# New Tirucallane-Type Triterpenes from *Dysoxylum variabile*

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Eight new tirucallane-type triterpenes, dyvariabilins A−H (**1**−**8**), three known tirucallanes, niloticin (**9**), dihydroniloticin (**10**), and tirucalla-7,24-diene-3$\beta$,23-diol (**11**), and two known sesquiterpenes, 1-(1-hydroxy-2-methylpropyl)-3a-methyl-7-methyleneoctahydroinden-4-ol and (+)-aphanamol I, were isolated from the stem bark of *Dysoxylum variabile*. Tirucallanes **1** and **11** with an allylic hydroxy group in the side chain and dyvariabilin C (**3**) with an α-epoxy group at positions 7 and 8 showed high instabilty in acidic medium and formed five hitherto unknown semisynthetic tirucallanes. Dyvariabilins B (**2**) and C (**3**) as well as the mixtures of dyvariabilins E and F (**5** and **6**) and dyvariabilins G and H (**7** and **8**) showed weak cytotoxicity against KB cells.

In our search for biologically active metabolites from plants that are employed in the traditional medicine of Papua New Guinea (PNG), *Dysoxylum variabile* Harms. (Meliaceae) was selected for phytochemical investigation. This is a small tree growing in disturbed foothills and montane rain forests. The bark of this plant is soaked in water and then squeezed to give a juice, which is used to treat constipation, stomachache, ischuria, and fever by the local people of PNG.[1] In a survey of New Guinea plants for alkaloids, Hartley et al. found the leaves and the bark of *D. variabile* to be alkaloid-negative.[2] No further chemical studies on this plant species have been reported. We describe herein the isolation and structure determination of terpenoids from the stem bark of *D. variabile*, which was collected in Morobe Province, PNG.

## Results and Discussion

The dried and pulverized bark of *D. variabile* was extracted in turn with MeOH and 70% aqueous MeOH. The crude MeOH extract was subjected to sequential solvent partition with *n*-hexane, $CHCl_3$, and 60% aqueous MeOH. The residue of the $CHCl_3$ phase showed significant cytotoxicity against KB nasopharyngal carcinoma cells (100% inhibition at 25 $\mu$g/mL) and was therefore subjected to fractionation by vacuum-liquid chromatography (VLC) on Si gel and RP-HPLC. Eight new tirucallane triterpenes, dyvariabilins A−H (**1**−**8**), together with three known tirucallane triterpenes, niloticin (**9**),[3−6] dihydroniloticin (**10**),[3,4,6] and tirucalla-7,24-diene-3$\beta$,23-diol (**11**) (Chart 1),[7] and two known sesquiterpenes, 1-(1-hydroxy-2-methylpropyl)-3a-methyl-7-methyleneoctahydroinden-4-ol[8] and (+)-aphanamol I,[9−11] were isolated. The structures of the isolates were elucidated on the basis of detailed spectral analysis, mainly HREIMS and 1D and 2D NMR spectroscopic methods. The spectral properties of the known compounds were in good agreement with literature data.[3−11]

Dyvariabilin A (**1**) gave a $M^+$ at *m*/*z* 454 in the positive EIMS. The $^{13}C$ NMR experiments including DEPT 135 sorted 30 signals into eight methyl, seven methylene, seven methine, and eight quaternary carbons, with shift values typical of a 3-keto tirucallane triterpene.[12] Signals of two quaternary carbons at $\delta_C$ 198.5 and $\delta_C$ 170.9 and one methine carbon at $\delta_C$ 124.9 pointed to the presence of an additional α,$\beta$-unsaturated keto group. Its location at C-6 was confirmed by long-range correlations between C-6 ($\delta_C$ 198.5) and H-5 at $\delta_H$ 2.47. The chemical shift values and multiplicities of the side chain carbons C-22 ($\delta_C$ 43.2, t), C-23 ($\delta_C$ 67.3, d), C-24 ($\delta_C$ 127.9, q), and C-25 ($\delta_C$ 135.9, q) resembled those of 23-hydroxy-24-ene tirucallanes and were corroborated by HMBC and DQF-COSY correlations.[12] Extensive 2D NMR measurements (DQF-COSY, HSQC, HMBC, and ROESY) confirmed the proposed structure and allowed the assignment of all signals in the $^1H$ and $^{13}C$ NMR spectra (see Tables1 and 2). In acidic $CDCl_3$ compound **1** decomposed completely to **1a** within a few days.

Dyvariabilin B (**2**) gave a $M^+$ peak at *m*/*z* 472.3550 in the HREIMS corresponding to the molecular formula $C_{30}H_{48}O_4$ (calcd 472.3553). The $^{13}C$ NMR spectrum showed close similarities to that of niloticin (**9**)[3−6] except for the upfield shifts of carbons 7 and 8 from $\delta_C$ 118.0 and $\delta_C$ 145.7 to $\delta_C$ 57.5 and $\delta_C$ 67.1, respectively. This information indicated the presence of an epoxy group at positions 7 and 8, which was confirmed by COSY correlations between H-7 ($\delta_H$ 3.23, d, $J$ = 4.2 Hz) and $H_2$-6 ($\delta_H$ 2.05, 1.93, each m) and long-range correlations between C-8 ($\delta_C$ 67.1) and both H-7 and H-9 ($\delta_H$ 2.13, m). A ROESY experiment showing NOEs between H-7 ($\delta_H$ 3.23) and $H_3$-18 ($\delta_H$ 1.07, s) clarified the $\beta$-position of the epoxy group.

The molecular formula of dyvariabilin C (**3**), $C_{30}H_{48}O_4$ (calcd 472.3553), which is identical to that of dyvariabilin B (**2**), was deduced from the $M^+$ peak in the HREIMS at *m*/*z* 472.3549. Its $^1H$ NMR and $^{13}C$ NMR spectra were also very similar to those of compound **2** except for the pronounced upfield shifts for C-8 (+3.6 ppm) and H-7 (+0.32 ppm), indicating that **2** and **3** are stereoisomers only differing in the stereochemistry of their epoxy group. In the case of dyvariabilin C (**3**), the α-position was clearly revealed by NOESY correlations between H-7 ($\delta_H$ 2.91, m) and $H_3$-30 ($\delta_H$ 1.10, s).

The HREIMS of dyvariabilin D (**4**) gave a $M^+$ peak at *m*/*z* 470.3395 corresponding to a molecular formula of $C_{30}H_{46}O_4$ (calcd 470.3396). The $^{13}C$ NMR spectrum closely resembled those of compounds **2** and **3**, but C-9 and C-11 were shifted downfield to $\delta_C$ 143.2 and 127.0, suggesting the presence of a double bond. The $^1H$ NMR, DQF-COSY, and HMBC spectra further confirmed the above inference

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**Chart 1**

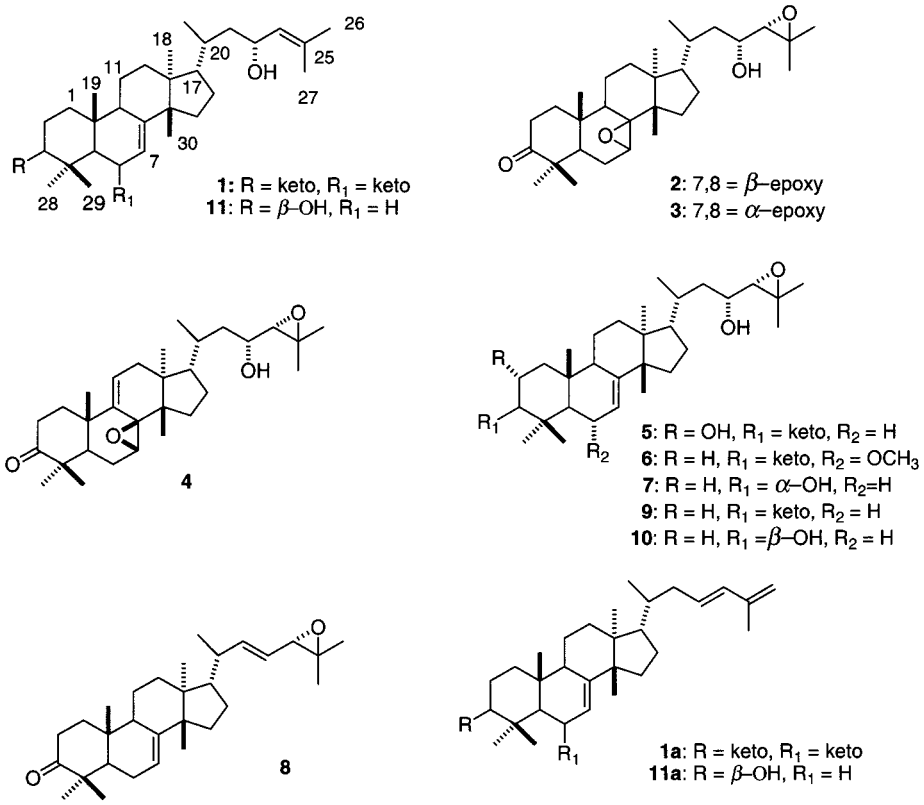


**Table 1.** $^1$H NMR Data for Compounds **1**−**8** ($\delta$, $CDCl_3$ for **1**, **2** and Acetone-$d_6$ **3**−**8**, $J$ in Hz)

| H | **1**[a] | **2**[b] | **3**[a] | **4**[b] | **5**[a] | **6**[a] | **7**[b] | **8**[b] |
|---|---|---|---|---|---|---|---|---|
| 1a | 1.98 m | 1.91 m | 1.97 m | 2.31 m | 2.29 dd (6, 12.7) | 1.88 m | 1.62 m | 2.00 m |
| 1b | 1.70 m | 1.32 m | 1.36 m | 1.62 m | 1.26 m | 1.65 m | 1.30 m | 1.46 m |
| 2a | 2.76 dt (6, 14) | 2.78 dt (6, 14) | 2.67 dt (5, 14) | 2.74 dt (6, 14) | 4.60 ddd (4, 6, 13) | 2.54 m | 1.90 m | 2.81 m |
| 2b | 2.34 m | 2.21 m | 2.05 m | 2.20 m | | 2.35 ddd (3, 6, 13) | 1.55 m | 2.11 m |
| 3 | | | | | | | 3.39 m | |
| 5 | 2.47 s | 1.49 br d (12) | 1.60 m | 1.98 d (12) | 1.77 dd (6, 11.5) | 2.01 d (10) | 1.86 m | 1.74 dd (6, 12) |
| 6a | | 2.05 m | 1.94 m, 2H | 1.97 m, 2H | 2.10 m, 2H | 4.05 dd (3, 10) | 2.01 m | 2.08 m, 2H |
| 6b | | 1.93 m | | | | | 1.94 m | |
| 7 | 5.78 d (2.6) | 3.23 d (4.2) | 2.91 m | 3.09 m | 5.34 m | 5.51 t (3) | 5.28 m | 5.33 m |
| 9 | 2.78 m | 2.13 m | 1.87 m | | 2.40 m | 2.49 m | 2.36 m | 2.38 m |
| 11a | 1.74 m, 2H | 1.75 m | 1.73 m, 2H | 5.84 dd (3, 5) | 1.64 m, 2H | 1.55 m, 2H | 1.55 m, 2H | 1.63 m, 2H |
| 11b | | 1.68 m | | | | | | |
| 12a | 1.87 m | 1.97 m | 1.84 m | 2.29 dd (5, 17) | 1.87 m | 1.87 m | 1.84 m | 1.82 m |
| 12b | 1.78 m | 1.68 m | 1.74 m | 2.19 m | 1.69 m | 1.69 m | 1.67 m | 1.68 m |
| 15a | 1.60 m, 2H | 1.38 m | 2.00 m | 1.69 m | 1.52 m, 2H | 1.52 m, 2H | 1.50 m, 2H | 1.50 m, 2H |
| 15b | | 1.10 m | 1.26 m | 0.94 m | | | | |
| 16a | 1.88 m | 1.99 m | 1.68 m | 2.05 m | 2.08 m | 2.08 m | 2.06 m | 2.06 m |
| 16b | 1.26 m | 1.20 m | 0.82 m | 1.34 m | 1.32 m | 1.32 m | 1.29 m | 1.29 m |
| 17 | 1.55 m | 1.68 m | 1.64 m | 1.70 m | 1.64 m | 1.64 m | 1.60 m | 1.60 m |
| 18 | 0.81 s | 1.07 s | 0.94 s | 0.91 s | 0.86 s | 0.89 s | 0.87 s | 0.87 s |
| 19 | 1.11 s | 1.18 s | 1.12 s | 1.21 s | 1.13 s | 0.92 s | 0.79 s | 1.04 s |
| 20 | 1.51 m | 1.45 m | 1.48 m | 1.51 m | 1.48 m | 1.48 m | 1.48 m | 2.10 m |
| 21 | 0.93 d (5.8) | 0.98 d (5.9) | 0.99 d (6.1) | 1.01 d (5.7) | 0.99 d (6.1) | 0.99 d (6.1) | 0.99 d (6.2) | 1.01 d (8) |
| 22a | 1.62 m | 1.61 m | 1.60 m | 1.62 m | 1.60 m | 1.60 m | 1.61 m | 5.57 dd (7, 16) |
| 22b | 1.11 m | 1.44 m | 1.35 m | 1.34 m | 1.30 m | 1.30 m | 1.32 m | |
| 23 | 4.47 dt (4, 9) | 3.58 m | 3.47 m | 3.49 m | 3.50 m | 3.50 m | 3.48 m | 5.50 dd (6, 16) |
| 24 | 5.11 br d (9) | 2.66 d (8.2) | 2.54 d (8.4) | 2.55 d (8.4) | 2.55 d (8.4) | 2.55 d (8.4) | 2.55 d (8.4) | 3.77 d (6) |
| 26 | 1.76 br s | 1.33 s | 1.26 s | 1.26 s | 1.26 s | 1.26 s | 1.26 s | 1.09 s |
| 27 | 1.72 br s | 1.34 s | 1.25 s | 1.25 s | 1.25 s | 1.25 s | 1.25 s | 1.11 s |
| 28 | 1.37 s | 1.01 s | 0.99 s | 1.02 s | 1.07 s | 1.21 s | 0.92 s | 1.09 s |
| 29 | 1.39 s | 1.08 s | 1.10 s | 1.07 s | 1.13 s | 1.18 s | 0.91 s | 1.10 s |
| 30 | 1.11 s | 1.09 s | 1.10 s | 0.99 s | 1.06 s | 1.07 s | 1.02 s | 1.04 s |
| $OCH_3$ | | | | | | 3.26 s | | |

[a] Spectra recorded at 300 MHz. [b] Spectra recorded at 500 MHz.

and together with the ROESY experiment revealed that dyvariabilin D (**4**) was the 9,11-didehydro derivative of dyvariabilin B (**2**).

Dyvariabilins E and F (**5** and **6**) were isolated as an inseparable mixture. The HREIMS of the mixture showed two $M^+$ peaks at $m/z$ 472.3550 and 486.3707, corresponding

**Table 2.** $^{13}C$ NMR Data of Compounds **1**−**8** ($\delta$, $CDCl_3$ for **1**, **2** and Acetone-$d_6$ for **3**−**8**)

| C | **1**[a] | **2**[a] | **3**[a] | **4**[b] | **5**[b] | **6**[b] | **7**[b] | **8**[b] |
|---|---|---|---|---|---|---|---|---|
| 1 | 37.6 | 40.4 | 38.8 | 37.5 | 48.8 | 36.3 | 31.9 | 38.8 |
| 2 | 34.0 | 34.1 | 34.9 | 35.1 | 69.6 | 34.6 | 26.4 | 35.1 |
| 3 | 214.8 | 216.0 | 214.7 | 214.6 | 216.2 | 215.8 | 75.6 | 215.0 |
| 4 | 47.1 | 48.0 | 47.2 | 47.1 | 47.7 | 47.7 | 37.9 | 48.1 |
| 5 | 65.3 | 52.0 | 47.3 | 42.6 | 53.5 | 55.6 | 45.0 | 52.9 |
| 6 | 198.5 | 24.0 | 24.1 | 23.8 | 24.8 | 77.4 | 24.5 | 24.9 |
| 7 | 124.9 | 57.5 | 55.2 | 53.3 | 118.4 | 118.9 | 118.9 | 118.6 |
| 8 | 170.9 | 67.1 | 63.5 | 61.6 | 146.4 | 150.1 | 146.6 | 146.6 |
| 9 | 49.7 | 48.4 | 49.4 | 143.2 | 49.3 | 47.9 | 49.5 | 49.0 |
| 10 | 43.2 | 36.7 | 35.2 | 36.9 | 36.1 | 33.6 | 35.3 | 35.6 |
| 11 | 17.7 | 18.4 | 18.6 | 127.0 | 18.9 | 18.8 | 18.6 | 18.7 |
| 12 | 32.5 | 35.4 | 33.6 | 39.4 | 34.2 | 34.1 | 34.5 | 34.1 |
| 13 | 43.1 | 44.3 | 45.6 | 44.8 | 44.2 | 44.0 | 44.2 | 44.1 |
| 14 | 52.4 | 50.3 | 49.9 | 48.6 | 51.8 | 51.8 | 51.9 | 51.9 |
| 15 | 33.0 | 31.5 | 28.3 | 26.7 | 34.6 | 34.4 | 34.6 | 34.6 |
| 16 | 29.7 | 28.6 | 28.2 | 28.3 | 29.2 | 29.1 | 29.2 | 29.1 |
| 17 | 52.3 | 54.4 | 54.1 | 52.5 | 54.1 | 53.9 | 54.1 | 53.4 |
| 18 | 21.9 | 23.9 | 20.6 | 23.1 | 22.0 | 22.0 | 22.0 | 22.3 |
| 19 | 13.9 | 15.5 | 14.5 | 16.8 | 13.9 | 13.9 | 13.3 | 12.8 |
| 20 | 36.4 | 33.5 | 34.0 | 34.0 | 34.3 | 34.3 | 34.4 | 41.0 |
| 21 | 19.0 | 20.0 | 20.3 | 20.3 | 20.5 | 20.5 | 20.5 | 20.5 |
| 22 | 43.2 | 40.5 | 41.7 | 41.8 | 41.9 | 41.9 | 41.9 | 139.0 |
| 23 | 67.3 | 69.3 | 69.9 | 69.9 | 69.9 | 69.9 | 69.9 | 128.3 |
| 24 | 127.9 | 68.3 | 69.1 | 69.1 | 69.1 | 69.1 | 69.1 | 79.8 |
| 25 | 135.9 | 60.2 | 58.8 | 58.8 | 58.8 | 58.8 | 58.8 | 72.7 |
| 26 | 25.9 | 19.8 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 24.6 |
| 27 | 18.3 | 24.9 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 26.2 |
| 28 | 25.2 | 24.2 | 25.2 | 25.6 | 24.7 | 29.4 | 28.4 | 24.9 |
| 29 | 21.7 | 20.4 | 22.9 | 22.8 | 21.6 | 22.4 | 22.1 | 21.6 |
| 30 | 24.9 | 21.3 | 22.9 | 21.4 | 27.6 | 27.1 | 27.6 | 27.7 |
| $OCH_3$ | | | | | | 52.9 | | |

[a] Spectra recorded at 75 MHz. [b] Spectra recorded at 125 MHz.

to the molecular formula $C_{30}H_{48}O_4$ (calcd 472.3553) for **5** and $C_{31}H_{50}O_4$ (calcd 486.3709) for **6**. The $^1H$ NMR and $^{13}C$ NMR resonances of dyvariabilins E and F (**5** and **6**) could be assigned clearly to each compound since their unequal concentrations in the mixture (~2:1, **5** and **6**) resulted in an obvious difference of signal intensities in the 1D NMR spectra. In comparison with niloticin (**9**),[3−6] C-1 and C-2 of compound **5** were shifted downfield to $\delta_C$ 48.8 and 69.6, respectively, and one methine proton signal which was assigned to H-2 by an HSQC experiment resonated at $\delta_H$ 4.60 (1H, ddd, $J$ = 4, 6 and 13 Hz), pointing to an additional hydroxyl group at C-2. The 2D NMR spectra confirmed the proposed structure, and with the help of a ROESY spectrum (NOE between H-2$\beta$ and $H_3$-19) dyvariabilin E (**5**) was identified as 2α-hydroxyniloticin. The presence of a methoxy group in compound **6** was suggested from the mass difference between **6** and niloticin (**9**). In accordance, the $^1H$ NMR spectrum showed an additional methyl singlet at $\delta_H$ 3.26 attributable to a carbon at $\delta_C$ 52.9, as determined by an HSQC experiment. The downfield shift of C-6 to $\delta_C$ 77.4 and the appearance of its corresponding proton signal at $\delta_H$ 4.05 (1H, dd, $J$ = 3 and 10 Hz) revealed the location of the methoxyl group to be at C-6. 2D NMR including a ROESY experiment confirmed that dyvariabilin F (**6**) was 6α-methoxyniloticin (NOE between H-6$\beta$ and $H_3$-29).

Due to the limited sample amount, the structures of dyvariabilins G (**7**) and H (**8**) were also elucidated from a mixture. The HREIMS gave two $M^+$ peaks at $m/z$ 458.3770 and 438.3496, corresponding to the molecular formula $C_{30}H_{50}O_3$ (calcd 458.3760) for compound **7** and $C_{30}H_{46}O_2$ (calcd 438.3498) for **8**. The $^1H$ and $^{13}C$ NMR signals were sorted according to their different intensities (~3:1, **7** and **8**) and the correlations displayed in the 2D NMR spectra. Compound **7**, which has the same molecular formula as dihydroniloticin (**10**), showed also very similar NMR behavior,[3,4,6] except for small changes of the chemical shifts

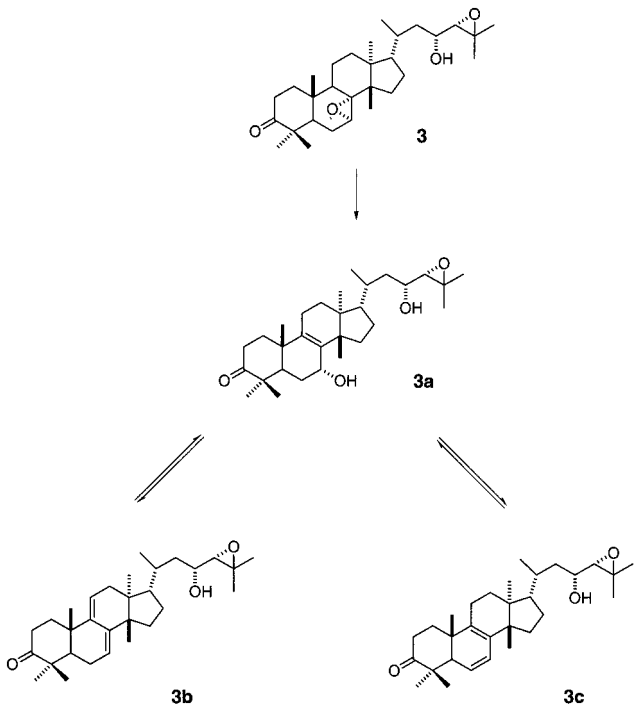


**Figure 1.** Transformation of **3** into **3a**, **3b**, and **3c** in acidic $CDCl_3$.

of some protons and carbons close to position 3. The ROESY experiment exhibited NOEs between H-3 ($\delta_H$ 3.39, m) and $H_3$-19 ($\delta_H$ 0.79, s), clearly revealing that dyvariabilin G (**7**) is an OH-3α isomer of dihydroniloticin (**10**). Compound **8** was proposed as a dehydrated derivative of niloticin (**9**) from the difference in their molecular mass. This was confirmed by detailed 1D and 2D NMR studies of **8** and the comparison with analogous data for niloticin (**9**).[3−6] Significant differences in terms of the chemical shift values and multiplicities between compound **8** and niloticin (**9**) were observed in the side chain. The $^{13}C$ NMR signals of C-22 ($\delta_C$ 40.7) and C-23 ($\delta_C$ 69.2) in compound **9** were replaced by two methine carbons in **8**, resonating at $\delta_C$ 139.0 (C-22) and 128.3 (C-23), that were indicative of a double bond. The $^1H$ NMR spectrum displayed two coupled double doublets at $\delta_H$ 5.57 (1H, dd, $J$ = 7 and 16 Hz, H-22) and 5.50 (1H, dd, $J$ = 6 and 16 Hz, H-23), accordingly.

Under mildly acidic conditions dyvariabilin A (**1**), dyvariabilin C (**3**), and the known compound **11**, tirucalla-7,24-diene-3$\beta$,23-diol, showed high instability. Compounds **1** and **11**, which have an allylic hydroxyl group in the side chain, completely eliminated the hydroxyl group at C-23 to form a 23,25-diene conjugated system in $CDCl_3$ at −20 °C within a few days, resulting in two stable products, **1a** and **11a**. The structural determination of **1a** and **11a** was carried out by NMR spectral analysis ($^1H$, $^{13}C$ NMR, DQF-COSY, HMQC, and HMBC) and was further confirmed by their HREIMS (see Experimental Section). Dyvariabilin C (**3**), with an α-epoxy group at positions 7 and 8, transformed in $CDCl_3$ at room temperature to a mixture of two dehydrated derivatives, **3b** and **3c**, via an intermediate, **3a** (Figure 1). The intermediate remained in an unchanged pure form for several days, so consequently all three structures could be determined from the successive 1D and 2D NMR experiments. In contrast, dyvariabilin B (**2**), the stereoisomer of **3**, showed no changes during storage.

Dyvariabilins B (**2**) and C (**3**), as well as the mixtures dyvariabilin E and F (**5** and **6**) and dyvariabilin G and H (**7** and **8**), showed weak cytotoxicity against KB cells, comparable in potency with niloticin (**9**) ($IC_{50}$ 10.0, 8.7, 10.1,

9.2, and 8.4 μg/mL, respectively) and hence contribute to the observed cytotoxicity of the initial $CHCl_3$ extract.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer model 241 polarimeter. IR spectra were recorded in KBr pellets on a Perkin-Elmer 2000 FT infrared spectrophotometer. NMR spectra were recorded in $CDCl_3$ or $(CD_3)_2CO$ on a Bruker AMX-300 (300.13 MHz for $^1H$, 75.47 MHz for $^{13}C$) and a Bruker DRX-500 (500.13 MHz for $^1H$, 125.76 MHz for $^{13}C$) at 295 K. The residual $CHCl_3$ ($\delta_H$ 7.27, $\delta_C$ 77.0) and $(CH_3)_2CO$ ($\delta_H$ 2.05, $\delta_C$ 206.6, 29.8) resonances were used as internal references. EIMS and HREIMS were obtained on a VG-Tribrid spectrometer at 70 eV. Si gel (particle size 40−63 μm, Merck) was used for VLC. RP-HPLC separations were performed on a Waters Spherisorb S10 ODS II column (250 × 20 mm, particle size 10 μm, Merck) with a Waters 590 programmable HPLC pump and Knauer differential refractometer.

**Plant Material.** The stem bark of *D. variabile* was collected in Morobe Province, on the road from Lae to Boana, Papua New Guinea, in September 1996. The plant was identified by Dr. M. M. J. van Balgooy, Rijksherbarium Leiden, The Netherlands, where a voucher specimen with the identification number ETH 96/26 21.9.1996 has been deposited.

**Extraction and Isolation.** Air-dried and powdered stem bark of *D. variabile* (700 g) was percolated successively with MeOH and 70% aqueous MeOH at room temperature. The MeOH extract was concentrated in vacuo and partitioned between *n*-hexane and 90% aqueous MeOH. The alcoholic phase was further partitioned between $CHCl_3$ and 60% aqueous MeOH. After removal of solvents under a vacuum, 16 g of the $CHCl_3$ soluble residue (60 g) was subjected to VLC (Si gel) using a step gradient of *n*-hexane−EtOAc−MeOH (*n*-hexane, *n*-hexane with increasing amounts of EtOAc (20% each step), EtOAc, EtOAc with increasing amounts of MeOH (20% each step), MeOH). The collected fractions were evaporated in vacuo and examined by TLC. Homogeneous fractions, showing similar spots on TLC, were pooled to give 10 major fractions (F1−F10). The white needle crystals (850 mg) of niloticin (**9**) precipitated from fraction F4 in EtOAc. Fraction F5 (1.9 g) was further fractionated on an open column of Si gel and eluted with $CH_2Cl_2$ containing increasing amounts of $Me_2CO$ to yield nine subfractions (F5.1−F5.9). Dihydroniloticin (158 mg, **10**) precipitated as white needle crystals from subfraction F5.9 in EtOAc. Subfractions F5.3 (115 mg), F5.7 (120 mg), and F5.8 (280 mg) were first treated by passage over a Waters $C_{18}$ Sep-Pak Vac column (MeOH−$H_2O$) and then subjected to RP-HPLC eluted with MeOH−$H_2O$ (95:5, 90:10). Dyvariabilin A (1.7 mg, **1**) was obtained from subfraction F5.3, dyvariabilin B (1.8 mg, **2**), and compound **11**, 1-(1-hydroxy-2-methylpropyl)-3a-methyl-7-methyleneoctahydroinden-4-ol, (+)-aphanamol I (2.8 mg, 3.5 mg, 12.1 mg) was obtained from subfraction F5.7, and dyvariabilins C−H (26 mg, **3**; 1.9 mg, **4**; 2.9 mg, **5** and **6**; 1.4 mg, **7** and **8**) were obtained from subfraction F5.8.

**Dyvariabilin A (1):** white, waxy solid; $[\alpha]^{20}_D$ −32° (*c* 0.1, $CHCl_3$); $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; DEIMS *m/z* 454 $[M]^+$ (8), 439 $[M - CH_3]^+$ (7), 421 $[439 - H_2O]^+$ (6).

**Tirucalla-7,23,25-triene-3,6-dione (1a):** white, waxy solid; $[\alpha]^{20}_D$ −38° (*c* 0.1, $CHCl_3$); $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 6.14 (1H, d, $J$ = 15.6 Hz, H-24), 5.79 (1H, d, $J$ = 2.5 Hz, H-7), 5.63 (1H, m, H-23), 4.88 (2H, br s, H-26), 2.78 (2H, m, H-9 and H-2a), 2.47 (1H, s, H-5), 2.34 (1H, m, H-2b), 2.30 (2H, m, H-22), 1.98 (1H, m, H-1a), 1.94 (1H, m, H-16a), 1.86 (2H, m, H-12), 1.85 (3H, s, H-27), 1.77 (2H, m, H-11), 1.71 (1H, m, H-1b), 1.60 (2H, m, H-15), 1.56 (1H, m, H-20), 1.55 (1H, m, H-17), 1.39 (3H, s, H-29), 1.37 (3H, s, H-28), 1.26 (1H, m, H-16b), 1.11 (3H, s, H-19), 1.10 (3H, s, H-30), 0.91 (3H, d, $J$ = 6 Hz, H-21), 0.85 (3H, s, H-18); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 214.8 (C-3), 198.5 (C-6), 170.9 (C-8), 142.2 (C-25), 134.4 (C-24), 129.0 (C-23), 124.9 (C-7), 114.3 (C-26), 65.3 (C-5), 52.4 (C-14), 52.3 (C-17), 49.7 (C-9), 47.1 (C-4), 43.2 (C-10), 43.1 (C-13), 39.3 (C-22), 37.6 (C-1), 36.5 (C-20), 34.0 (C-2), 33.0 (C-15), 32.5 (C-12), 29.7 (C-16), 25.2 (C-28), 24.9 (C-30), 21.9 (C-18), 21.7 (C-29), 18.7 (C-27), 18.5 (C-21), 17.7 (C-11), 13.9 (C-19); DEIMS *m/z* 436 $[M]^+$ (25), 421 $[M - CH_3]^+$ (15), 355 (55), 325 (100); HREIMS *m/z* found 436.3340 ($C_{30}H_{44}O_2$ requires 436.3341).

**Dyvariabilin B (2):** white, waxy solid; $[\alpha]^{20}_D$ −42° (*c* 0.05, $CHCl_3$); $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; DEIMS *m/z* 472 $[M]^+$ (95), 457 $[M - CH_3]^+$ (45), 147 (100); HREIMS (positive mode) *m/z* found 472.3550 ($C_{30}H_{48}O_4$ requires 472.3553).

**Dyvariabilin C (3):** white, waxy solid; $[\alpha]^{20}_D$ −58° (*c* 0.2, $CHCl_3$); $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; DEIMS *m/z* 472 $[M]^+$ (100), 457 $[M - CH_3]^+$ (27), 147 (11); HREIMS (positive mode) *m/z* found 472.3549 ($C_{30}H_{48}O_4$ requires 472.3553).

**24*S*,25-Epoxy-7α,23*R*-dihydroxytirucalla-8-en-3-one (3a)**: $[\alpha]^{20}_D$ −50° (*c* 0.6, $CHCl_3$); $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 4.17 (1H, m, H-7), 3.59 (1H, dt, $J$ = 5, 8 Hz, H-23), 2.67 (1H, d, $J$ = 8 Hz, H-24), 2.54 (2H, m, H-2), 2.16 (1H, dd, $J$ = 2.5, 12.5 Hz, H-5), 2.08 (2H, m, H-11), 2.05 (1H, m, H-16a), 1.96 (1H, m, H-1a), 1.77 (2H, m, H-12), 1.71 (1H, m, H-6a), 1.71 (1H, m, H-15a), 1.69 (1H, m, H-22a), 1.69 (1H, m, H-1b), 1.58 (1H, m, H-17), 1.48 (1H, m, H-20), 1.45 (1H, m, H-22b), 1.35 (1H, m, H-15b), 1.34 (3H, s, H-27), 1.33 (3H, s, H-26), 1.30 (1H, m, 16b), 1.13 (3H, s, H-28), 1.07 (3H, s, H-29), 1.01 (3H, d, $J$ = 5.9, H-21), 0.99 (3H, s, H-19), 0.92 (3H, s, H-30), 0.81 (3H, s, H-18); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 217.7 (C-3), 138.9 (C-9), 135.9 (C-8), 69.2 (C-23), 68.4 (C-24) 64.9 (C-7), 60.3 (C-25), 50.4 (C-17), 50.0 (C-14), 46.6 (C-4), 44.5 (C-5), 43.9 (C-13), 40.8 (C-22), 37.9 (C-10), 34.9 (C-1), 34.3 (C-2), 33.9 (C-20), 30.6 (C-12), 29.9 (C-15), 29.8 (C-6), 28.8 (C-16), 26.9 (C-28), 25.6 (C-30), 24.9 (C-27), 21.5 (C-11), 21.1 (C-29), 20.3 (C-21), 19.8 (C-26), 18.5 (C-18), 15.7 (C-19); DEIMS *m/z* 472 $[M]^+$ (21), 454 $[M - H_2O]^+$, 439 $[454 - CH_3]^+$ (59), 367 (100).

**Δ⁹-Niloticin (3b):** $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.40 (1H, m, H-7), 5.24 (1H, m, H-11), 3.59 (1H, dt, $J$ = 5, 8 Hz, H-23), 2.87 (1H, $J$ = 5.2, 14.6 Hz, H-2a), 2.67 (1H, d, $J$ = 8.2 Hz, H-24), 2.33 (1H, m, H-2b), 2.17 (2H, m, H-6), 2.15 (2H, m, H-12), 2.11 (1H, m, H-1a), 2.08 (1H, m, H-16a), 1.77 (1H, m, H-1b), 1.69 (1H, m, H-22a), 1.65 (1H, m, H-5), 1.65 (1H, m, H-15a), 1.65 (1H, m, H-17), 1.58 (1H, m, H-17), 1.45 (1H, m, H-22b), 1.42 (1H, m, H-20), 1.38 (1H, m, H-15b), 1.34 (3H, s, H-26), 1.33 (3H, s, H-27), 1.27 (1H, m, H-16b), 1.14 (3H, s, H-29), 1.06 (3H, s, H-28), 0.99 (3H, s, H-19), 0.96 (3H, d, $J$ = 6.3, H-21), 0.89 (3H, s, H-30), 0.63 (3H, s, H-18); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 216.6 (C-3), 143.8 (C-9), 141.3 (C-8), 118.4 (C-7), 116.0 (C-11), 69.2 (C-23), 68.4 (C-24), 60.3 (C-25), 51.2 (C-17), 50.1 (C-5), 49.6 (C-14), 47.8 (C-4), 44.1 (C-13), 40.8 (C-22), 38.1 (C-12), 36.9 (C-1), 36.1 (C-10), 34.9 (C-2), 33.6 (C-20), 31.1 (C-15), 28.4 (C-16), 24.9 (C-27), 24.4 (C-28), 24.2 (C-6), 23.0 (C-30), 22.1 (C-29), 19.9 (C-19 and C-21), 19.8 (C-26), 15.9 (C-18); DEIMS *m/z* 454 $[M]^+$.

**24*S*,25-Epoxy-23*R*-hydroxytirucalla-6,8-dien-3-one (3c):** $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 5.98 (1H, dd, $J$ = 2.9, 10.2 Hz, H-6), 5.68 (1H, br d, $J$ = 10.2 Hz, H-7), 3.59 (1H, dt, $J$ = 5, 8 Hz, H-23), 2.87 (1H, $J$ = 5.2, 14.6 Hz, H-2a), 2.67 (1H, d, $J$ = 8.2 Hz, H-24), 2.41 (1H, br s, H-5), 2.33 (1H, m, H-2b), 2.15 (2H, m, H-11), 2.05 (1H, m, H-16a), 1.98 (1H, m, H-1a), 1.79 (2H, m, H-12), 1.70 (1H, m, H-1b), 1.69 (1H, m, H-22a), 1.58 (1H, m, H-17), 1.50 (1H, m, H-15a), 1.45 (1H, m, H-22b), 1.40 (1H, m, H-15b), 1.40 (1H, m, H-20), 1.34 (3H, s, H-26), 1.33 (3H, s, H-27), 1.30 (1H, m, 16b), 1.20 (3H, s, H-29), 1.13 (3H, s, H-28), 0.99 (3H, s, H-19), 0.98 (3H, d, $J$ = 6.3 Hz, H-21), 0.95 (3H, s, H-30), 0.81 (3H, s, H-18);$^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 216.1 (C-3), 136.7 (C-9), 134.3 (C-8), 126.2 (C-6), 124.9 (C-7), 69.2 (C-23), 68.4 (C-24), 60.3 (C-25), 52.8 (C-5), 50.4 (C-17), 48.6 (C-14), 47.4 (C-4), 44.0 (C-13), 40.8 (C-22), 37.3 (C-10), 34.7 (C-2), 34.1 (C-1), 33.8 (C-20), 30.6 (C-15), 30.3 (C-12), 28.8 (C-16), 25.5 (C-30), 24.9 (C-27), 24.6 (C-28), 22.8 (C-29), 21.1 (C-11), 20.2 (C-21), 19.8 (C-26), 15.4 (C-18), 13.6 (C-19); DEIMS *m/z* 454 $[M]^+$.

**Dyvariabilin D (4):** white, waxy solid; $[\alpha]^{20}_D$ −27° (*c* 0.1, $CHCl_3$); $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; DEIMS *m/z* 470 $[M]^+$ (16), 452 $[M - H_2O]^+$ (2), 437 $[452 - CH_3]^+$ (3), 398 (98), 149 (100); HREIMS (positive mode) *m/z* found 470.3395 ($C_{30}H_{46}O_4$ requires 470.3396).

**Dyvariabilin E and F (5 and 6):** $^1$H and $^{13}$C NMR data, see Tables 1 and 2; DEIMS *m/z* 472 [**5**, M]$^+$ (2) and 486 [**6**, M]$^+$ (1); HREIMS (positive mode) *m/z* found 472.3550 (**5**, $C_{30}H_{48}O_4$ requires 472.3553) and 486.3707 (**6**, $C_{31}H_{50}O_4$ requires 486.3709).

**Dyvariabilin G and H (7 and 8):** $^1$H and $^{13}$C NMR data, see Tables 1 and 2; DEIMS *m/z* 458 [**7**, M]$^+$ (10), 443 [**7**, M − $CH_3$]$^+$ (13), 425 [**7**, 443 − $H_2O$]$^+$ (34) and 438 [**8**, M]$^+$ (4), 423 [**8**, M − $CH_3$]$^+$ (21); HREIMS (positive mode) *m/z* found 458.3770 (**7**, $C_{30}H_{50}O_3$ requires 458.3760) and 438.3496 (**8**, $C_{30}H_{46}O_2$ requires 438.3498).

**Tirucalla-7,23,25-trien-3*β*-ol (11a):** white, waxy solid; $[\alpha]^{20}_D$ −13° (*c* 0.2, $CHCl_3$); $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 6.12 (1H, d, $J$ = 15.8 Hz, H-24), 5.64 (1H, m, H-23), 5.27 (1H, br s, H-7), 4.86 (2H, br s, H-26), 3.26 (1H, dd, $J$ = 4.3, 10.6 Hz, H-3α), 2.44 (H, br dd, $J$ = 5.9, 12.7, H-22a), 2.20 (1H, m, H-9), 2.10 (1H, m, H-6a), 1.97 (1H, m, H-6b), 1.94 (1H, m, H-16a), 1.85 (3H, s, H-27), 1.84 (1H, m, H-12a), 1.75 (1H, m, H-22b), 1.68 (1H, m, H-1a), 1.65 (2H, m, H-2), 1.65 (1H, m, H-12b), 1.52 (2H, m, H-11), 1.50 (1H, m, H-17), 1.50 (1H, m, H-20), 1.33 (1H, m, H-5), 1.28 (1H, m, H-16b), 1.15 (1H, m, H-1b), 1.00 (3H, s, H-30), 0.98 (3H, s, H-28), 0.87 (3H, s, H-29), 0.86 (3H, s, H-18), 0.85 (3H, d, $J$ = 5.5 Hz, H-21), 0.76 (3H, s, H-19); $^{13}$C NMR (75 MHz) $\delta$ 145.7 (C-8), 142.2 (C-25), 133.8 (C-24), 129.9 (C-23), 117.9 (C-7), 114.0 (C-26), 79.2 (C-3), 53.2 (C-17), 51.3 (C-14), 50.6 (C-5), 48.9 (C-9), 43.6 (C-13), 39.0 (C-4), 38.6 (C-22), 37.2 (C-1), 36.5 (C-20), 34.9 (C-10), 33.9 (C-15), 33.8 (C-12), 28.3 (C-16), 27.7 (C-2), 27.6 (C-28), 27.3 (C-30), 23.9 (C-6), 22.2 (C-18), 19.0 (C-21), 18.8 (C-27), 18.2 (C-11), 14.7 (C-29), 13.1 (C-19); DEIMS *m/z* 424 [M]$^+$ (43), 409 [M − $CH_3$]$^+$ (100), 391 [409 − $H_2O$]$^+$ (21), 327 (36), 313 (76); HREIMS (positive mode) *m/z* found 424.3681 ($C_{30}H_{48}O$ requires 424.3705).

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