Saponins from the Bark of *Nephelium maingayi*[⊥]

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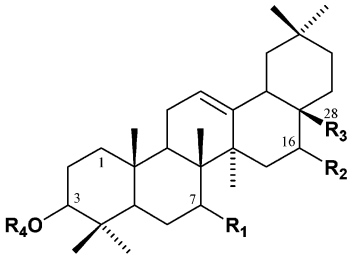
Activity-guided fractionation of the bark of *Nephelium maingayi*, collected in Indonesia, led to the isolation of six new saponins (**1**−**6**). The aglycon of **4** was determined to be a new compound, 7α-methoxyerythrodiol, and those of **1**−**3** and of **5** and **6** were identified as erythrodiol and maniladiol (16*β*-hydroxyamyrin), respectively. The structures of **1**−**6** were determined on the basis of spectral data interpretation, and the absolute configurations of their component monosaccharides were determined as their thiazolidine derivatives after acid hydrolysis. Of the isolates, only compounds **1** and **5** showed very weak cytotoxic activity against a panel of human tumor cell lines.

Nephelium maingayi Hiern (Sapindaceae) is a tree indigenous to both Malaysia and Sumatra and Kalimantan in Indonesia, for which there is no information in the literature on its biological activity or constituents, although the pulp is known to be edible. The fruits of some species of this genus, such as *N. lappaceum* and *N. mutabile*, are commonly known as "rambutan" and are cultivated widely and consumed as a tropical dessert for their pleasant flavor.[1] In previous phytochemical work on *N. lappaceum*, an extract of the dried seeds afforded several cyanolipids.[2] Crude extracts of *N. lappaceum* have been reported to exhibit antioxidant activity[3] and an inhibitory effect against plaque formation by herpes simplex virus type 1 (HSV-1).[4] Polyphenols,[5,6] tannins,[7] fatty acids,[8] and several volatile oil components[9] have been reported from various members of this genus.

As a part of our ongoing program on the discovery of new anticancer agents from plants,[10] a chloroform-soluble extract of the bark of *Nephelium maingayi* was found to exhibit cytotoxic activity when evaluated against a panel of human cancer cell lines. Bioassay-guided phytochemical investigation of this extract, using a human lung cancer cell line (Lu1) to monitor fractionation, led to the isolation of six new saponins, namely, nepheliosides I−VI (**1**−**6**). The structures of compounds **1**−**6** were determined on the basis of various 1D- and 2D-NMR experiments and by HRESI-MS. The absolute configurations of the component monosaccharides of saponins **1**−**6** were determined as their thiazolidine derivatives after acid hydrolysis. These purified isolates were evaluated against a human cancer cell line panel.

Results and Discussion

Compound **1** was determined to have the elemental composition $C_{46}H_{76}O_{15}$ from its HRESIMS and ^{13}C NMR spectral data. In the ^{13}C NMR spectrum of **1**, the presence of three sugar moieties was indicated, along with 30 carbon signals for the aglycon, which was identified as erythrodiol.[11] The ESIMS-MS of **1** gave informative fragment peaks at *m/z* 759 $[(M + Na) - 132]^+$ and 729 $[(M + Na) - 162]^+$, corresponding to the loss of a terminal pentose and



	R_1	R_2	R_3	R_4
1	H	H	CH_2OH	–Xyl$^{2}_{3}$–Glc, Ara
2	H	H	CH_2OH	–Glc$^{2}_{3}$–Glc, Ara
3	H	H	CH_2OH	–Xyl^{3}–Glc^{6}–Ac
4	α-OMe	H	CH_2OH	–Glc$^{2}_{3}$–Glc, Ara
5	H	β-OH	CH_3	–Xyl$^{2}_{3}$–Glc, Ara
6	H	β-OH	CH_3	–Glc$^{2}_{3}$–Glc, Ara
7	H	H	CH_2OH	H
8	H	β-OH	CH_3	H
9	α-OMe	H	CH_2OH	H

[⊥] Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of Research Triangle Institute for their pioneering work on bioactive natural products.

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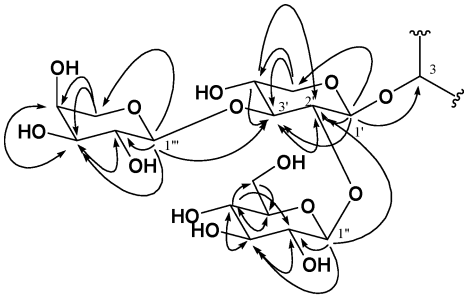

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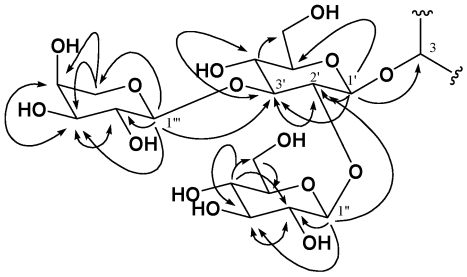

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Figure 1. HMBC correlations of sugar moieties of **1** and **2**.

a terminal hexose unit, respectively. The sugar composition was determined by TLC to be glucose, arabinose, and xylose, after a microscale acid hydrolysis with 10% HCl in dioxane. The absolute configurations of the component monosaccharides were determined as D-glucose, L-arabinose, and D-xylose by GC analysis of their thiazolidine derivatives.[13] Also obtained on acid hydrolysis was the aglycon, erythrodiol (**7**), which was identified by spectral data comparison to literature values.[11] The ^{13}C NMR chemical shift of C-3 (δ_C 89.0) suggested that **1** has a glycosyl linkage at C-3. The HMBC spectrum showed cross-peaks between the anomeric protons of xylose (H-1′), glucose (H-1″), and arabinose (H-1‴) with C-3, C-2′, C-3′, and C-5′; C-2′, C-2″, and C-3″; and C-3′, C-3‴, and C-5‴, respectively. The other HMBC correlations observed for these sugar moieties of **1** are shown in Figure 1. The assignments of the sugar moieties and linkages were confirmed from the COSY spectrum, which showed cross-peaks between H-1′ and H-2′, H-2′ and H-3′, H-4′ and H-5′, H-1″ and H-2″, H-4″ and H-5″, H-5″ and H-6″, H-1‴ and H-2‴, H-2‴ and H-3‴, and H-4‴ and H-5‴. Other COSY and HMBC correlations were consistent with the aglycon of **1** being erythrodiol (**7**). Thus, compound **1** was determined to be erythrodiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-xylopyranoside and has been accorded the trivial name nephelioside I.

The spectral data of compound **2** were similar to those of **1**. Compound **2** exhibited a molecular formula of $C_{47}H_{78}O_{16}$ from its HRESIMS, suggesting the presence of another hexose unit instead of a pentose unit in **2**. The sugar composition was determined to be D-glucose and L-arabinose by microscale acid hydrolysis and GC analysis of the thiazolidine derivatives obtained, and erythrodiol (**7**) was also obtained on acid hydrolysis. The assignments of the sugar unit and their linkages were confirmed by HMBC (Figure 1) and COSY experiments. The structure of **2** was characterized, therefore, as erythrodiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (nephelioside II).

Compound **3** exhibited a molecular formula of $C_{43}H_{70}O_{12}$ from its HRESIMS. Diagnostic ESIMS fragments occurred at *m*/*z* 597 $[(M + Na) - 162 - Ac]^+$ and 465 $[597 - 132]^+$, indicating that the molecule contained a pentose and a hexose moiety and an acetyl group. Acid hydrolysis of **3** afforded D-glucose and D-xylose, which were identified by GC analysis as indicated previously, in addition to erythrodiol (**7**). The HMBC spectrum showed cross-peaks between the anomeric proton of xylose (H-1′) and C-3, C-2′, and C-5′, H-3′ and C-2′ and C-5′, and H-5′ and C-4′. These interactions supported the presence of a xylose unit and its linkage to the C-3 position of erythrodiol. Further correlations were observed in the HMBC spectrum between the anomeric proton of glucose (H-1″) and C-3′ and C-3″, and H-2″ and C-1″ and C-3″. Other correlations between H-3″ and C-5″, H-4″ and C-5″, H-6″ and C-5″, and the acetyl proton (δ_H 2.00) and C″-6 and the acetyl carbonyl carbon (δ_C 170.9) supported the presence of a C-6″ acetylated glucose unit and its linkage to C-3′ of the xylose unit. Therefore, the structure of **3** was established as erythrodiol 3-*O*-(6″-acetyl)-*β*-D-glucopyranosyl-(3→1)-*β*-D-xylopyranoside, to which the name nephelioside III was given.

The HRESIMS of compound **4** showed a sodiated molecular ion at *m*/*z* 951.5302 $[M + Na]^+$, indicating an elemental formula of $C_{48}H_{80}O_{17}$. The ESIMS-MS of **4** gave fragment peaks at *m*/*z* 819 $[(M + Na) - 132]^+$ and 789 $[(M + Na) - 162]^+$, corresponding to the loss of a terminal pentose and a terminal hexose unit, respectively. Further ESIMS fragments at *m*/*z* 496 $[(M + Na) - 2\times$hexose $-$ pentose $+ H]^+$ indicated that the molecule contains a pentose moiety and two hexose units. The sugar composition was determined by TLC to be arabinose and glucose. The absolute configurations of the component monosaccharides were determined as D-glucose and L-arabinose by GC analysis of their thiazolidine derivatives.[13] In the ^{13}C NMR spectrum, 31 carbons were observed in the aglycon moiety, including a methoxy group at δ_C 54.0. The HMBC spectrum showed cross-peaks between a methoxy proton (δ_H 3.21) and C-7 (δ_C 76.0), H-7 and C-9, H-9 and C-8, the H-25 methyl protons (δ_H 1.04) and C-9 and C-10, the H-26 methyl protons (δ_H 1.01) and C-9 and C-14, the H-27 methyl proton (δ_H 1.45) and C-8, and H-5 and C-4 and C-8. Also, the correlations between H-5 and H-6, H-6 and H-7, and a methoxy proton and the H-7 proton were observed in the ^{1}H−^{1}H COSY spectrum. These interactions gave evidence for the linkage adjacent to the methoxy group which was attached at C-7. The assignments of the sugar moieties and linkages were also confirmed from the HMBC and COSY spectra. The methoxy group attached at C-7 was determined as in the α-configuration through a NOESY experiment. Correlations were observed between the methoxy proton and the C-27 methyl protons, as well as the H-9 proton. On enzymatic hydrolysis of **4** with *β*-glucosidase in 5% EtOH−H_2O for 72 h, the new compound 7α-methoxyerthrodiol (**9**) was produced and characterized from its ^{1}H NMR and EIMS data. Thus, compound **4** was determined as 7α-methoxyerythrodiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (nephelioside IV). The possibility of compound **4** being an extraction artifact was investigated, but this seems unlikely since the non-methoxylated compound **2** was found to be stable in MeOH for more than a week.

Compound **5** exhibited the same elemental formula ($C_{46}H_{76}O_{15}$) as **1**. The ^{1}H and ^{13}C NMR spectra of **5** were similar to those of **1**, except for one more methyl signal instead of the hydroxymethyl signal at C-28. The aglycon was therefore identified as maniladiol (**8**)[14] and confirmed

Table 1. Cytotoxic Activity of Compounds **1** and **5**[a–c]

compound	Lu1	LNCaP	MCF-7	HUVEC
1	19.5	>20	>20	>20
5	13.6	11.7	13.9	17.7

[a] Results are expressed as ED_{50} values (μg/mL). [b] Compounds **2**–**4** and **6** were inactive against all cell lines tested (ED_{50} >20 μg/mL). [c] Key to cell lines used: Lu1 = human lung cancer; LNCaP = hormone-dependent human prostate cancer; MCF-7 = breast adenocarcinoma; HUVEC = human umbilical vein endothelial cells.

from the HMBC and COSY spectra of **5** and the generation of this compound by acid hydrolysis. Accordingly, compound **5** was determined structurally as maniladiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-xylopyranoside (nephelioside V).

On examination of its NMR and mass spectral data and by acid hydrolysis, compound **6** was found to possess maniladiol (**8**) as the aglycon and the same sugar moiety as **2**. Thus, **6** was characterized as maniladiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (nephelioside VI).

Compounds **1**–**6** were evaluated against a panel of human tumor cell lines.[15,16] Compound **5** exhibited a weak cytotoxic effect against all of the cell lines in which it was tested. Compound **1** mediated a weak response only against the Lu1 (human lung cancer) cell line (Table 1). All other isolates were inactive.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. UV spectra were measured on a Beckman DU-7 spectrometer. IR spectra were taken on a JASCO FT/IR-410 spectrophotometer. ^{1}H and ^{13}C NMR data (including DEPT, HMQC, HMBC, NOESY, and ^{1}H–^{1}H COSY spectra) were measured on a Bruker DRX-500 instrument operating at 500.1 and 125.7 MHz, respectively. Compounds were analyzed in pyridine-d_5, with tetramethylsilane (TMS) as internal standard. ^{13}C NMR multiplicity was determined using DEPT experiments. ESIMS and HRESIMS were recorded on a Finnigan LCQ spectrometer. GC analysis was carried out on a JEOL GC Mate II instrument with a HP5-MS column (0.25 mm i.d. × 30 m) (Agilent).

Plant Material. The stem bark of *Nephelium maingayi* Hiern was collected at Timpah District, Kapuas, Indonesia, in August 2000. A voucher specimen (A5077) has been deposited at the Field Museum of Natural History, Chicago, IL, and the Herbarium Bogoriense, Indonesian Institute of Science, Bogor, Indonesia.

Extraction and Isolation. The dried stem bark of *N. maingayi* (670 g) was extracted three times with MeOH at room temperature. The resultant extracts were combined, concentrated under vacuum, dissolved in MeOH (500 mL), and washed with hexane (3 × 500 mL). The lower layer was concentrated to dryness under reduced pressure and partitioned between 5% MeOH/H_2O (500 mL) and $CHCl_3$ (3 × 500 mL). The $CHCl_3$-soluble extract [4.2 g, ED_{50} 18.2 μg/mL against the Lu1 cell line (human lung cancer cell line)][17] was subjected to Si gel column chromatography and eluted with a gradient mixture of hexane–Me_2CO–MeOH (8:1:0.1 → 2:1:0.1, 100 mL per fraction) to give 10 pooled fractions. Fraction 8 was weakly active when tested against the Lu1 cell line (ED_{50} 19.4 μg/mL). Additional chromatographic separation of fraction 8 over reversed-phase Si gel with 75% MeOH–H_2O yielded seven subfractions (fractions 8A–8G). Further purification of subfraction 8B, by HPLC with an ODS-AQ column (250 × 20 mm; YMC, Inc., Willington, NC) using 85% MeOH–H_2O as eluant at a flow rate of 6 mL/min, afforded compound **4** (6 mg; t_R 17.8 min). Compounds **2** (8 mg) and **6** (7 mg) were obtained from subfraction 8D using HPLC with 87% MeOH–H_2O (t_R 18.2 and 23.4 min, respectively). Subfraction 8F was subjected to HPLC with an ODS-AQ column using 87% MeOH–H_2O as eluant at a flow rate of 6 mL/min, to afford **1** (9 mg) and **5** (6 mg) (t_R 18.3 and 22.7 min, respectively). From fraction 5, compound **3** (10 mg) was obtained using reversed-phase Si gel column chromatography eluted with 80% MeOH–H_2O and was purified by HPLC using 85% MeOH–H_2O at a flow rate of 6 mL/min (t_R 27.5 min).

Erythrodiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-xylopyranoside (nephelioside I, 1): amorphous powder; $[\alpha]_D$ +79.3° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (3.60), 245 (3.24), 251 (3.30), 261 (3.18) nm; IR (film) ν_{max} 3386 (br), 2945, 2922, 2869, 2359, 2343, 1650, 1460, 1369, 1254, 1072, 1049, 1003 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.45 (1H, d, J = 7.8 Hz, H-1″), 5.23 (1H, dd, J = 3.9, 3.8 Hz, H-12), 5.19 (1H, d, J = 7.0 Hz, H-1‴), 4.85 (1H, d, J = 6.8 Hz, H-1′), 4.75 (1H, dd, J = 6.8, 6.7 Hz, H-2′), 4.50 (2H, m, H-2‴, 4‴), 4.36 (2H, m, H-3′, 6″), 4.30 (1H, m, H-4′), 4.27 (3H, m, H-5′, 6″, 5‴), 4.16 (3H, m, H-3″, 4″, 3‴), 4.04 (1H, m, H-2″), 3.85 (1H, d, J = 10.6 Hz, H-28), 3.76 (2H, m, H-5′, 5‴), 3.62 (1H, m, H-5″), 3.57 (1H, d, J = 10.6 Hz, H-28), 3.24 (1H, dd, J = 11.7, 4.4 Hz, H-3), 2.29 (1H, dd, J = 13.4, 3.9 Hz, H-18), 2.05 (1H, m, H-2), 1.98 (2H, m, H-11, 16), 1.96 (1H, m, H-22), 1.88 (1H, m, H-19), 1.82 (1H, m, H-2), 1.70 (1H, m, H-22), 1.60 (1H, m, H-9), 1.51 (1H, m, H-11), 1.46 (2H, m, H-1, 6), 1.43 (2H, m, H-7, 21), 1.32 (1H, m, H-6), 1.29 (3H, s, Me-27), 1.24 (3H, s, Me-23), 1.22 (1H, m, H-21), 1.19 (2H, m, H-15, 19), 1.09 (3H, s, Me-24), 1.02 (1H, m, H-16), 0.98 (3H, s, Me-30), 0.96 (3H, s, Me-29), 0.93 (3H, s, Me-26), 0.90 (1H, m, H-1), 0.84 (3H, s, Me-25), 0.74 (1H, brd, J = 11.7 Hz, H-5); ^{13}C NMR, see Table 2; ESIMS *m/z* 891 $[M + Na]^+$, 759 $[M + Na - 132]^+$, 729 $[M + Na - 162]^+$; HRESIMS *m/z* 891.5105 $[M + Na]^+$ (calcd for $C_{46}H_{76}O_{15}Na$, 891.5082); GC analysis of sugar components, t_R 16.55, 17.03, and 34.19 min.

Erythrodiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (nephelioside II, 2): amorphous powder; $[\alpha]_D$ +44.3° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (3.59), 251 (3.13) nm; IR (film) ν_{max} 3729 (br), 2921, 2861, 2358, 2335, 1460, 1369, 1079, 1049, 1003 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.72 (1H, d, J = 7.8 Hz, H-1″), 5.26 (1H, d, J = 7.7 Hz, H-1‴), 5.23 (1H, dd, J = 3.8, 3.8 Hz, H-12), 4.87 (1H, d, J = 7.7 Hz, H-1′), 4.48 (2H, m, H-6″, 2‴), 4.42 (2H, m, H-5′, 6′), 4.34 (1H, m, H-6″), 4.28 (3H, m, H-6′, 3″, 5‴), 4.26 (2H, m, H-3′, 4‴), 4.12 (3H, m, H-2″, 4″, 3‴), 4.03 (1H, dd, J = 12.4, 12.4 Hz, H-4′), 3.88 (2H, m, H-2′, 5″), 3.84 (1H, m, H-5‴), 3.83 (1H, d, J = 10.5 Hz, H-28), 3.58 (1H, d, J = 10.5 Hz, H-28), 3.30 (1H, dd, J = 11.1, 4.4 Hz, H-3), 2.31 (1H, dd, J = 12.9, 3.3 Hz, H-18), 2.05 (1H, m, H-2), 1.98 (2H, m, H-11, 16), 1.96 (1H, m, H-22), 1.88 (1H, m, H-19), 1.82 (1H, m, H-2), 1.70 (1H, m, H-22), 1.61 (1H, dd, J = 10.3, 7.6 Hz, H-9), 1.51 (1H, m, H-11), 1.46 (2H, m, H-1, 6), 1.43 (2H, m, H-7, 21), 1.33 (1H, m, H-6), 1.31 (3H, s, Me-27), 1.27 (3H, s, Me-23), 1.22 (1H, m, H-21), 1.19 (2H, m, H-15, 19), 1.10 (3H, s, Me-24), 1.02 (1H, m, H-16), 1.00 (3H, s, Me-30), 0.97 (3H, s, Me-29), 0.92 (3H, s, Me-26), 0.84 (1H, m, H-1), 0.83 (3H, s, Me-25), 0.74 (1H, d, J = 11.1 Hz, H-5); ^{13}C NMR, see Table 2; ESIMS *m/z* 921 $[M + Na]^+$, 789 $[M + Na - 132]^+$, 759 $[M + Na - 162]^+$; HRESIMS *m/z* 921.5192 $[M + Na]^+$ (calcd for $C_{47}H_{78}O_{16}Na$, 921.5188); GC analysis of sugar components, t_R 17.04 and 34.26 min.

Erythrodiol 3-*O*-(6″-acetyl)-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranoside (nephelioside III, 3): amorphous powder; $[\alpha]_D$ +23.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (3.49), 250 (2.75) nm; IR (film) ν_{max} 3401 (br), 2921, 2860, 2358, 2343, 2328, 1734, 1453, 1369, 1247, 1079, 1034 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.38 (1H, d, J = 7.8 Hz, H-1″), 5.25 (1H, dd, J = 3.4, 3.4 Hz, H-12), 4.78 (1H, d, J = 7.4 Hz, H-1′), 4.93 (1H, brd, J = 11.4 Hz, H-6″), 4.77 (1H, m, H-6″), 4.61 (1H, m, H-2′), 4.51 (1H, m, H-4′), 4.29 (1H, m, H-5′), 4.24 (2H, m, H-3′, 3″), 4.04 (3H, m, H-2″, 4″, 5″), 3.87 (1H, m, H-28), 3.84 (1H, dd, J = 11.2, 10.0 Hz, H-5′), 3.59 (1H, brd, J = 9.5 Hz, H-28), 3.37 (1H, dd, J = 11.6, 4.4 Hz, H-3), 2.33 (1H, dd, J = 13.4, 4.1 Hz, H-18), 2.19 (1H, m, H-2), 2.00 (3H, m, H-11, 16, 22), 1.97 (3H, s, Ac), 1.92 (2H, m, H-2, 19), 1.70 (1H, ddd,

Table 2. ^{13}C NMR Spectral Data (δ) of Compounds **1**−**6** (C_5D_5N, 125 MHz)

C	1	2	3	4	5	6	C	1	2	3	4	5	6
1	38.9	38.8	38.9	39.9	38.9	38.8	26	16.9	16.9	17.0	18.3	17.1	17.1
2	26.6	26.9	26.8	26.9	26.6	26.6	27	26.1	26.2	26.2	25.3	27.4	27.4
3	89.0	89.2	88.7	89.5	88.9	89.2	28	68.7	68.7	68.7	68.8	22.5	22.5
4	39.7	39.6	39.7	40.0	39.7	39.6	29	33.4	33.5	33.5	33.4	33.5	33.6
5	55.7	55.7	55.8	55.8	55.7	55.7	30	23.8	23.9	23.9	23.8	24.2	24.2
6	18.4	18.5	18.5	18.6	18.4	18.5	OMe				54.0		
7	32.9	32.9	33.0	76.0	33.0	33.0	1′	105.3	105.2	107.6	105.2	105.4	105.2
8	40.1	40.1	40.1	43.4	40.2	40.2	2′	77.1	77.9	71.9	77.9	77.1	77.9
9	47.9	47.9	48.0	52.5	47.2	47.2	3′	82.3	87.2	84.2	87.3	82.3	87.2
10	36.8	36.8	36.9	38.1	38.0	38.0	4′	69.4	69.8	69.4	69.7	69.5	69.7
11	22.8	22.9	22.9	22.7	23.9	23.9	5′	65.8	79.6	67.1	79.5	65.9	79.6
12	122.4	122.4	122.5	122.5	122.4	122.4	6′		62.6		62.4		62.6
13	145.0	145.1	145.1	149.3	144.5	144.6	1″	104.3	103.8	106.3	103.8	104.3	103.8
14	42.0	42.0	42.0	42.0	43.9	43.9	2″	76.0	76.6	75.5	76.6	76.1	76.6
15	30.0	30.0	30.0	30.0	36.4	36.5	3″	78.6	78.6	78.2	78.6	78.7	78.6
16	26.0	26.1	26.1	26.2	64.4	64.4	4″	72.2	72.4	71.5	72.5	72.2	72.5
17	37.6	37.6	37.7	37.5	36.8	36.8	5″	77.5	77.8	75.3	77.7	77.4	77.8
18	42.7	42.7	42.7	42.4	49.5	49.6	6″	63.1	63.6	64.7	63.3	63.0	63.3
19	47.0	47.1	47.1	46.9	47.0	47.1	1‴	105.3	105.2		105.2	105.4	105.2
20	31.2	31.3	31.3	31.3	31.1	31.3	2‴	72.6	72.9		72.9	72.6	72.9
21	34.7	34.7	34.7	34.6	34.7	34.8	3‴	74.4	74.8		74.8	74.5	74.8
22	31.8	31.8	31.8	31.7	31.2	31.1	4‴	68.8	69.7		69.7	68.7	69.7
23	28.0	28.0	28.1	28.1	28.0	28.0	5‴	67.0	68.0		68.0	67.1	68.0
24	16.7	16.8	16.9	16.8	16.8	16.8	Ac			170.9			
25	15.6	15.7	15.7	17.2	15.7	15.7	Ac			20.8			

J = 13.9, 3.3, 3.3 Hz, H-22), 1.65 (1H, dd, J = 10.2, 7.4 Hz, H-9), 1.55 (5H, m, H-1, 6, 7, 11), 1.45 (1H, dd, J = 13.5, 3.5 Hz, H-21), 1.33 (1H, m, H-15), 1.33 (3H, s, Me-23), 1.32 (3H, s, Me-27), 1.28 (4H, m, H-7, 15, 21), 1.21 (2H, m, H-19), 1.04 (1H, m, H-16), 1.01 (3H, s, Me-24), 1.00 (3H, s, Me-30), 0.97 (3H, s, Me-29), 0.95 (3H, s, Me-26), 0.85 (3H, s, Me-25), 0.83 (1H, d, J = 11.6 Hz, H-5); ^{13}C NMR, see Table 2; ESIMS *m/z* 801 $[M + Na]^+$, 639 $[M + Na - 162]^+$, 597 $[M + Na - 162\text{-}(Ac)]^+$, 465 $[597 - 132]^+$; HRESIMS *m/z* 801.4760 $[M + Na]^+$ (calcd for $C_{43}H_{70}O_{12}Na$, 801.4765); GC analysis of sugar components, t_R 16.55 and 34.23 min.

7-Methoxyerythrodiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (nephelioside IV, 4): amorphous powder; $[\alpha]_D$ +19.6° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 (3.65), 251 (3.15) nm; IR (film) ν_{max} 3417 (br), 2929, 2853, 2358, 2332, 1650, 1460, 1368, 1163, 1079, 1049, 996 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 5.74 (1H, d, J = 7.7 Hz, H-1″), 5.49 (1H, d, J = 3.3 Hz, H-12), 5.27 (1H, d, J = 7.6 Hz, H-1‴), 4.90 (1H, d, J = 7.7 Hz, H-1′), 4.49 (2H, m, H-6″, 2‴), 4.42 (2H, m, H-5′, 6′), 4.34 (1H, m, H-6″), 4.28 (3H, m, H-6′, 3″, 5‴), 4.26 (2H, m, H-3′, 4‴), 4.15 (1H, m, H-4″), 4.11 (2H, m, H-2″, 3‴), 4.03 (1H, dd, J = 9.2, 9.2 Hz, H-4′), 3.86 (2H, m, H-2′, 5″), 3.80 (1H, m, H-28), 3.79 (1H, m, H-5‴), 3.75 (1H, dd, J = 8.5, 3.4 Hz, H-7), 3.62 (1H, m, H-28), 3.39 (1H, dd, J = 11.9, 4.3 Hz, H-3), 3.27 (3H, s, OMe), 2.38 (1H, brd, J = 12.1 Hz, H-18), 2.22 (1H, m, H-2), 2.02 (1H, m, H-11), 1.99 (1H, m, H-22), 1.90 (3H, m, H-1, 16, 19), 1.88 (1H, m, H-2), 1.82 (1H, d, J = 8.5 Hz, H-9), 1.71 (1H, m, H-22), 1.52 (2H, m, H-6, 11), 1.44 (1H, m, H-21), 1.36 (1H, m, H-1), 1.31 (3H, m, H-6, 15, 19), 1.38 (3H, s, Me-27), 1.28 (3H, s, Me-23), 1.22 (2H, m, H-15, 21), 1.13 (3H, s, Me-24), 1.02 (1H, m, H-16), 0.98 (3H, s, Me-30), 0.97 (3H, s, Me-25), 0.95 (3H, s, Me-26), 0.93 (3H, s, Me-29), 0.79 (1H, d, J = 11.9 Hz, H-5); ^{13}C NMR, see Table 2; ESIMS *m/z* 951 $[M + Na]^+$, 819 $[M + Na - 132]^+$, 789 $[M + Na - 162]^+$; 496 $[(M + Na) - 2$ hexose $-$ pentose $+ H]^+$; HRESIMS *m/z* 951.5302 $[M + Na]^+$ (calcd for $C_{48}H_{80}O_{17}Na$, 951.5293); GC analysis of sugar components, t_R 17.04 and 34.18 min.

Maniladiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-xylopyranoside (nephelioside V, 5): amorphous powder; $[\alpha]_D$ +47.8° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (3.62), 250 (3.51), 256 (3.31) nm; IR (film) ν_{max} 3370 (br), 2945, 2366, 2343, 1650, 1452, 1384, 1171, 1079, 1026 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 5.57 (1H, d, J = 7.7 Hz, H-1″), 5.32 (1H, dd, J = 3.3, 3.3, H-12), 5.21 (1H, d, J = 7.1 Hz, H-1‴), 4.86 (1H, d, J = 6.8 Hz, H-1′), 4.76 (1H, dd, J = 6.7, 6.6 Hz, H-2′), 4.53 (1H, m, H-16), 4.50 (2H, m, H-2‴, 4‴), 4.30 (6H, m, H-3′, 4′, 5′, 6″, 5‴), 4.17 (3H, m, H-3″, 4″, 3‴), 4.06 (1H, dd, J = 6.8, 6.8 Hz, H-2″), 3.79 (2H, m, H-5′, 5‴), 3.23 (1H, dd, J = 11.2, 3.9 Hz, H-3), 2.44 (1H, brd, J = 13.7, H-22), 2.33 (1H, dd, J = 13.3, 3.7 Hz, H-18), 2.07 (2H, m, H-2, 15), 1.88 (1H, d, J = 13.7 Hz, H-19), 1.87 (2H, m, H-11), 1.82 (1H, m, H-2), 1.63 (2H, m, H-15, 21), 1.60 (1H, m, H-9), 1.50 (3H, m, H-1, 6, 7), 1.32 (3H, m, H-6, 7, 22), 1.37 (3H, s, Me-27), 1.25 (3H, s, Me-23), 1.20 (2H, m, H-19, 21), 1.17 (3H, s, Me-28), 1.07 (3H, s, Me-24), 1.03 (3H, s, Me-26), 1.00 (3H, s, Me-30), 0.95 (3H, s, Me-29), 0.90 (1H, m, H-1), 0.88 (3H, s, Me-25), 0.74 (1H, d, J = 11.2 Hz, H-5); ^{13}C NMR, see Table 2; ESIMS *m/z* 891 $[M + Na]^+$, 759 $[M + Na - 132]^+$, 729 $[M + Na - 162]^+$; HRESIMS *m/z* 891.5064 $[M + Na]^+$ (calcd for $C_{46}H_{76}O_{15}Na$, 891.5082); GC analysis of sugar components, t_R 16.55, 17.04, and 34.18 min.

Maniladiol 3-*O*-[α-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (nephelioside VI) (6): amorphous powder; $[\alpha]_D$ +40.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (3.57), 250 (3.02) nm; IR (film) ν_{max} 3386 (br), 2921, 2853, 2366, 2336, 1650, 1460, 1361, 1163, 1079, 1049, 996 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) δ 5.72 (1H, d, J = 7.8 Hz, H-1″), 5.33 (1H, dd, J = 3.3, 3.2 Hz, H-12), 5.26 (1H, d, J = 7.6 Hz, H-1‴), 4.87 (1H, d, J = 7.8 Hz, H-1′), 4.56 (1H, m, H-16), 4.50 (2H, m, H-6′, 2‴), 4.43 (1H, m, H-5′), 4.34 (2H, m, H-6″), 4.29 (1H, m, H-3″), 4.25 (4H, m, H-3′, 6′, 4‴, 5‴), 4.19 (1H, m, H-4″), 4.11 (2H, m, H-2″, 3‴), 4.00 (1H, m, H-4′), 3.87 (2H, m, H-2′, 5″), 3.78 (1H, m, H-5‴), 3.30 (1H, dd, J = 11.7, 4.4 Hz, H-3), 2.45 (1H, brd, J = 13.8 Hz, H-22), 2.33 (1H, dd, J = 13.8, 4.1 Hz, H-18), 2.19 (1H, m, H-2), 2.07 (1H, dd, J = 12.0, 12.0 Hz, H-15), 1.92 (1H, d, J = 13.7 Hz, H-19), 1.87 (2H, m, H-11), 1.82 (1H, m, H-2), 1.63 (2H, m, H-15, 21), 1.58 (1H, m, H-9), 1.50 (2H, m, H-6, 7), 1.42 (1H, m, H-1), 1.32 (3H, m, H-6, 7, 22), 1.37 (3H, s, Me-27), 1.26 (3H, s, Me-23), 1.22 (1H, m, H-21), 1.20 (1H, m, H-19), 1.16 (3H, s, Me-28), 1.11 (3H, s, Me-24), 1.03 (3H, s, Me-26), 0.99 (3H, s, Me-30), 0.94 (3H, s, Me-29), 0.90 (1H, m, H-1), 0.86 (3H, s, Me-25), 0.74 (1H, d, J = 11.4 Hz, H-5); ^{13}C NMR, see Table 2; ESIMS *m/z* 921 $[M + Na]^+$, 789 $[M + Na - 132]^+$, 759 $[M + Na - 162]^+$; HRESIMS *m/z* 921.5196 $[M + Na]^+$ (calcd for $C_{47}H_{78}O_{16}Na$, 921.5188); GC analysis of sugar components, t_R 17.03 and 34.23 min.

Determination of Sugar Components. Compounds **1**−**6** (1 mg each) were heated in 10% HCl−dioxane (1:1, 2 mL) at 80 °C for 2 h, and the solution was evaporated under N_2. The residue was dissolved in anhydrous pyridine (100 μL), 0.1 M L-cysteine methyl ester hydrochloride (200 μL) was added, and the mixture was warmed at 60 °C for 1 h. The trimethylsilyl-

ation reagent HMDS-TMCS (hexamethyldisilazane−trimethylchlorosilane−pyridine, 2:1:10) (Acros Organics, Geel, Belgium) was added, and warming at 60 °C was continued for another 30 min. The thiazolidine derivatives were subjected to GC analysis to identify the sugars. Column temperature 200 °C; injection temperature 280 °C; carrier gas He at flow rate of 50 mL/min; D-xylose, L-arabinose, and D-glucose: 16.55, 17.03, and 34.18 min, respectively.

Acid Hydrolysis of Compounds. A solution of each saponin (4−6 mg each) in 10% HCl−dioxane (1:1, 2 mL) was heated at 80 °C for 4 h. The reaction mixture was neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$. After concentration, the H_2O layer was examined by TLC with $CHCl_3$−MeOH−H_2O (6:4:1) and compared with authentic samples. Glucose, arabinose, and xylose: R_f 0.24, 0.34, and 0.37, respectively. The $CHCl_3$ extract of **1**−**3** afforded erythrodiol (**7**) as aglycon after purification by preparative Si gel TLC ($CHCl_3$−MeOH, 9:1). Also, maniladiol (**8**) was obtained as aglycon from the $CHCl_3$ extract of **5** and **6**. Erythrodiol (**7**)[11,12] and maniladiol (**8**)[14] were identified by comparison of physical data ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, EIMS) with reported values.

Enzymatic Hydrolysis of 4. Compound **4** (3 mg) was incubated with β-glucosidase (1 mg) in 5% EtOH-aqueous solution at 37 °C for 72 h. Extraction of the reaction mixture with $CHCl_3$ followed by preparative Si gel TLC using $CHCl_3$−MeOH (9:1) afforded 7α-methoxyerthyrodiol (**9**, 0.6 mg).

7α-Methoxyerthyrodiol (9): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.33 (1H, d, J = 3.2 Hz, H-12), 3.83 (1H, dd, J = 8.3, 3.1 Hz, H-7), 3.63 (1H, brd, J = 11.1 Hz, H-28), 3.50 (1H, brd, J = 11.1 Hz, H-28), 3.32 (1H, dd, J = 11.0, 4.3 Hz, H-3), 3.23 (3H, s, OMe), 1.26 (3H, s, Me-27), 1.23 (3H, s, Me-23), 1.04 (3H, s, Me-24*), 0.97 (3H, s, Me-30*), 0.90 (6H, s, Me-25*, 26*), 0.84 (3H, s, Me-29*), 0.78 (1H, d, J = 12.8 Hz, H-5) (*assignments may be interchanged); EIMS m/z 472 $[M]^+$.

Bioassay Evaluation. Compounds **1**−**6** were evaluated for cytotoxicity against a panel of human cancer cell lines according to established protocols.[15,16]

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References and Notes

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(17) Although it was intended that the activity-guided fractionation of the weakly cytotoxic $CHCl_3$ extract of *N. maingayi* bark would lead to more potent purified isolates, this was not the case (Table 1), as has been our experience on numerous prior occasions with cytotoxic plants, including collaborative work with Drs. Wall and Wani (e.g., Long, L.; Lee, S. K.; Chai, H.-B.; Rasoanaivo, P.; Gao, Q.; Navarro, H.; Wall, M. E.; Wani, M. C.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **1997**, *53*, 15663−15670). The reasons for the lack of increase in potency of biological activity on concentration of the mass of plant extract are uncertain, but could include the lability of bioactive principles on chromatographic purification and/or the synergistic effect of individual cytotoxic constituents when tested in the form of a complex mixture.

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