Bidesmosidic Oleanane Saponins from *Xerospermum noronhianum*

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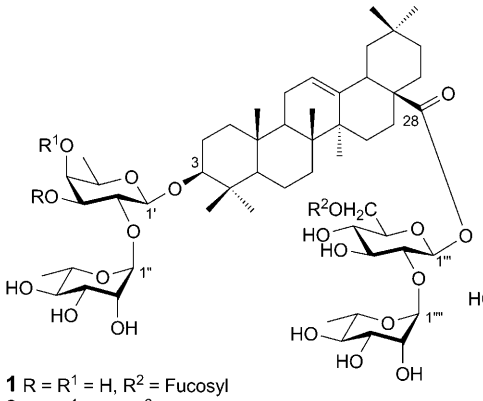
Three new oleanane-type triterpenoid saponins, 3-*O*-(α-L-rhamnopyranosyl(1→2)-β-D-fucopyranosyl)-28-*O*-{[α-L-rhamnopyranosyl(1→2)][β-D-fucopyranosyl(1→6)]-β-D-glucopyranosyl} oleanolic acid (**1**), 3-*O*-[α-L-rhamnopyranosyl(1→3)-β-D-fucopyranosyl]-28-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl] oleanolic acid (**2**), and 3-*O*-{α-L-rhamnopyranosyl(1→2)-[3′,4′-diacetoxy-β-D-fucopyranosyl]}-28-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl] oleanolic acid (**3**) have been isolated from the stems of *Xerospermum noronhianum*. The structures of the saponins were determined as a series of bidesmosidic oleanane saponins based on spectral evidence. The anticholinesterase activity of the saponins **1**–**3** was also evaluated.

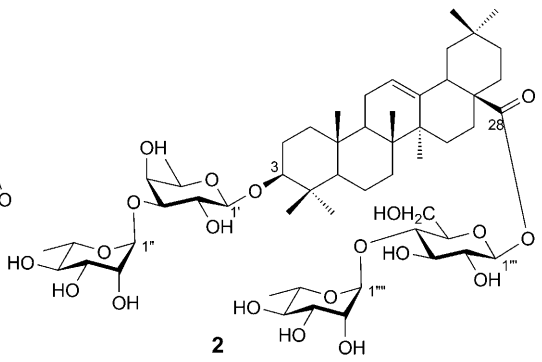
Introduction. – *Xerospermum noronhianum* BL. belongs to a small genus within the family Sapindaceae. It is a tree mainly found in south-eastern Asia and Malaysia, where it is known as *Rambutan pachat* [1]. The fruits of *X. noronhianum* are edible, with a sweet and pleasant taste, resembling those of *Nephellium lappaceum*, another Sapindaceae species which yields the tropical fruit *Rambutan* of commercial importance. While the fruit of *X. noronhianum* is too small to be of any commercial value, the species has some medicinal value. An infusion of the pulped stone of the fruit is prescribed as a drink to cure severe stomach pains, and a decoction of the leaves is used if the previous treatment fails [2]. Species of the Sapindaceae family are known to be a rich source of saponins [3–6] and have also been documented to yield other classes of compounds such as diterpenes [7][8], triterpenes [9][10], flavonoids [7][11–13], benzoic acid derivatives [14], and long-chain fatty acids and cyanolipids [15–17]. In a preliminary bioactivity screening, the crude MeOH extract of *X. noronhianum* showed anticholinesterase activity. Previously, we reported the isolation of flavonoid glycosides and simple aromatic compounds from the species [18]. Here, we further report the isolation and structural elucidation of three bidesmosidic oleanane saponins. The anticholinesterase activity of the saponins was also evaluated using a simple bioautographic method.

Results and Discussion. – The AcOEt-soluble fraction of the 10% aqueous MeOH extract of the stems of *X. noronhianum* was fractionated on a *RP-18* Solid Phase Extraction (SPE) cartridge, to yield two sub-fractions. The MeOH/H_2O 1:1 fraction

contains complex polymeric constituents and tannins, while the 100% MeOH fraction contains the crude saponins. Further isolation and purification of the saponins from the latter fraction was carried out by semi-preparative reverse phase HPLC, yielding three pure saponins, **1**–**3**. The structures were established on the basis of acid hydrolysis, NMR (*Table*), and MS data.



1 R = R^1 = H, R^2 = Fucosyl
3 R = R^1 = Ac, R^2 = H



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Compound **1** was obtained as white needles (118.5 mg), determined to have the elemental composition $C_{60}H_{98}O_{24}$ by ^{13}C-NMR analysis and FT-ICR-MS which gave a *pseudo*-molecular ion peak at *m*/*z* 1225.6415 ($[M+Na]^+$; calc. 1225.6346, $C_{60}H_{98}NaO_{24}^+$). Further, the APCI-MS (positive-ion mode) spectrum gave a *quasi*-molecular ion peak at *m*/*z* 1220 ($[M+NH_4]^+$), while the negative-ion mode spectrum exhibited a molecular ion peak at *m*/*z* 1201 ($[M-H]^-$). The negative-ion mode APCI-MS also exhibited prominent fragment ions at *m*/*z* 1055 ($[M-H-146]^-$, loss of a deoxyhexose), 909 ($[M-H-(146\times 2)]^-$, loss of two deoxyhexose units), 747 ($[M-(146\times 2)-162]^-$, loss of a hexose and two deoxyhexose units), 601 ($[M-H-(146\times 3)-162]^-$, loss of a hexose and three deoxyhexose units), and 455 ($[M-H-(146\times 4)-162]^-$, loss of a hexose and four deoxyhexose units), respectively. A fragment ion at *m*/*z* 439 in the positive-ion mode APCI-MS spectrum suggested that the aglycone moiety was oleanolic acid [19], which was confirmed by acid hydrolysis of the saponin yielding oleanolic acid and a mixture of sugars. The ^{13}C-NMR spectrum showed 60 signals, of which 30 were assigned to the triterpenoidal moiety and another 30 to the saccharide portion (*Table*). As predicted from the APCI-MS analysis, the saccharide portion of saponin **1** consisted of five residues. The ^{1}H-NMR spectrum exhibited five anomeric H-atoms at δ(H) 5.39 (*d*, $J=7.7$), 5.38 (*d*, $J=1.4$), 5.34 (*d*, $J=2.1$), 4.31 (*dd*, $J=4.5$, 2.8), and 4.37 (*d*, $J=7.7$) which correlated to five anomeric C-atoms at δ(C) 95.2, 101.6, 102.0, 104.8, and 105.9, respectively, indicating that **1** is an oleanolic pentasaccharide (*Table*). Meanwhile, Me *doublet*s at δ(H) 1.21 ($J=6.3$), 1.24 ($J=6.3$), 1.25 ($J=5.6$), and 1.26 ($J=6.3$) in the ^{1}H-NMR spectrum supported the presence of four 6-deoxyhexose sugar units. The sequential assignments of H- and C-atom resonances of each monosaccharide was carried out by analysis of the 2D-NMR data from COSY, TOCSY, ROESY, HSQC, and HMBC experiments, as well as their

multiplet pattern and coupling constants in the ^{1}H-NMR spectrum. The sugars were revealed to consist of two α-L-rhamnopyranosyl (Rha I and Rha II), a β-D-glucopyranosyl (Glc), and two β-D-fucopyranosyl (Fuc I and Fuc II) moieties. Analysis by HPLC of the component sugars obtained from acid hydrolysis confirmed the identity of the monosaccharides to be α-L-rhamnose, β-D-glucose and β-D-fucose. In the ^{13}C-NMR spectrum, glycosidation shifts were observed for the δ values of the signals due to C(3) (90.3) and C(28) (178.0) in the oleanolic moiety, establishing **1** as a 3,28-bidesmoside [20].

The structure of the sugar chain at C(3) was unambiguously confirmed by HMBC correlations (*Fig.*). Clear ^3J correlation was observed between C(3) (δ(C) 90.3) on the aglycone to the Fuc I H–C(1) (δ(H) 4.37). The ROESY spectrum also further supported this linkage by exhibiting cross-peaks between H–C(3) (δ(H) 3.12) of the aglycone and Fuc I H–C(1) (δ(H) 4.37). Meanwhile, Rha I H–C(1) (δ(H) 5.34) was a

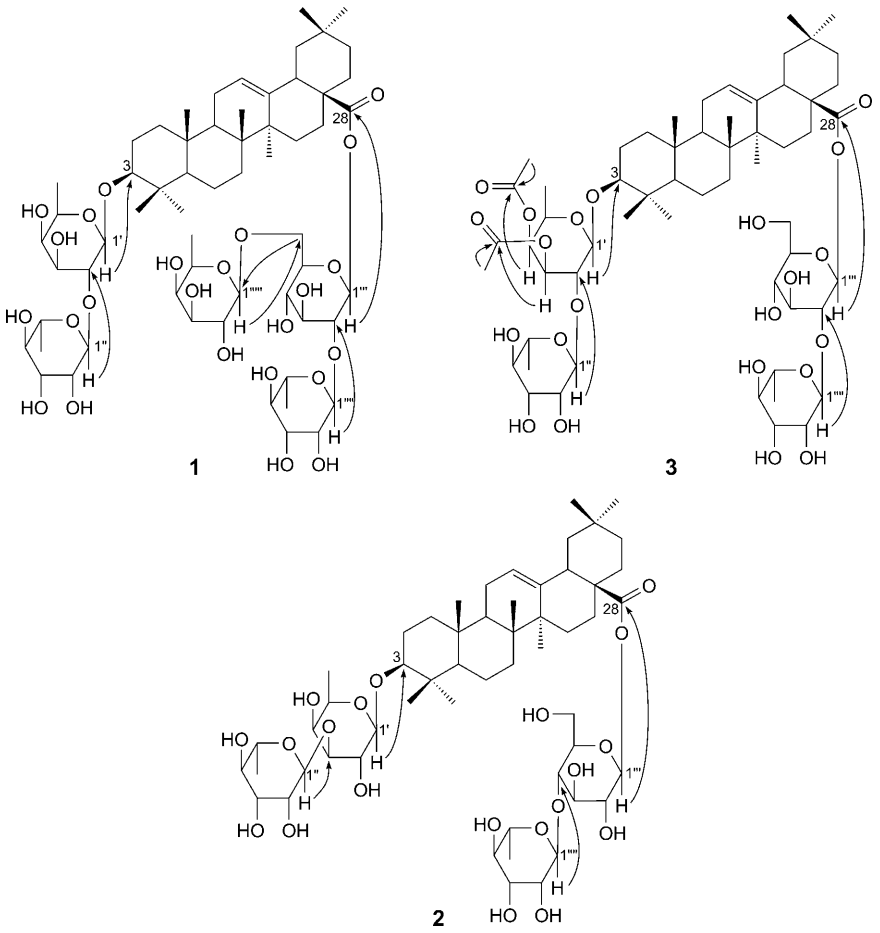

Figure. *Selected HMBCs for Saponins* **1**–**3**

Table. *^{1}H- and ^{13}C-NMR Data of Saponins* **1**–**3** (CD_3OD 700 MHz)[a])

	1		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
Aglycone						
CH_2(1)	40.2	0.97–0.98 (*m*, H_a), 1.62–1.63 (*m*, H_b)	40.2	0.98, (*dd*, *J* = 13.0, 3.5, H_a), 1.63–1.65 (*m*, H_b)	40.2	0.95–0.97 (*m*, H_a), 1.59–1.61 (*m*, H_b)
CH_2(2)	27.3	1.68–1.69 (*m*, H_a), 1.91–1.92 (*m*, H_b)	27.3	1.65–1.68 (*m*, H_a), 1.89–1.91[b]) (*m*, H_b)	27.3	1.73–1.75[b]) (*m*, H_a), 1.90–1.95 (*m*, H_b)
H–C(3)	90.3	3.12 (*dd*, *J* = 11, 3.5)	90.3	3.12 (*dd*, *J* = 12, 4.2)	90.7	3.19 (*dd*, *J* = 11.2, 4.2)
C(4)	40.3	–	40.3	–	40.3	–
H–C(5)	57.5	0.76 (*d*, *J* = 11)	57.5	0.77 (*d*, *J* = 12)	57.4	0.78 (*d*, *J* = 11.2)
CH_2(6)	19.4	1.21–1.23 (*m*, H_a), 1.44–1.46 (*m*, H_b)	19.4	1.39–1.40 (*m*, H_a), 1.56–1.58 (*m*, H_b)	19.4	1.41–1.42 (*m*, H_a), 1.54–1.56[b]) (*m*, H_b)
CH_2(7)	34.1	1.43–1.44 (*m*, H_a), 1.48–1.50 (*m*, H_b)	34.1	1.43–1.44 (*m*, H_a), 1.47 (*dd*, *J* = 13, 3.5, H_b)	34.1	1.44–1.45 (*m*, H_a), 1.48–1.49 (*m*, H_b)
C(8)	40.8	–	40.8	–	40.8	–
H–C(9)	49.6	1.53–1.56 (*m*)	49.6	1.57–1.58 (*m*)	48.1	1.54–1.56[b]) (*m*)
C(10)	37.9	–	37.9	–	37.9	–
CH_2(11)	24.6	1.91–1.92 (*m*)	24.6	1.89–1.91 (*m*)[b])	24.6	1.93–1.95 (*m*)
H–C(12)	123.8	5.27 (br. *t*, *J* = 3.5)	123.8	5.27 (br. *t*, *J* = 3.5)	123.7	5.27 (br. *t*, *J* = 2.8)
C(13)	144.8	–	144.9	–	144.9	–
C(14)	43.1	–	43.1	–	43.1	–
CH_2(15)	29.3	1.23–1.24 (*m*)	29.3	1.19–1.20 (*m*)[b])	29.3	1.19–1.20 (*m*)
CH_2(16)	24.1	1.65–1.68 (*m*, H_a), 2.08 (*dt*, *J* = 9.8, 6.3, H_b)	24.1	1.62–1.63 (*m*, H_a), 2.07 (*dd*, *J* = 14, 10.5, H_b)	24.1	1.67–1.68 (*m*, H_a), 2.06–2.08 (*m*, H_b)
C(17)	48.1	–	48.1	–	49.6	–
H–C(18)	42.9	2.54 (*dd*, *J* = 14, 4.2)	42.9	2.82 (*dd*, *J* = 14, 4.2)	42.9	2.82 (*dd*, *J* = 14, 4.2)
CH_2(19)	47.4	1.13–1.14 (*m*, H_a), 1.75–1.77 (*m*, H_b)	47.4	1.14–1.16 (*m*, H_a), 1.73 (*t*, *J* = 14, H_b)	47.4	1.14–1.15 (*m*, H_a), 1.73–1.75[b]) (*m*, H_b)
C(20)	31.6	–	31.6	–	31.6	–
CH_2(21)	34.9	1.22–1.23 (*m*, H_a), 1.41–1.44 (*m*, H_b)	34.9	1.19–1.20 (*m*, H_a)[b]), 1.40–1.41 (*m*, H_b)	34.9	1.25–1.26 (*m*, H_a), 1.41–1.44 (*m*, H_b)
CH_2(22)	33.1	1.57–1.60 (*m*, H_a), 1.73–1.76 (*m*, H_b)	33.0	1.51–1.54 (*m*, H_a), 1.80 (*dt*, *J* = 14, 4.2, H_b)	33.0	1.58–1.60 (*m*, H_a), 1.80 (*dt*, *J* = 15, 4.2, H_b)
Me(23)	28.6	1.05 (*s*)	28.6	1.05 (*s*)	28.5	1.06 (*s*)
Me(24)	17.3	0.85 (*s*)	17.3	0.85 (*s*)	17.1	0.87 (*s*)
Me(25)	16.3	0.96 (*s*)	16.2	0.96 (*s*)	16.2	0.97 (*s*)
Me(26)	17.9	0.80 (*s*)	17.8	0.81 (*s*)	17.8	0.81 (*s*)
Me(27)	26.2	1.16 (*s*)	26.2	1.16 (*s*)	26.2	1.16 (*s*)
C(28)	178.0	–	178.0	–	178.0	–
Me(29)	33.5	0.91 (*s*)	33.6	0.91 (*s*)	33.6	0.91 (*s*)
Me(30)	24.2	0.93 (*s*)	24.1	0.93 (*s*)	24.1	0.93 (*s*)
3-*O*-Sugar						
Fuc I						
H–C(1)	105.9	4.37 (*d*, *J* = 7.7)	105.9	4.34 (*d*, *J* = 7.7)	105.6	4.55 (*d*, *J* = 7.7)
H–C(2)	76.9	3.63 (*dd*, *J* = 9.1, 7.9)	76.6	3.63 (*dd*, *J* = 9.1, 7.7)	74.9	3.72 (*dd*, *J* = 9.8, 7.7)
H–C(3)	76.5	3.59–3.60 (*m*)	77.3	3.57 (*dd*, *J* = 9.1, 5.6)	75.5	4.98 (*dd*, *J* = 9.8, 3.5)
H–C(4)	73.9	3.53 (*dd*, *J* = 3.5, 1.0)	79.2	3.55 (*d*, *J* = 8.4)	72.5	5.17 (*dd*, *J* = 3.5, 1.4)
H–C(5)	71.5	3.59–3.60 (*m*)	71.4	3.39 (*dd*, *J* = 12, 7)	69.7	3.88 (*dd*, *J* = 6.4, 4.2)
Me(6)	18.0	1.26 (*d*, *J* = 6.3)	18.3	1.24 (*d*, *J* = 6.3)	16.5	1.14 (*d*, *J* = 6.3)
3-AcO		–		–	171.7, 20.9	2.03 (*s*)
4-AcO		–		–	172.5, 20.6	2.15 (*s*)

Table (cont.)

	1		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
Rha I						
H–C(1)	102.0	5.34 (*d*, *J* = 2.1)	101.7	5.37 (*d*, *J* = 0.7)	102.5	4.98 (br. *s*)
H–C(2)	72.0	3.95 (*dd*, *J* = 3.5, 2.1)	71.9	3.94 (*dd*, *J* = 3.5, 1.4)	71.7	3.79 (*dd*, *J* = 4.2, 1.4)
H–C(3)	72.1	3.73–3.77 (*m*)	72.4	3.66 (*dd*, *J* = 6.3, 3.5)	72.1	3.60 (*dd*, *J* = 4.9, 3.5)
H–C(4)	74.0	3.38 (*dd*, *J* = 9.1, 2.1)	73.7	3.53 (*d*, *J* = 2.8)	73.8	3.37 (*dd*, *J* = 9.8, 5.6)
H–C(5)	70.0	3.98 (*m*)	70.3	3.75 (*dd*, *J* = 9.1, 2.8)	70.6	3.86 (*d*, *J* = 5.6)
Me(6)	18.3	1.21 (*d*, *J* = 6.3)	16.8	1.25 (*d*, *J* = 6.3)	18.1	1.22 (*d*, *J* = 6.3)
28-*O*-Sugar						
Glc						
H–C(1)	95.2	5.39 (*d*, *J* = 7.7)	95.2	5.44 (*d*, *J* = 7.7)	95.2	5.44 (*d*, *J* = 7.7)
H–C(2)	77.0	3.59–3.60 (*m*)	71.6	3.60 (*dd*, *J* = 7, 1.4)	77.3	3.58 (*d*, *J* = 7.7)
H–C(3)	79.1	3.55 (*dd*, *J* = 8.4, 8.4)	74.1	3.58–3.59 (*m*)	71.4	3.40–3.41 (*m*)
H–C(4)	71.4	3.44 (*dd*, *J* = 8.4, 9.8)	76.9	3.39 (*dd*, *J* = 12, 7)	79.2	3.55–3.56 (*m*)
H–C(5)	77.9	3.49, (*ddd*, *J* = 9.8, 4.9, 2.1)	78.5	3.32–3.34 (*m*)	78.5	3.34–3.35 (*m*)
CH_2(6)	69.3	3.73 (*dd*, *J* = 12.6, 4.9), 4.04 (*dd*, *J* = 12.6, 2.1)	62.5	3.68 (*dd*, *J* = 4.9, 3.5), 3.79 (*dd*, *J* = 12, 2.8)	62.5	3.67 (*dd*, *J* = 7, 2.8), 3.79 (*dd*, *J* = 4.2, 2.1)
Rha II						
H–C(1)	101.6	5.38 (*d*, *J* = 1.4)	102.0	5.34 (*d*, *J* = 0.7)	101.7	5.37 (*d*, *J* = 1.4)
H–C(2)	72.1	3.93 (*dd*, *J* = 3.5, 1.4)	72.1	3.95 (*dd*, *J* = 3.5, 2.1)	71.9	3.94 (*dd*, *J* = 3.5, 2.1)
H–C(3)	72.3	3.66 (*dd*, *J* = 9.1, 3.5)	72.2	3.75 (*dd*, *J* = 9.1, 2.8)	72.2	3.66 (*dd*, *J* = 2.1, 1.4)
H–C(4)	73.8	3.38 (*dd*, *J* = 9.1, 2.1)	73.9	3.37 (*dd*, *J* = 9.8, 0.7)	73.8	3.39 (*dd*, *J* = 8.4, 1.4)
H–C(5)	70.3	3.75–3.77 (*m*)	69.9	3.98 (*dd*, *J* = 9.8, 6.3)	70.3	3.75 (*dd*, *J* = 6.3, 3.5)
Me(6)	16.8	1.25 (*d*, *J* = 5.6)	18.0	1.21 (*d*, *J* = 6.3)	18.3	1.25 (*d*, *J* = 6.3)
Fuc II						
H–C(1)	104.8	4.31 (*dd*, *J* = 4.5, 2.8)	–	–	–	–
H–C(2)	75.2	3.47 (*m*)[b])	–	–	–	–
H–C(3)	71.9[c])	3.59–3.60 (*m*)	–	–	–	–
H–C(4)	72.5	3.47 (*m*)[b])	–	–	–	–
H–C(5)	73.1[c])	3.59–3.60 (*m*)	–	–	–	–
Me(6)	18.0	1.24 (*d*, *J* = 6.3)	–	–	–	–

[a]) *J* values are in Hz; chemical shifts are given in ppm; assignments were confirmed by COSY, TOCSY, HSQC and HMBC experiments. [b]) Overlapped signals. [c]) Interchangeable signals.

terminal sugar unit, being glycosidically linked to Fuc I C(2) (δ(C) 76.9) based on the ^3J correlation observed between the two nuclei, and the reverse correlation observed between Fuc I H–C(2) (δ(H) 3.63) and Rha I C(1) (δ(C) 102.0) in the HMBC spectrum.

Similarly, intense ^3J correlation was observed between C(28) of the aglycone with Glc H–C(1) (δ(H) 5.39). The anomeric H-atoms of the other rhamnopyranosyl and fucopyranosyl moieties, Rha II (δ(H) 5.38) and Fuc II (δ(H) 4.31), both showed ^3J correlations to the only glucopyranosyl moiety; Rha II H–C(1) with Glc C(2) (δ(C) 77.0) and Fuc II H–C(1) with Glc C(6) (δ(C) 69.3). The Fuc II anomeric H-atom appeared as a *dd*, believed to be due to long range correlations with the other Fuc II H-atoms, possibly Fuc II H–C(5) or Fuc II H–C(3). The glycosidic linkage of Fuc II H–C(1) to Glc C(6) was further supported by the reverse correlations observed

between Fuc II C(1) (δ(C) 104.8) and the O-bearing CH_2 group, Glc H_a–C(6) (δ(H) 4.04, *dd*, $J = 12.6$, 2.1) and Glc H_b–C(6) (δ(H) 3.73, *dd*, $J = 12.6$, 4.9). Similarly, the glycosidic linkage of Rha II H–C(1) to Glc C(2) was supported by the reverse correlations observed between Glc H–C(2) (δ(H) 3.59–3.60) and Rha II C(1) (δ(C) 101.6). On the basis of the above results, **1** was elucidated as 3-*O*-(α-L-rhamnopyranosyl(1 → 2)-β-D-fucopyranosyl)-28-*O*-{[α-L-rhamnopyranosyl(1 → 2)][β-D-fucopyranosyl(1 → 6)]-β-D-glucopyranosyl} oleanolic acid.

The FT-ICR-MS spectra of **2** gave a *pseudo*-molecular ion peak at *m*/*z* 1079.5761 ($[M + Na]^+$; calc. 1079.5767), in agreement with the molecular formula $C_{54}H_{88}NaO_{20}^+$ for a saponin with one 6-deoxyhexose unit less than saponin **1**. The APCI-MS (positive-ion mode) of **2** gave a *quasi*-molecular ion peak at *m*/*z* 1074 ($[M + NH_4]^+$), while the negative-ion mode exhibited a molecular ion peak at *m*/*z* 1055 ($[M - H]^-$). The negative-ion mode APCI-MS further exhibited prominent fragment ions at *m*/*z* 909 ($[M - H - 146]^-$), 747 ($[M - H - 146 - 162]^-$), 601 ($[M - H - (2 \times 146) - 162]^-$), and 455 ($[M - H - (3 \times 146) - 162]^-$), corresponding to the losses of a deoxyhexose, a hexose and a deoxyhexose, a hexose and two deoxyhexose units, and a hexose and three deoxyhexose units, respectively. The NMR spectral features were very similar to **1**, which confirmed that the aglycone was oleanolic acid, substituted at C(3) and C(28) (*Table*). As predicted from the APCI-MS analysis, **2** contained four sugar residues, which was also obvious from the ^{1}H- and ^{13}C-NMR spectra which exhibited four anomeric H-atoms at δ(H) 5.44 (*d*, $J = 7.7$), 5.37 (*d*, $J = 0.7$), 5.34 (*d*, $J = 0.7$), and 4.34 (*d*, $J = 7.7$), directly correlated to C-atoms at δ(C) 95.2, 101.7, 102.0, and 105.9, respectively. Sugar Me *doublet*s at δ(H) 1.24 ($J = 6.3$), 1.25 ($J = 6.3$), and 1.21 ($J = 6.3$) in the ^{1}H-NMR spectrum supported the presence of three 6-deoxyhexose sugar units. Analysis of 1D- and 2D-NMR data revealed the sugar moieties to be two terminal α-L-rhamnopyranoses (Rha I and Rha II), a 4-substituted β-D-glucopyranose (Glc), and a 3-substituted β-D-fucopyranose (Fuc). The sugar chain structures at C(3) and C(28) as well as the glycosidic linkages at each terminal were again deduced from HMBC and ROESY correlations. Cross-peaks were observed between C(3) (δ(C) 90.3) and Fuc H–C(1) (δ(H) 4.34), and between Rha I H–C(1) (δ(H) 5.37) and Fuc C(3) (δ(C) 77.3), as well as the reverse correlations between Fuc H–C(3) (δ(H) 3.57) and Rha I C(1) (δ(C) 101.7) (*Fig.*). On the carboxy terminus, correlations were seen between C(28) (δ(C) 178.0) with Glc H–C(1) (δ(H) 5.44), and between Rha II H–C(1) (δ(H) 5.34) with Glc C(4) (δ(C) 76.9), as well as the reverse correlation between Glc H–C(4) (δ(H) 3.58–3.59) with Rha II C(1) (δ(C) 102.0). The same conclusion was deduced from the ROESY cross-peaks seen across the glycosidic linkages. Thus **2** was characterized as 3-*O*-[α-L-rhamnopyranosyl(1 → 3)-β-D-fucopyranosyl]-28-*O*-[α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranosyl] oleanolic acid.

The molecular formula for **3** was determined to be $C_{58}H_{92}O_{22}$ based on FTI-CR-MS which exhibited a *pseudo*-molecular ion peak at *m*/*z* 1163.5973 ($[M + Na]^+$, calc. 1163.5978) in agreement with the molecular formula $C_{58}H_{92}NaO_{22}^+$. The positive-ion mode APCI-MS showed a *quasi*-molecular ion peak at *m*/*z* 1158 ($[M + NH_4]^+$) and a characteristic fragment ion at *m*/*z* 439, indicating the presence of the same aglycone moiety as **1** and **2** and representing a loss of one hexose, three deoxyhexose units, a H_2O molecule and two AcO groups ($[M + H - (3 \times 146) - 162 - (2 \times 42) - H_2O]^+$). This was also apparent from the negative-ion-mode APCI-MS, where the aglycone could

also be identified from the fragment ion at *m/z* 455 for oleanolic acid. In the negative-ion-mode APCI-MS, daughter ions were also observed at *m/z* 976 ($[M-146-H_2O]^-$), 831 ($[M-H-146-162]^-$), 789 ($[M-H-146-162-42]^-$), 747 ($[M-H-146-162-(2\times42)]^-$), 643 ($[M-H-(2\times146)-162-42]^-$), and 455 ($[M-(3\times146)-162-(2\times42)]^-$). From these fragment ions, it was concluded that **3** contained three deoxyhexose units and a hexose, as well as two AcO units. The ^{1}H-NMR spectrum of **3** was very similar to that of saponin **2**, except for the presence of AcO Me *singlet*s at δ(H) 2.03 and 2.15 (*Table*). As predicted from APCI-MS, the saccharide part of **3** also consisted of four residues, based on the anomeric H-atom resonances at δ(H) 5.44 (*d*, $J=7.7$), 5.37 (*d*, $J=1.4$), 4.98 (br. *s*), and 4.55 (*d*, $J=7.7$). Apart from the AcO H-atoms, sugar Me *doublet*s were observable at δ(H) 1.14 ($J=6.3$), 1.22 ($J=6.3$), and 1.25 ($J=6.3$), again consistent with the presence of three 6-deoxyhexose sugar units. Assignment of the four sugar spin systems was once again achieved by careful analysis of the 1D- and 2D-NMR data, which identified the sugars to be two terminal α-L-rhamnopyranoses (Rha I and Rha II), a 2-substituted β-D-glucopyranose (Glc), and 2-substituted diacetoxy β-D-fucopyranose. The two AcO groups were deduced to be on β-D-fucose on the basis of the deshielded δ values of two of its H-atoms, Fuc H–C(3) (δ(H) 4.98) and Fuc H–C(4) (δ(H) 5.17). Each of these two H-atoms was also shown to correlate to the carbonyl C-atoms at δ(C) 171.7 and 172.5, respectively (*Fig.*). The diacetoxy β-D-fucopyranose was shown to be linked to C(3) of the aglycone by the ^3J correlations from Fuc H–C(1) (δ(H) 4.55) to C(3) (δ(C) 90.7) and the reverse correlations from H–C(3) (δ(H) 3.19) to Fuc C(1) (δ(C) 105.6). Similarly, ^3J correlations between Glc H–C(1) (δ(H) 5.44) with C(28) of the aglycone provided the C(28) link to the other sugar part of the bidesmoside. The glycosidic linkages between the sugars were again obtained from HMBC correlations. Cross-peaks were observed between Rha I H–C(1) (δ(H) 4.98) with Fuc C(2) (δ(C) 74.9), while Rha II H–C(1) (δ(H) 5.37) was correlated to Glc C(2) (δ(C) 77.3). ROESY Interactions across the glycosidic linkages further supported the assignments. Based on all these informations, the structure of **3** was thus characterized as 3-*O*-{α-L-rhamnopyranosyl(1 → 2)-[3′,4′-diacetoxy-β-D-fucopyranosyl]}-28-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranosyl] oleanolic acid.

The three saponins were evaluated for their anticholinesterase activity against the two enzymes acetylcholinesterase and butyrylcholinesterase. Evaluation of anticholinesterase activity was performed using a simple thin layer chromatography (TLC) bioautographic method [21], where the bioactivity was estimated from the calculated pMIQ values [22]. The pMIQ value is the negative logarithm of the minimum inhibitory quantity (in mol) that produced a spot with the least observable whiteness. In comparison to the positive controls (tacrine and galanthamine), the purified triterpenes and the bidesmosides exhibited only weak activity against both cholinesterases, giving pMIQ values of less than 10.5. Tacrine and galanthamine gave pMIQ values of 11.37 and 11.57, respectively.

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* and silica gel (SiO_2; 70–230 mesh and 230–400 mesh, *Merck*). TLC: pre-coated SiO_2 aluminium plates (*Merck 60 F-254*); the spots were

detected by spraying with 5% H_2SO_4 and heating to 100°. Semi-prep. HPLC separations: reverse phase column (*Inertsil ODS-3* column, 5 μm, 250 × 7.6 mm i.d.) on a *Shimadzu LC-8A* series pumping system equipped with a UV/VIS detector *SPC-20A*, detection wavelength set to 210 nm. HPLC analysis of the acid hydrolysate was carried out using an NH_2 column (*Alltech Econosphere NH_2*, 5 μm, 150 × 4.6 mm i.d.), on a *Jasco* HPLC apparatus equipped with a refractive index detector *RI-1530*. Optical rotation: *Jasco* (Tokyo, Japan) *DIP-370 Digital Polarimeter* equipped with a sodium lamp (589 nm). ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance II AV-700*, equipped with a cryoplatform and a 5 mm TCI cryoprobe controlled by Topspin ver. 2.0. All NMR experiments were carried in CD_3OD as solvent out, using standard *Bruker* pulse programs. APCI-MS Spectra: *LCQ Deca ThermoFinnigan* Spectrometer equipped with Xcalibur software. HR-MS: FT-ICR Mass spectrometer (*Bruker Apex-Qe*, equipped with *Apollo-II ESI* ion source, ApexControl ver. 2.0.0, capillary voltage 4.5 kV, spray shield voltage 4.0 kV, dry gas temp. 150°, dry gas flow 5.0 l/min).

Plant Material. Xerospermum noronhianum was collected from the Universiti Putra Malaysia Forest Reserve in Ayer Hitam, Selangor, Malaysia. The plant was identified by Mr. *Shamsul Khamis* and a voucher specimen (SK1013/05) has been deposited with the herbarium of Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia.

Extraction and Isolation. The dried powdered stems (1.7 kg) of *X. noronhianum* were defatted with petroleum ether (PE) 30–40°, and macerated at r.t. for 3 d with MeOH/H_2O 9:1 (10 l). The extraction procedure was repeated three times, each time with fresh solvent. The collected MeOH extracts were pooled, evaporated under reduced pressure, and lyophilized, yielding 141 g of crude extract which was further fractionated by liquid-liquid partitioning into hexane (5 g), AcOEt (24 g), and aq. (29 g) fractions. The AcOEt fraction was fractionated (in 1-g batches) into two sub-fractions using a commercial *RP-18* Solid Phase Extraction (SPE) cartridge (*IST ISOLUTE Flash C18*, 5 g sorbent mass, 25-ml reservoir volume). The cartridge was first preconditioned with 10 ml H_2O followed by 10 ml of MeOH/H_2O 1:1. The loaded cartridge was eluted first with 15 ml of MeOH/H_2O 1:1, followed by 35 ml of 100% MeOH. LC/MS Analysis of the collected eluates showed that the MeOH/H_2O 1:1 eluate contained largely polymeric constituents and tannins, while the 100% MeOH eluate contained saponins (7.3 g). About 1 g of this crude saponin fraction, dissolved in 2 ml of MeCN, was subjected to further purification by semi-prep. reverse phase HPLC, injected in 10-mg portions (20-μl injection volume) eluted in a gradient manner using a flow rate of 3 ml/min, with first H_2O (0–10 min), then MeCN/H_2O 40:60 (10–20 min), MeCN/H_2O 50:50 (20–30 min), and finally 100% MeCN. Three saponins were successfully isolated: **1** (39.0 mg, t_R 14.24 min), **2** (9.3 mg, t_R 14.82 min), and **3** (8.8 mg, t_R 23.19 min).

Acid Hydrolysis of **1**–**3**. A soln. of each saponin (5 mg) in 5% H_2SO_4 (1 ml) was stirred at 80° in a stoppered reaction vial for 8 h. After cooling, the reaction product was passed through an *SPE RP-18* 500 mg cartridge (*Silicycle*), eluted with 20 ml of dist. H_2O, followed by 20 ml of MeOH. The aglycone was collected in the MeOH fraction and co-TLC against a standard sample of oleanolic acid (*Sigma*) confirmed the identity of the aglycone. The H_2O fraction, containing the mixed sugars, was analyzed by HPLC on an NH_2 column (*Alltech Econosphere NH_2*, 5 μm, 150 × 4.6 mm i.d.), isocratically eluted with MeCN/H_2O 8:2 at a flow rate of 1 ml/min. In the hydrolysate, the individual monosaccharides were detected by comparison with the retention times of authentic samples (*Sigma-Aldrich*) of D-glucose (t_R 6.97 min), D-fucose (t_R 4.25 min), and L-rhamnose (t_R 3.77 min). After purification by semi-prep. HPLC using the same column and eluent conditions, the optical rotation of each purified sugar was measured on a polarimeter.

TLC Bioautographic Assay for Anticholinesterase Activity. The TLC bioautographic assay was carried out using the method described in [20]. Stock solns. of the compounds were prepared in MeOH at a concentration of 1 mg/ml, and from this stock soln. 1 μl was applied on the TLC plate for the bioautographic assay. The inhibitory activity of the compounds was gauged from the detection limit or the pMIQ values, representing the negative logarithm of the minimal inhibitory amount of the test sample (in mol), that produced an inhibition spot with the least observable whiteness. To measure these pMIQ values, serial 2-fold dilutions of each test compound were bioautographically assayed. The cut-off limit for bioactivity was set at a pMIQ of 10.5. Compounds having pMIQ values below this are considered as inactive or not sufficiently active to be of interest for further evaluation as a lead compound [22].

*6-Deoxy-β-*D*-galactopyranosyl-(1 → 6)-[6-deoxy-α-*L*-mannopyranosyl-(1 → 2)]-1-*O*-[(3β)-3-[[6-deoxy-2-*O*-(6-deoxy-α-*L*-mannopyranosyl)-β-*D*-galactopyranosyl]oxy}-28-oxoolean-12-en-28-yl]-β-*D*-glucopyranose* (**1**). White solid from MeOH. $[\alpha]_D^{23} = -40$ ($c = 1.0$, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD, 700 MHz): *Table*. APCI-MS (pos.): 1220 ($[M + NH_4]^+$), 439 ($[M + H - (4 \times 146) - 162 - H_2O]^+$). APCI-MS (neg.): 1201 ($[M - H]^-$), 1055 ($[M - H - 146]^-$), 909 ($[M - H - (2 \times 146)]^-$), 747 ($[M - H - (2 \times 146) - 162]^-$), 601 ($[M - H - (3 \times 146) - 162]^-$), 455 ($[M - H - (4 \times 146) - 162]^-$ or [aglycone – H]$^-$). FT-ICR-MS (pos.): 1225.6415 ($[M + Na]^+$, $C_{60}H_{98}NaO_{24}^+$; calc. 1225.6346).

*1-O-[(3β)-3-{[6-Deoxy-3-O-(6-deoxy-α-*L*-mannopyranosyl)-β-*D*-galactopyranosyl]oxy}-28-oxoolean-12-en-28-yl]-4-O-(6-deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranose* (**2**). White solid from MeOH. $[\alpha]_D^{23} = +23.9$ ($c = 0.46$, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD, 700 MHz): *Table*. APCI-MS (pos.): 1074 ($[M + NH_4]^+$), 439 ($[M - H_2O - (3 \times 146) - 161]^+$). APCI-MS (neg.): 1055 ($[M - H]^-$), 909 ($[M - H - 146]^-$), 747 ($[M - H - 146 - 162]^-$), 601 ($[M - H - (2 \times 146) - 162]^-$), 455 ($[M - H - (3 \times 146) - 162]^-$ or [aglycone – H]$^-$). FT-ICR-MS (pos.): 1079.5761 ($[M + Na]^+$, $C_{54}H_{88}NaO_{20}^+$; calc. 1079.5767).

*2-O-(6-Deoxy-α-*L*-mannopyranosyl)-1-O-[(3β)-3-{[3,4-di-O-acetyl-6-deoxy-2-O-(6-deoxy-α-*L*-mannopyranosyl)-β-*D*-galactopyranosyl]oxy}-28-oxoolean-12-en-28-yl]-β-*D*-glucopyranose* (**3**). White solid from MeOH. $[\alpha]_D^{23} = -11.7$ ($c = 0.51$, MeOH). ^{1}H- and ^{13}C-NMR (CD_3OD, 700 MHz): *Table*. APCI-MS (pos.): 1158 ($[M + NH_4]^+$), 439 ($[M + H - (3 \times 146) - 162 - (2 \times 42) - H_2O]^+$). APCI-MS (neg.): 1139 ($[M - H]^-$), 976 ($[M - 146 - H_2O]^-$, 831 ($[M - H - 146 - 162]^-$), 789 ($[M - H - 146 - 162 - 42]^-$), 747 ($[M - H - 146 - 162 - (2 \times 42)]^-$), 643 ($[M - H - (2 \times 146) - 162 - 42]^-$), 455 ($[M - (3 \times 146) - 162 - (2 \times 42)]^-$). FT-ICR-MS (pos.): 1163.5973 ($[M + Na]^+$, $C_{58}H_{92}NaO_{22}^+$; calc. 1163.5978).

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REFERENCES

[1] E. J. H. Corner, 'Wayside Trees of Malaya Volume 2', The Malayan Nature Society, Malaysia, 1988, p. 673.
[2] I. H. Burkill, 'A Dictionary of the Economic Products of the Malay Peninsular Volume 2 (I–Z)', Government of Malaysia and Singapore, 1966, p. 2313.
[3] C. Dizes, F. Gerald, C. Lavaud, R. Elias, R. Faure, G. Massiot, G. Balansard, *Phytochemistry* **1998**, *48*, 1229.
[4] C. Lavaud, L. Voutquenne, G. Massiot, L. Le Men-Olivier, B. C. Das, O Laprévote, L. Serani, C. Delaude, M. Becchi, *Phytochemistry* **1998**, *47*, 441.
[5] L. Voutquenne, C. Lavaud, G. Massiot, C. Delaude, *Phytochemistry* **1998**, *49*, 2081.
[6] L. Voutquenne, P. Guinot, O. Thoison, T. Sevenet, C. Lavoud, *Phytochemistry* **2003**, *64*, 781.
[7] J. P. David, I. D. dos Santos, J. M. David, *Fitoterapia* **2004**, *75*, 795.
[8] A. Ortega, P. E. García, J. Cárdenas, C. Mancera, S. Marquina, M. L. C. Garduño, E. Maldonado, *Tetrahedron* **2001**, *57*, 2981.
[9] E. L. Ghisalberti, P. R. Jefferies, M. A. Sefton, *Phytochemistry* **1973**, *12*, 1125.
[10] R. Savoir, B. Tursch, M. Cession, *Tetrahedron Lett.* **1967**, *8*, 2129.
[11] F. R. van Heerden, A. M. Viljoen, B. E. van Wyk, *Fitoterapia* **2000**, *71*, 602.
[12] K. Sachdev, D. K. Kulshreshtha, *Phytochemistry* **1983**, *22*, 1253.
[13] K. Sachdev, D. K. Kulshreshtha, *Phytochemistry* **1986**, *25*, 1967.
[14] Z. Ali, V. U. Ahmad, M. Zahid, R. B. Tareen, *Phytochemistry* **1998**, *48*, 1271.
[15] M. Nishizawa, K. Adachi, S. Sastrapradja, Y. Hayashi, *Phytochemistry* **1983**, *22*, 2853.
[16] D. S. Seigler, M. Cortes, J. M. Aguilera, *Biochem. Syst. Ecol*. **1987**, *15*, 71.
[17] L. Voutquenne, C. Lavaud, G. Massiot, T. Sevenet, H. A. Hadi, *Phytochemistry* **1999**, *50*, 63.
[18] P. J. Tan, I. Intan Safinar, A. Faridah, L. Nordin, S. Khozirah, *ACGC Chem. Res. Commun.* **2008**, *22*, 63

[19] J.-L. Wolfender, K. Hostettmann, F. Abe, T. Nagao, H. Okabe, T. Yamauchi, *J. Chromatogr., A* **1995**, *712*, 155.
[20] S. B. Mahato, A. P. Kundu, *Phytochemistry* **1994**, *37*, 1517.
[21] I. K. Rhee, M. van de Meent, K. Ingkaninan, R. Verpoorte, *J. Chromatogr., A* **2001**, *915*, 217.
[22] S. Di Giovanni, A. Borloz, A. Urbain, A. Marston, K. Hostettmann, P.-A. Carrupt, M. Reist, *Eur. J. Pharm. Sci.* **2008**, *33*, 109.

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