Full Papers

Phragmalin Limonoids from the Madagascan Meliaceae *Neobeguea leandreana*

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The combined hexane−CH$_2$Cl$_2$ extract of the stembark of the Madagascan Meliaceae *Neobeguea leandreana* yielded three novel phragmalin limonoids, leandreanins A (**1**), B (**2**), and C (**3**). Leandreanins A (**1**) and B (**2**) are rare *seco*-ring D 17-keto compounds related to pseudrelone B, while leandreanin C (**3**) has an unprecedented acetoxy functionality at C-19.

First described in 1958 by Leroy, *Neobeguea* is a relatively recent addition to the Meliaceae family, where it is placed in the tribe Swietenieae, subfamily Swietenioideae.[1] It is considered to be botanically closely related to the genus *Khaya*.[2] The genus comprises only three species, all of which are endemic to the dry thorny forests of the deep south of Madagascar, where the arid climate has resulted in the reduced leaves, shortened branches, and underground water storage organs which characterize its species.[3] *Neobeguea leandreana* J.-F.Leroy and *Neobeguea mahafalensis* J.-F.Leroy are both known to the local populace by the name of "Handy" and are reported to have medicinal properties; literature reports, however, on their medicinal usage are nonspecific and anecdotal only.[3,4]

Studies on *N. mahafalensis*, the only species that has previously been investigated, yielded the known phragmalin class limonoids pseudrelone A$_2$,[4] previously found in *Pseudocedrela kotschyii* (Schweinf.) Harms,[5] and, more recently, the novel compound neobeguin.[6]

Results and Discussion

A methanol extract of the stembark of *N. leandreana* was shown by ^{1}H NMR spectroscopy to contain only sugars and was not investigated further, while the hexane and CH$_2$-Cl$_2$ extracts were sufficiently similar, by ^{1}H NMR and TLC

analysis, to be combined. Three novel phragmalin group limonoids, viz., leandreanin A (**1**), leandreanin B (**2**), and leandreanin C (**3**), were isolated.

An HRMS of leandreanin A (**1**) gave an [M]$^+$ at *m/z* 732.2612, corresponding to the molecular formula C$_{36}$H$_{44}$O$_{16}$ and 15 double-bond equivalents. The ^{1}H and ^{13}C NMR spectra showed a carbonyl carbon signal at δ_C 198.6 and unusual downfield furanyl ring resonances (δ_H 7.98s, H-21, δ_C 146.6 (CH), C-21; δ_H 7.40s, H-23, δ_C 143.0 (CH), C-23; δ_H 6.73s, H-22, δ_C 110.7 (CH), H-22; and δ_C 125.5 (C), C-20). This inferred the presence of a *seco*-ring D 17-keto limonoid, which was supported by correlation of the C-17 resonance in the HMBC spectrum to multiplets at δ_H 3.32, ascribed to H-14 (δ_C 44.2 (CH), C-14), and δ_H 2.35, assigned to 2H-12 (δ_C 36.0 (CH$_2$), C-12), and to a quaternary methyl signal (δ_H 1.52s, δ_C 28.0 (CH$_3$)) assigned to C-18. Repeated attempts to obtain an HMBC correlation between H-21 and/or H-22 and the ^{13}C NMR ketonic carbon resonance, which would irrefutably confirm its placement at C-17, proved unsuccessful. However, given that the other evidence for our structure is convincing, the lack of such a correlation was not crucial.

Correlations in the COSY spectrum between the H-14 resonance and a pair of double doublets at δ_H 2.95 (dd, *J* = 17.2, 3.5 Hz) and 3.43 (dd, *J* = 17.2, 7.3 Hz) allowed assignment of these as H-15a and H-15b, respectively, with the corresponding C-15 resonance occurring at δ_C 30.7 (CH$_2$). A COSY correlation between a two-proton multiplet at δ_H 2.35 (2H-12) and a broad singlet at δ_H 5.40, coupled

leandreanin A (1)

leandreanin B (2)

A

leandreanin C (3)
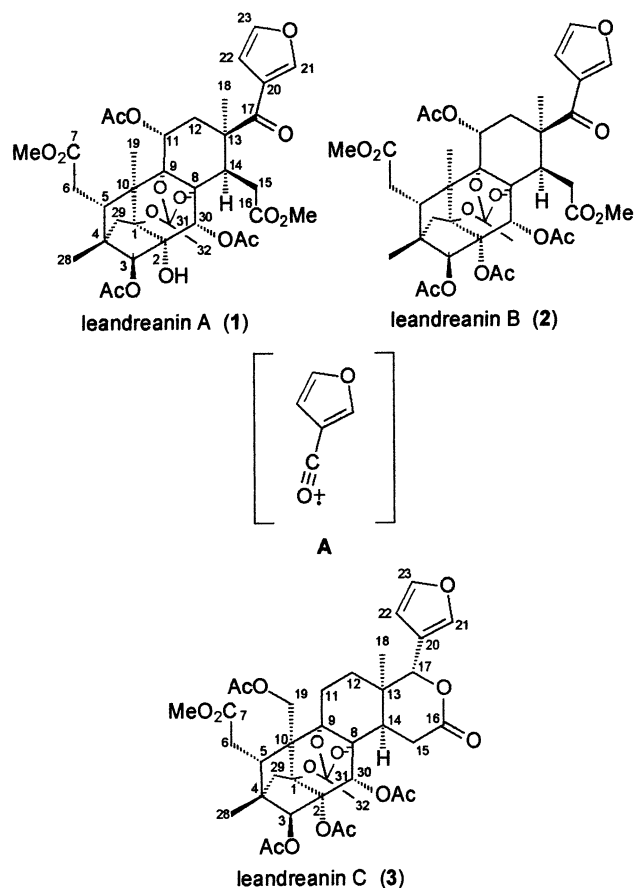
in the HMBC spectrum to a oxymethine resonance at δ_C 67.2 and ascribed to H-11, established an acetate ester at C-11. Likewise, an HMBC correlation to the two H-15 resonances established the C-16 carbomethoxy carbonyl carbon at δ_C 175.7 (C) and attached methyl ester at δ_H 3.66 (s, 3H) and δ_C 51.6 (CH$_3$). NOESY correlations between H-14 and 3H-18, and between H-11 and a methyl signal at δ_H 1.18, assigned to 3H-19 (δ_C 15.9 (CH$_3$), C-19), revealed the stereochemistry of the protons at these positions to be H-14α and H-11β, respectively.

That leandreanin A (**1**) possesses a phragmalin-type 4,-29,1-bridge and a 1,8,9-orthoacetate linkage was deduced from the characteristic pair of coupled doublets (δ_H 1.81d, δ_H 1.65d, $J = 10.8$ Hz), assigned to the two H-29, and the quaternary carbon signal at δ_C 118.9, which was ascribed to the orthoacetate carbon C-31. The C-29 resonance appears characteristically at δ_C 39.2.[7] The quaternary C−O resonance at δ_C 85.8 was assigned to C-1 by virtue of HMBC correlations to both 2H-29 and 3H-19, while that at δ_C 78.1, which lacks the 3H-19 correlation, was ascribed to C-2. The remaining fully substituted C−O signals at δ_C 86.6/δ_C 86.4 are thus those of C-8/C-9, which could not be distinguished. An HMBC correlation to H-14α of the oxymethine signal at δ_C 70.5 established this as C-30, and thus that the remaining oxymethine resonance at δ_C 82.6 is C-3. The methyl signal at δ_H 0.79 was assigned to 3H-28 by its NOESY correlation to the H-3 singlet at δ_H 4.63. In turn, 3H-28 displayed a NOESY correlation to H-5 at δ_H 2.55m (δ_C 36.3 (CH), C-5) and 2H-6 at δ_H 2.25m (δ_C 34.1 (CH$_2$), C-6). An HMBC correlation between 2H-6 and a second carbomethoxy signal at δ_C 172.5 confirmed this as C-7, with the accompanying methyl ester resonances at δ_H 3.52 (3H, s) and δ_C 51.7 (CH$_3$). The three acetate moieties (δ_H 1.96s, 3H; δ_C 170.4 (C) and 21.3 (CH$_3$); δ_H 2.17s, 3H, δ_C 167.7 (C) and 21.1 (CH$_3$); δ_H 2.17s, 3H, δ_C 169.7 (C) and

21.0 (CH$_3$)) were placed at C-3, C-30, and C-11α, respectively, with NOESY correlations to the H-29a and H-5 resonances establishing that H-3 is α and H-30 is β, as expected. Leandreanin A thus has structure **1**.

Only one 17-keto *seco*-ring D limonoid with the 1,8,9-orthoacetate linkage has previously been characterized.[8] The original structure of pseudrelone B[9] from *Pseudocedrela kotschyii* was subsequently revised[10] after X-ray crystallographic analysis. The 17-keto *seco*-ring D limonoid febrinolide with a 8,9,14-orthoacetate has been reported from *Soymida febrifuga* A. Juss.,[11] while the much simpler obacunone-type compound oriciopsin[12] has been isolated from *Oriciopsis glaberrima* Engl. (Rutaceae). In this publication[12] the appearance of a peak at *m/z* 95 in the mass spectrum, corresponding to the fragment **A**, is considered as convincing support for the proposed *seco*-ring D 17-keto structure. We observed an identical fragment in the mass spectrum of **1** and consider that evidence for the structure given for leandreanin A is conclusive.

An HRMS of leandreanin B (**2**) gave an [M]$^+$ at *m/z* 774.2750, corresponding to the molecular formula C$_{38}$H$_{46}$O$_{17}$ and a difference, relative to leandreanin A (**1**), of C$_2$H$_2$O. Comparison of the ^{1}H and ^{13}C NMR spectra of these two compounds revealed them to be very similar, with the major differences being the appearance, in the ^{13}C NMR spectrum, of a fourth ester carbonyl signal and methyl carbon resonance, and in the ^{1}H NMR spectrum, of a fourth acetyl methyl singlet. In addition, the singlets at δ_H 4.63 and 5.02, ascribed to H-3 and H-30 in **1**, were shifted downfield to δ_H 5.03 and 5.59 in leandreanin B (**2**), while the corresponding C-3 and C-30 ^{13}C NMR resonances were shifted upfield to δ_C 80.7 and 67.9. The fully substituted C−O signal at δ_C 86.4 displayed HMBC correlations to multiplets at δ_H 2.62 and 2.35, ascribed to H-5 and 2H-12, respectively (C-12 assigned by comparison with **1**), and is assigned to C-9. Both this C-9 resonance and that at δ_C 85.3 correlated to 3H-19, establishing this as C-1. The remaining signal at δ_C 85.7 displayed an HMBC correlation to a multiplet at δ_H 3.26, ascribed to 2H-15 (C-15 assigned by comparison with **1**), and thus must be C-8. However, it also displayed correlations to both H-3 and H-29b (δ_H 1.80, by comparison with **1**), suggesting that the resonances for C-8 and C-2 are superimposed. The intensity of this signal was approximately double that of the signals for C-1 and C-9. Comparison of the ^{13}C NMR spectra for chukrasins B and E from *Chukrasia tabularis* A. Juss[13] revealed that acetylation at C-2 shifts the C-3 resonance upfield from δ_C 83.2 to 80.2, while leaving that for C-30 unchanged at δ_C 68.5 and, significantly, causing a sharp downfield shift in the C-2 signal from δ_C 77.1 to 83.0. Thus, if it is accepted that the signals for C-2 and C-8 are superimposed at δ_C 85.7, then leandreanin B (**2**) is the novel compound 2-acetylleandreanin A.

Although they are not as rare as the *seco*-ring D compounds, phragmalin-class limonoids are not widespread in the Meliaceae. They currently number some 30 examples from only six species, all of which are members of the tribe Swietenieae, subfamily Swietenioideae. The 12 busseins A−M were reported from *Entandrophragma bussei* Engl.,[14] while *Chukrasia tabularis* has yielded chukrasins A−E[13] and a number of other phragmalin derivatives.[7,15] Besides febrinolide,[10] *Soymida febrifuga* A.Juss. has yielded febrinins A and B[16] and two epoxy febrinin B derivatives.[11]

An HRMS of leandreanin C (**3**) gave an [M]$^+$ at *m/z* 744.2631, corresponding to the molecular formula C$_{37}$H$_{44}$O$_{16}$ and 16 double-bond equivalents. Inspection of the ^{1}H and ^{13}C NMR spectra of this compound revealed the charac-

teristic signals of the 4,29,1-bridge and 1,8,9-orthoacetate (δ_C 119.4 (C), C-31; δ_H 2.24m/1.66m, 2H, H-29, δ_C 38.8 (CH$_2$), C-29; δ_H 1.62s, 3H, 3H-32, by HMBC correlation to C-31). In contrast to leandreanins A (**1**) and B (**2**), however, the furan ring protons were no longer shifted downfield (δ_H 7.65s, δ_C 141.9 (CH), H-21; δ_H 7.36s, δ_C 143.3 (CH), H-23; δ_H 6.38s, δ109.1 (CH), H-22; and δ_C 122.3 (C), C-20). Also, C-21 and C-22 both correlated in the HMBC spectrum to a one proton resonance at δ_H 5.71, which was coupled to an oxymethine signal at δ_C 69.6 and is, hence, assigned to H-17. Consequently, leandreanin C (**4**) has a "normal" ring D lactone, within which are C-14 (δ_C 47.5 (CH), HMBC correlation to H-17), H-14 (δ_H 2.24m, HSQC correlation to C-14), and C-15 (δ_C 30.2 (CH$_2$), HMBC correlation to H-14), with H-15β (by NOESY correlation to H-17) at δ_H 2.81 (dd, J = 15.4, 3.7 Hz) and H-15α at δ_H 2.28m. The C-16 resonance occurred at δ_C 174.1 (C) and was assigned by HMBC correlation to both H-14 and 2H-15, the singlet signal at δ_H 1.20 to 3H-18, by NOESY correlation to H-17, and that at δ_C 38.9 (C) to C-13, by HMBC correlations to H-17, 2H-15, and 3H-18.

Further examination of these spectra, however, revealed that leandreanin C (**3**) had only three quaternary methyl resonances rather than the expected four; furthermore, it possessed an oxymethylene signal at δ_C 68.8 correlating in the HSQC spectrum to a pair of doublets (J = 13.8 Hz) at δ_H 4.29 and 4.73. An HMBC correlation between 2H-15 and a fully substituted C–O resonance at δ_C 86.2 established this as C-8. The C-8 signal was correlated to a singlet resonance at δ_H 5.95, ascribed to H-30, and from which a further HMBC correlation to an oxymethine signal at δ_C 81.3 established this as C-3; HSQC correlations placed C-30 at δ_C 68.6 (CH) and H-3 as a singlet at δ_H 5.16. The C-3 resonance displayed HMBC correlations to H-30, H-29, a multiplet at δ_H 2.50, assigned to H-5, and to a 3H methyl singlet signal at δ_H 0.92, which correlated also to C-29, and can therefore only be 3H-28; HSQC correlations placed the C-5 resonance at δ_C 32.9 (CH) and that of C-28 at δ_C 13.7 (CH$_3$). This suggests that it is the C-19 methyl group that is missing and, consequently, that the signals at δ_H 4.29 and 4.73 can be ascribed to the two protons on C-19. HMBC correlations between both the H-3 and 3H-28 resonances and a fully substituted signal at δ_C 46.1 established this as C-4 and, therefore, that the fully substituted signal at δ_C 45.1 can be ascribed to C-10. This latter assignment was further supported by HMBC correlations between the C-10 resonance and H-29 and also to a pair of coupled multiplets at δ_H 2.34 and 2.50, which were ascribed to 2H-6 by HMBC correlation to C-5. Final confirmation that it was indeed C-19 that had been oxidized was seen from HMBC correlations between 2H-19 and both C-10 and C-5. The remaining fully substituted C–O resonances at δ_C 86.0 and 85.2 were then assigned to C-9 and C-2, respectively, on the basis of an HMBC correlation between the H-5 and C-9 resonances on one hand and between the H-29 and C-2 signals on the other.

The quaternary methyl signals and corresponding ester carbonyl resonances in the ^{13}C NMR spectrum of **3** were superimposed to the extent that they could not be distinguished. Despite the fact that the acetate ester methyl signals were well separated in the ^{1}H NMR spectrum, they were also superimposed on underlying signals, and it could not be determined with certainty which signal was responsible for a given HMBC correlation. The fact that three of the acetate esters occurred at the adjacent C-2, C-3, and C-30 made it virtually impossible to assign them on the basis of NOESY correlations. Thus the only acetate ester

methyl signal that could be unequivocally assigned was that at δ_H 2.11, which was placed at C-19 on the basis of NOESY correlations to the 2H-19 resonance at δ_H 4.29 and to the H-11α resonance at δ_H 1.88m. To our knowledge, this is the first report of a phragmalin limonoid with an oxygenated C-19 methyl group, and leandreanin C thus has the novel structure **3**.

Experimental Section

CH_3O), 3.54 (3H, s, 7-CH_3O), 3.26 (2H, m, 2H-15), 3.21 (1H, m, H-14), 2.62 (1H, m, H-5), 2.35 (2H, m, 2H-12), 2.32 (2H, m, 2H-6), 2.16 (3H, s, 11-OCOCH_3), 2.07, 2.04 (each 3H, s, 2-OCOCH_3, 3-OCOCH_3), 1.98 (3H, s, 30-OCOCH_3), 1.80, 1.55 (each 1H, m, H-29), 1.56 (3H, s, 3H-32), 1.47 (3H, s, 3H-18), 1.20 (3H, s, 3H-19), 0.79 (3H, s, 3H-28); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 199.5 (s, C-17), 175.8 (s, C-16), 172.3 (s, C-7), 170.0, 170.0, 169.7, 167.8 (each s, 2-OCOCH$_3$, 3-OCOCH$_3$, 11-OCOCH3, 30-OCOCH$_3$), 146.3 (d, C-21), 142.5 (d, C-23), 126.0 (s, C-20), 118.9 (s, C-31), 111.0 (d, C-22), 86.4 (s, C-9), 85.7, 85.7 (each s, C-2, C-8), 85.3 (s, C-1), 80.7 (d, C-3), 67.9 (d, C-30), 67.0 (d, C-11), 51.8 (q, 7-CH$_3$O), 51.5 (q, 16-CH$_3$O), 47.2 (s, C-13), 46.1, 45.7 (each s, C-4, C-10), 44.5 (d, C-14), 40.0 (t, C-29),), 35.8 (t, C-12), 35.7 (d, C-5), 34.0 (t, C-6), 30.2 (t, C-15), 28.8 (q, C-18), 21.6, 21.4 (each q, 2-OCOCH$_3$, 30-OCOCH$_3$), 21.0, 20.8 (each q, 3-OCOCH$_3$, 11-OCOCH$_3$), 20.3 (q, C-32), 16.1 (q, C-19), 14.6 (q, C-28); FABMS *m/z* 797 [M + Na]$^+$, 775 [M + H]$^+$, 731, 715, 613, 581, 539, 511, 479, 451, 399, 357, 339, 307, 279, 213, 107, 95, 69, 55; HRMS found [M]$^+$ *m/z* 774.2750 (calc. for [C$_{38}$H$_{46}$O$_{17}$]$^+$ 774.2730).

Leandreanin C (3): pale yellow gum (10.0 mg); [α]$_D$ $-30°$ (*c*, 0.164 in CHCl$_3$); IR (NaCl) ν_{max} 2953, 1754, 1379, 1251, 1063 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.65 (1H, s, H-21), 7.36 (1H, s, H-23), 6.38 (1H, s, H-22), 5.95 (1H, s, H-30), 5.71 (1H, s, H-17), 5.16 (1H, s, H-3), 4.73, 4.29 (each 1H, d, *J* = 13.8 Hz, 2H-19), 3.66 (3H, s, 7-CH_3O), 2.81 (1H, dd, *J* = 15.4, 3.7 Hz, H-15β), 2.50 (2H, m, H-5, H-6b), 2.34 (1H, m, H-6a), 2.28, 2.06, 1.94 (each 3H, s, 2-OCOCH_3, 3-OCOCH_3), 2.28 (1H, m, H-15α), 2.24 (2H, m, H-14, H-29b), 2.12 (1H, m, H-11β), 2.11 (3H, s, 19-OCOCH_3), 1.88 (1H, m, H-11α), 1.66 (1H, m, H-29a), 1.62 (3H, s, 3H-32), 1.20 (3H, s, 3H-18), 1.14, 1.08 (2H, m, 2H-12), 0.92 (3H, s, 3H-28); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 174.1 (s, C-16), 171.3 (s, C-7), 170.3, 169.7, 169.4, 169.0 (each s, 2-OCOCH3, 3-OCOCH$_3$, 19-OCOCH$_3$, 30-OCOCH$_3$), 143.3 (d, C-23), 141.9 (d, C-21), 122.3 (s, C-20), 119.4 (s, C-31), 109.1 (d, C-22), 86.2 (s, C-8), 86.0 (s, C-9), 85.7 (s, C-1), 85.2 (s, C-2), 81.3 (d, C-3), 69.6 (d, C-17), 68.8 (t, C-19), 68.6 (d, C-30), 51.6 (q, 7-CH$_3$O), 47.5 (d, C-14), 46.1 (s, C-4), 45.1 (s, C-10), 38.9 (s, C-13), 38.8 (t, C-29), 32.9 (d, C-5), 31.6 (t, C-12), 30.9 (t, C-6), 30.2 (t, C-15), 25.8 (t, C-11), 21.6, 21.6, 21.4, 21.3 (each q, 2-OCOCH$_3$, 3-OCOCH$_3$, 19-OCOCH$_3$, 30-

OCOCH$_3$), 21.1, 20.6 (each q, C-18, C-32), 13.7 (q, C-28); HRMS found [M]$^+$ *m/z* 744.2631 (calc for [C$_{37}$H$_{44}$O$_{16}$]$^+$ 744.2629).

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