4 and 1, H-15), 3.26 (1H, br d, *J* 4, H-14), 3.35 (1H, dt, *J* 13 and 1, H-3), 3.43 (1H, ddd, *J* 14, 13 and 5, H-6), 5.21 (1H, ddd, *J* 11, 5.5 and 2, H-16), 6.40 (1H, br s, 16-OH), 7.09 (1H, td, *J* 7.5 and 1, H-10), 7.13 (1H, td, *J* 7.5 and 1, H-11), 7.29 (1H, dd, *J* 7.5 and 1, H-12), 7.48 (1H, br d, 7.5, H-9) and 7.81 (1H, br s, NH); δ_C (100 MHz; $CDCl_3$; Me_4Si) 7.4 (C-18), 24.3 (C-6), 32.5 (C-19), 37.4 (C-20), 42.2 (C-17), 52.8 (C-14), 53.1 (C-5), 54.9 (C-3), 58.6 (C-15 and C-21), 68.9 (C-16), 109.1 (C-12), 110.4 (C-7), 117.8 (C-9), 119.0 (C-10), 121.2 (C-11), 129.3 (C-8), 135.4 (C-13) and 139.3 (C-2); *m*/*z* (EI) 312 (M$^+$, 100%, $C_{19}H_{24}N_2O_2$), 296 (35), 265 (20), 156 (52) and 144 (40).

N(1)-Methylvoafinine **9**, was obtained as a light yellowish oil, $[\alpha]_D$ −39 (*c* 0.069, $CHCl_3$), ν_{max} (film)/cm^{-1} 3363; λ_{max} (EtOH)/nm 231 (log ε/dm^3 mol^{-1} cm^{-1} 4.46), 287 (4.08) and 295 (4.06); δ_H (400 MHz; $CDCl_3$; Me_4Si) 0.66 (3H, t, *J* 7.5, H-18), 1.24 (2H, m, 2 × H-19), 1.45 (1H, dd, *J* 12 and 1, H-21), 1.96 (1H, dd, *J* 15 and 2, H-17), 2.36 (1H, td, *J* 13 and 3, H-5), 2.51 (1H, ddd, *J* 15, 5.5 and 1, H-17), 2.58 (1H, br d, *J* 12, H-21), 2.64 (1H, dd, *J* 13 and 1, H-3), 2.69 (1H, ddd, *J* 13, 5 and 3, H-5), 2.88 (1H, dt, *J* 14 and 3, H-6), 2.90 (1H, dd, *J* 4 and 1, H-15), 3.27 (1H, br d, *J* 4, H-14), 3.37 (1H, br d, *J* 13, H-3), 3.49 (1H, ddd, *J* 14, 13 and 5, H-6), 3.68 (3H, s, NMe), 5.30 (1H, ddd, *J* 11, 5.5 and 2, H-16), 6.83 (1H, d, *J* 11, 16-OH), 7.10 (1H, td, *J* 7.5 and 1, H-10), 7.18 (1H, td, *J* 7.5 and 1, H-11), 7.27 (1H, dd, *J* 7.5 and 1, H-12) and 7.50 (1H, dd, 7.5 and 1, H-9); δ_C (100 MHz; $CDCl_3$; Me_4Si) 7.3 (C-18), 24.5 (C-6), 30.3 (NMe), 32.7 (C-19), 37.6 (C-20), 40.6 (C-17), 52.9 (C-14), 53.1 (C-5), 54.8 (C-3), 58.4

(C-21), 58.8 (C-15), 67.6 (C-16), 108.6 (C-7 and C-12), 117.7 (C-9), 118.8 (C-10), 120.9 (C-11), 128.0 (C-8), 137.2 (C-13) and 140.1 (C-2); *m/z* (EI) 326 (M^+, 100%, $C_{20}H_{26}N_2O_2$), 310 (37), 279 (22), 170 (56), 157 (62) and 144 (43).

Voafinidine **10**, was obtained as a light yellowish oil, $[\alpha]_D$ +57 (*c* 0.185, $CHCl_3$), ν_{max} (film)/cm^{-1} 3384; λ_{max} (EtOH)/nm 233 (log ε/dm^3 mol^{-1} cm^{-1} 4.49), 287 (4.08) and 295 (4.07); δ_H (400 MHz; $CDCl_3$; Me_4Si) 0.94 (3H, t, *J* 7.5, H-18), 1.41 (1H, dq, *J* 15 and 7.5, H-19), 1.60 (1H, dq, *J* 15 and 7.5, H-19), 1.67 (1H, d, *J* 12, H-21), 1.77 (2H, m, 2 × H-17), 2.22 (1H, t, *J* 9.5, H-3), 2.30 (1H, dt, *J* 12 and 8, H-5), 2.55 (1H, dt, *J* 12 and 3, H-5), 2.69 (1H, ddd, *J* 9.5, 6.5 and 2, H-3), 2.71 (1H, ddd, *J* 15.5, 6 and 3, H-16), 2.84 (1H, ddd, *J* 15.5, 9 and 3, H-16), 2.90 (2H, m, 2 × H-6), 3.20 (1H, d, *J* 9.5, H-15), 3.36 (1H, dd, *J* 12 and 2, H-21), 3.52 (1H, td, *J* 9.5 and 6.5, H-14), 3.66 (3H, s, NMe), 7.03 (1H, td, *J* 7.5 and 1, H-10), 7.10 (1H, td, *J* 7.5 and 1, H-11), 7.21 (1H, br d, *J* 7.5, H-12) and 7.44 (1H, br d, 7.5, H-9); δ_C (100 MHz; $CDCl_3$; Me_4Si) 8.4 (C-18), 18.6 (C-16), 22.7 (C-6), 29.4 (C-19), 29.5 (NMe), 31.6 (C-17), 42.5 (C-20), 53.0 (C-5 and C-21), 60.0 (C-3), 70.7 (C-14), 80.8 (C-15), 107.8 (C-7), 108.6 (C-12), 117.2 (C-9), 118.4 (C-10), 120.0 (C-11), 127.3 (C-8), 136.2 (C-13) and 141.4 (C-2); *m/z* (EI) 328 (M^+, 100%, $C_{20}H_{28}N_2O_2$), 213 (24), 171 (77), 157 (48), 156 (29) and 111 (15).

Voalenine **11**, was obtained as a light yellowish oil, $[\alpha]_D$ −445 (*c* 0.04, $CHCl_3$), ν_{max} (film)/cm^{-1} 3472 and 1660; λ_{max} (EtOH)/nm 204 (log ε/dm^3 mol^{-1} cm^{-1} 4.21), 226 (4.12) and 296 (3.79); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.05 (3H, t, *J* 7.5, H-18), 1.43 (1H, ddd, *J* 14, 9 and 3, H-5), 1.52 (1H, dq, *J* 15 and 7.5, H-19), 1.59 (1H, dq, *J* 15 and 7.5, H-19), 2.13 (1H, dd, *J* 12 and 1.5, H-21), 2.30 (2H, m, 2 × H-6), 2.34 (1H, dd, *J* 14 and 3, H-5), 2.56 (1H, br d, *J* 12, H-21), 2.57 (1H, dd, *J* 11 and 1.5, H-3), 2.60 (1H, dd, *J* 11 and 1.5, H-17), 3.14 (1H, d, *J* 4, H-15), 3.16 (1H, dd, *J* 4 and 1.5, H-14), 3.34 (2H, d, *J* 11, H-3 and H-17), 4.43 (1H, br s, 7-OH), 7.32 (1H, td, *J* 7.5 and 1, H-10), 7.40 (1H, td, *J* 7.5 and 1, H-11), 7.43 (1H, ddd, *J* 7.5, 1.5 and 0.7, H-9) and 7.61 (1H, ddd, *J* 7.5, 1.5 and 0.7, H-12); δ_C (100 MHz; $CDCl_3$; Me_4Si) 7.6 (C-18), 30.6 (C-19), 41.7 (C-6), 42.6 (C-20), 42.7 (C-17), 51.5 (C-5), 51.8 (C-3 and C-14), 58.4 (C-15), 58.8 (C-21), 86.1 (C-7), 122.1 (C-9), 122.8 (C-12), 128.2 (C-10), 129.7 (C-11), 140.4 (C-8), 152.5 (C-13), 177.3 (C-2) and 197.5 (C-16); *m/z* (EI) 326 (M^+, 100%, $C_{19}H_{22}N_2O_3$), 298 (9), 225 (15), 201 (17), 185 (20), 144 (40), 130 (61) and 108 (27); *m/z* (FABMS, glycerol) 327.19 (MH^+, $C_{19}H_{22}N_2O_3$ + H requires 327.17).

Conophyllinine **13**, was obtained as a light yellowish oil, $[\alpha]_D$ −109 (*c* 0.09, $CHCl_3$), ν_{max} (film)/cm^{-1} 3383, 1672 and 1609; λ_{max} (EtOH)/nm 204 (log ε/dm^3 mol^{-1} cm^{-1} 3.43), 239 (3.23), 311 (3.25) and 334 (3.31); δ_H (400 MHz; $CDCl_3$; Me_4Si) 0.70 (3H, t, *J* 7.5, H-18), 0.81 (3H, t, *J* 7.5, H-18′), 0.86 (1H, m, H-19), 1.15 (1H, dq, *J* 14 and 7.5, H-19), 1.28 (1H, m, H-19′), 1.34 (1H, m, H-19′), 1.70 (1H, ddd, *J* 11.5, 7.5 and 4, H-6), 1.76 (1H, m, H-6′), 1.99 (1H, td, *J* 11.5 and 6, H-6), 2.09 (1H, td, *J* 11.5 and 6.5, H-6′), 2.40 (1H, d, *J* 15.5, H-17), 2.45 (1H, t, *J* 10, H-3′), 2.50 (1H, d, *J* 15.5, H-17′), 2.64 (1H, d, *J* 1, H-21), 2.68 (1H, m, H-5), 2.71 (1H, s, H-21′), 2.73 (1H, d, *J* 15.5, H-17), 2.77 (1H, br d, *J* 15.5, H-17′), 2.83 (1H, ddd, *J* 11.5, 9 and 5, H-5′), 2.92 (1H, br t, *J* 7.5, H-5), 2.98 (1H, dd, *J* 9 and 6.5, H-5′), 3.34 (1H, dd, *J* 10 and 5, H-3′), 3.55 (1H, d, *J* 9, H-15′), 3.77 (6 H, s, CO_2Me and CO_2Me'), 3.81 (3H, s, 11-OMe), 3.86 (3H, s, 12-OMe), 3.92 (1H, td, *J* 10 and 5, H-14′), 4.15 (1H, br s, H-15), 4.79 (1H, d, *J* 8, H-3), 5.03 (1H, dd, *J* 8 and 4, H-14), 5.33 (1H, s, 10-OH), 5.56 (1H, s, H-9), 6.36 (1H, s, H-12′), 7.15 (1H, s, H-9′), 8.77 (1H, br s, *N*H) and 8.91 (1H, br s, *N*H′); δ_C (100 MHz; $CDCl_3$; Me_4Si) 7.4 (C-18), 8.9 (C-18′), 22.2 (C-17), 23.3 (C-17′), 26.5 (C-19), 27.4 (C-19′), 42.0 (C-6), 43.6 (C-20′), 44.7 (C-20), 46.0 (C-5), 46.5 (C-6′), 50.9 (CO_2Me, C-5′), 51.1 (CO_2Me'), 53.9 (C-3′), 54.8 (C-7), 55.2 (C-7′), 59.5 (C-3), 60.4 (12-OMe), 60.9 (11-OMe), 65.2 (C-21), 69.7 (C-15), 69.9 (C-14′), 70.1 (C-21′), 81.3 (C-15′), 85.2 (C-14), 90.7 (C-16), 92.3 (C-16′), 93.5 (C-12′), 104.3 (C-9), 114.0 (C-10′), 118.8 (C-9′), 128.7 (C-13), 130.0 (C-8′), 133.5 (C-8), 136.8 (C-12), 138.8 (C-11), 143.6 (C-10), 145.2 (C-13′), 161.0 (C-11′), 164.8 (C-2), 167.4 (C-2′), 168.7 (CO_2Me) and 169.1 (CO_2Me'); *m/z* (APIMS) 813 (MH^+, $C_{44}H_{52}N_4O_{11}$ + H); *m/z* (FABMS, NBA) 813.3726 (MH^+, $C_{44}H_{52}N_4O_{11}$ + H requires 813.3711).

Oxidation of conophylline 2, conophyllidine 3 and conofoline 4

Anodic oxidation of conophylline **2** (50 mg, 0.06 mmol) was carried out at a platinum gauze anode (0.69 V *versus* Ag/AgCl, 30% CH_2Cl_2–MeCN, 0.1 M Et_4NClO_4) in a divided cell under nitrogen. The reaction proceeded smoothly until consumption of 2 F mol^{-1}. In the course of the reaction, the solution turned orange and the colour intensified as more of the product was formed. The solution was then evaporated to dryness and CH_2Cl_2 (10 mL) was added. The precipitated electrolyte was then filtered off and the residue washed with CH_2Cl_2. The product mixture was then chromatographed over SiO_2 with 2% MeOH–$CHCl_3$ as eluent to afford conophylline quinone (48 mg, 97%).

Conophylline quinone **14** was obtained as a reddish-orange amorphous solid, $[\alpha]_D$ −138 (*c* 0.25, $CHCl_3$), ν_{max} (film)/cm^{-1} 3373, 1679, 1634 and 1610; λ_{max} (EtOH)/nm 202 (log ε/dm^3 mol^{-1} cm^{-1} 4.63), 248 (4.46), 329 (4.56) and 393 (4.17); δ_H (400 MHz; $CDCl_3$; Me_4Si) 0.81 (3H, t, *J* 7.5, H-18), 0.81 (1H, m, H-19), 0.90 (3H, t, *J* 7.5, H-18′), 1.16 (1H, dq, *J* 14 and 7.5, H-19), 1.26 (1H, m, H-19′), 1.28 (1H, m, H-19′), 1.68 (1H, dd, *J* 11.5 and 4, H-6′), 1.73 (1H, dd, *J* 11.5 and 4, H-6), 2.06 (1H, m, H-6′), 2.08 (1H, m, H-6), 2.37 (1H, br s, H-21), 2.47 (1H, br s, H-21′), 2.51 (1H, d, *J* 15.5, H-17′), 2.69 (1H, d, *J* 15.5, H-17), 2.72 (2H, m, H-5 and H-5′), 2.75 (1H, br d, *J* 15.5, H-17′), 2.93 (1H, d, *J* 13, H-3′), 2.97 (1H, br d, *J* 15.5, H-17), 3.07 (2H, m, H-5 and H-5′), 3.13 (1H, d, *J* 4, H-15′), 3.25 (1H, d, *J* 4, H-14′), 3.59 (1H, d, *J* 13, H-3′), 3.78 (3H, s, CO_2Me'), 3.87 (3H, s, 12-OMe), 3.89 (3H, s, CO_2Me), 4.14 (1H, d, *J* 4, H-15), 4.17 (3H, s, 11-OMe), 4.82 (1H, d, *J* 8, H-3), 4.99 (1H, s, H-9), 5.06 (1H, dd, *J* 8 and 4, H-14), 6.33 (1H, s, H-12′), 7.06 (1H, s, H-9′) and 9.02 (1H, br s, *N*H′); δ_C (100 MHz; $CDCl_3$; Me_4Si) 7.4 (C-18′), 7.6 (C-18), 23.3 (C-17′), 26.9 (C-19′), 27.1 (C-17 and C-19), 37.1 (C-20′), 40.1 (C-6), 44.4 (C-6′), 45.8 (C-5), 48.8 (C-20), 49.5 (C-3′), 51.1 (C-5′ and CO_2Me'), 52.3 (C-14′), 53.1 (C-7 and CO_2Me), 54.1 (C-7′), 56.3 (C-15′), 59.4 (C-3), 61.1 (11-OMe), 61.3 (12-OMe), 65.6 (C-21), 69.1 (C-15), 72.1 (C-21′), 84.7 (C-14), 92.0 (C-16′), 92.9 (C-12′), 112.7 (C-10′), 118.0 (C-9), 119.1 (C-9′), 121.6 (C-16), 131.1 (C-8′), 144.0 (C-12), 145.3 (C-11), 145.9 (C-13′), 154.3 (C-8), 161.0 (C-11′), 163.6 (C-2′), 164.6 (C-2), 166.4 (C-13), 166.4 (CO_2Me), 168.8 (CO_2Me') and 182.8 (C-10); *m/z* (APIMS, MeOH) 793 MH^+; (FABMS, NBA) 793 (MH^+, $C_{44}H_{48}N_4O_{10}$ + H).

Conophylline quinone **14** was instantaneously and quantitatively reduced by $NaBH_4$ or ascorbic acid in CH_2Cl_2 to conophylline **2**.

Oxidation of conophylline **2**, conophyllidine **3** (from *T. divaricata*, single flower variety[5]) and conofoline **4**, with MnO_2 in CH_2Cl_2 afforded the corresponding quinones in near quantitative yields.

Conophyllidine quinone **15** was obtained as a reddish-orange amorphous solid, $[\alpha]_D$ −270 (*c* 0.037, $CHCl_3$), ν_{max} (film)/cm^{-1} 3371, 1679, 1631 and 1611; λ_{max} (EtOH)/nm 203 (log ε/dm^3 mol^{-1} cm^{-1} 4.60), 248 (4.47), 329 (4.49) and 394 (4.13); δ_H (400 MHz; $CDCl_3$; Me_4Si) 0.80 (3H, t, *J* 7.5, H-18), *ca.* 0.80 (1H, m, H-19), 0.81 (3H, t, *J* 7.5, H-18′), 1.12 (1H, dq, *J* 14 and 7.5, H-19), 1.20 (1H, dq, *J* 14 and 7.5, H-19′), 1.27 (1H, m, H-19′), 1.65 (1H, m, H-6), 1.75 (1H, m, H-6′), 2.04 (1H, td, *J* 11.5 and 6, H-6), 2.08 (1H, td, *J* 11.5 and 6.5, H-6′), 2.37 (1H, d, *J* 1, H-21), 2.40 (1H, d, *J* 15.5, H-17′), 2.62 (1H, dd, *J* 15.5 and 1, H-17′), 2.68 (1H, br s, H-21′), 2.68 (2H, m, H-5 and H-5′), *ca.* 2.70 (1H, m, H-17), 2.97 (1H, dd, *J* 15.5 and 1, H-17), 3.05 (2H, m, H-5 and H-5′), 3.22 (1H, d, *J* 16, H-3′), 3.48 (1H, dd,

J 16 and 3, H-3′), 3.77 (3H, s, CO_2Me'), 3.86 (3H, s, 12-OMe), 3.89 (3H, s, CO_2Me), 4.16 (1H, d, *J* 4, H-15), 4.16 (3H, s, 11-OMe), 4.81 (1H, d, *J* 8, H-3), 4.94 (1H, s, H-9), 5.06 (1H, dd, *J* 8 and 4, H-14), 5.78 (2H, br s, H-14′ and H-15′), 6.34 (1H, s, H-12′), 7.11 (1H, s, H-9′) and 9.01 (1H, br s, *N*H′); δ_C (100 MHz; $CDCl_3$; Me_4Si) 7.6 (C-18 and C-18′), 26.8 (C-19′), 26.9 (C-19), 27.2 (C-17), 28.3 (C-17′), 40.1 (C-6), 41.1 (C-20′), 44.8 (C-6′), 45.8 (C-5), 48.7 (C-20), 50.5 (C-5′), 50.9 (CO_2Me and CO_2Me'), 51.1 (C-3′), 53.1 (C-7), 54.5 (C-7′), 59.4 (C-3), 61.1 (11-OMe), 61.3 (12-OMe), 65.5 (C-21), 69.1 (C-15), 70.8 (C-21′), 84.8 (C-14), 92.9 (C-12′ and C-16′), 112.5 (C-10′), 118.0 (C-9), 119.0 (C-9′), 121.4 (C-16), 124.2 (C-14′), 131.4 (C-8′), 133.3 (C-15′), 143.9 (C-12), 145.4 (C-11), 145.8 (C-13′), 154.2 (C-8), 160.8 (C-11′), 163.6 (C-2′), 164.9 (C-2), 166.4 (C-13 and CO_2Me), 168.8 (CO_2Me') and 182.7 (C-10); *m*/*z* (APIMS, MeOH) 777 (MH^+, $C_{44}H_{48}N_4O_9$ + H).

Conofoline quinone **16** was obtained as a reddish-orange amorphous solid, $[a]_D$ −302 (*c* 0.282, $CHCl_3$), ν_{max} (film)/cm^{-1} 1699, 1633 and 1579; λ_{max} (EtOH)/nm 207 (log ε/dm^3 mol^{-1} cm^{-1} 4.42), 267 (4.21), 305 (3.85) and 393 (3.95); δ_H (400 MHz; $CDCl_3$; Me_4Si) 0.75 (3H, t, *J* 7.5, H-18′), 0.93 (3H, t, *J* 7.5, H-18), 1.00 (1H, dq, *J* 14 and 7.5, H-19), 1.13 (1H, dq, *J* 14 and 7.5, H-19), 1.24 (1H, m, H-16′ and H-19′), 1.34 (1H, dq, *J* 14 and 7.5, H-19′), 1.50 (1H, br dd, *J* 14 and 4, H-17′), 1.60 (1H, dd, *J* 11.5 and 4, H-6), 1.62 (1H, ddd, *J* 13, 9 and 2, H-6′), 1.81 (1H, td, *J* 14 and 3, H-17′), 1.87 (1H, m, H-16′), 2.06 (1H, td, *J* 11.5 and 6, H-6), 2.18 (1H, q, *J* 9, H-5′), 2.24 (1H, s, H-21′), 2.34 (1H, m, H-6′), 2.35 (1H, d, *J* 13, H-3′), 2.39 (1H, m, H-5), 2.59 (1H, d, *J* 1, H-21), 2.81 (3H, s, *N*Me′), 2.89 (1H, d, *J* 15.5, H-17), 2.94 (1H, d, *J* 4, H-15′), 2.95 (1H, br d, *J* 15.5, H-17), 2.96 (1H, m, H-5), 3.20 (1H, td, *J* 9 and 2, H-5′), 3.27 (1H, d, *J* 4, H-15), 3.34 (1H, br d, *J* 4, H-14′), 3.42 (1H, dd, *J* 11 and 5, H-2′), 3.43 (1H, dd, *J* 4 and 1, H-14), 3.58 (1H, dd, *J* 13 and 1, H-3′), 3.90 (3H, s, CO_2Me), 3.92 (3H, s, 12-OMe), 4.19 (3H, s, 11-OMe), 4.50 (1H, s, H-3), 5.20 (1H, s, H-9), 6.40 (1H, d, *J* 8, H-12′), 6.89 (1H, d, *J* 1.5, H-9′) and 6.97 (1H, dd, *J* 8 and 1.5, H-11′); δ_C (100 MHz; $CDCl_3$; Me_4Si) 7.6 (C-18), 7.7 (C-18′), 20.1 (C-16′), 23.6 (C-17′), 27.1 (C-19), 28.1 (C-19′), 28.3 (C-17), 31.4 (*N*Me′), 34.6 (C-20′), 40.5 (C-20), 40.9 (C-6), 41.4 (C-6′), 47.5 (C-5), 51.3 (C-7′), 52.2 (CO_2Me), 52.8 (C-14′), 52.9 (C-7), 53.0 (C-3′), 53.6 (C-5′), 54.7 (C-14), 56.0 (C-15), 57.1 (C-15′), 58.4 (C-3), 61.0 (11-OMe), 61.3 (12-OMe), 61.6 (C-21), 67.6 (C-21′), 73.4 (C-2′), 105.8 (C-12′), 117.4 (C-9), 121.3 (C-10′), 121.5 (C-16), 122.8 (C-9′), 128.2 (C-11′), 136.9 (C-8′), 144.0 (C-12), 146.0 (C-11), 150.0 (C-13′), 154.8 (C-8), 163.0 (C-13), 165.6 (C-2), 166.6 (CO_2Me) and 183.1 (C-10); *m*/*z* (APIMS, MeOH) 735 (MH^+, $C_{43}H_{50}N_4O_7$ + H).

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