Full Papers

Limonoids Showing Selective Toxicity to DNA Repair-Deficient Yeast and Other Constituents of *Trichilia emetica*

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Bioactivity-directed fractionation of the MeCOEt extract of *Trichilia emetica* (Meliaceae) resulted in the isolation of the limonoids nymania 1 (**1**), drageana 4 (**3**), trichilin A (**4**), rohituka 3 (**5**), and Tr-B (**7**) and the novel *seco*-A protolimonoid **8**. Of these, nymania 1 and Tr-B showed selective inhibitory activity toward DNA repair-deficient yeast mutants. The isolation, structure elucidation, ^{13}C NMR spectral assignments, and biological activities of these compounds are reported.

As a part of our ongoing collaborative effort to identify novel naturally occurring anticancer agents,[1] we have screened a number of extracts derived from plants collected in Ethiopia employing a mechanism-based bioassay utilizing genetically engineered yeast strains.[2,3] Of those investigated, a MeCOEt extract of *Trichilia emetica* Vahl. (Meliaceae) was found to exhibit selective activity against the *rad52* yeast strain RS 322YK compared with the wild-type *RAD*$^+$ (RS 188N) strain. Bioassay-guided fractionation of this extract afforded the bioactive limonoids nymania 1 (**1**) and Tr-B (**7**). The known inactive limonoids drageana 4 (**3**), trichilin A (**4**), and rohituka 3 (**5**) were also isolated, together with a new *seco*-A-protolimonoid that was determined to have the structure **8**. In this paper, we report the isolation, structure elucidation, NMR spectral assignments, and biological activity of these limonoids. ^{13}C NMR spectral assignments of **2**−**3**, **5**, and **7** have been made for the first time, and those for **4** have been revised based on 2D NMR data. This also constitutes the first report of the activity of limonoids against DNA repair-deficient yeast.

T. emetica [syn. *T. somalensis* Choiv., *T. jubensis* Chiov., *T. roka* (Forssk. *nom*. nud.) Chiov, *Rochetia choensis* Del.], a plant native to Africa,[4] finds uses in indigenous medicine for the treatment of a variety of human disorders such as pneumonia and colds; it is also used as an emetic, diuretic, and purgative and to induce labor in pregnant women.[5] Previous studies on *T. emetica* have led to the isolation of a number of limonoids,[6−10] some of which have a wide range of biological activities including insect antifeedant and growth regulating properties[11,12] and antifungal, bactericidal and antiviral activities.[11]

Results and Discussion

The dried and powdered stem bark of *T. emetica* was sequentially extracted with cold MeCOEt and MeOH. Of these extracts, only the MeCOEt extract exhibited significant selective activity in our yeast bioassay.[1,3] A portion of this extract was fractionated by solvent−solvent partition with hexane and 80% aqueous MeOH, followed by dilution of the 80% aqueous MeOH fraction to 60% aqueous MeOH with water and extraction of the resulting aqueous phase with $CHCl_3$. The bioactivity was traced through 80% aqueous MeOH to the $CHCl_3$ fraction (see Table 1). Flash chromatography of the bioactive $CHCl_3$ fraction afforded 14 combined fractions, of which five were bioactive. These were further fractionated by normal and reversed-phase column chromatography, normal and reversed-phase preparative TLC, and reversed-phase HPLC to furnish the two bioactive compounds nymania 1 (**1**) and Tr-B (**7**) and several inactive compounds **3**−**5** and **8**, all of which were found to have ^1H NMR[6,10] and MS[13] data characteristic of limonoids. The bioactivities of the crude extract, the semipure fractions, and the two bioactive compounds are given in Table 1.

The less polar bioactive compound was identified as nymania 1 (**1**) by comparison of its ^1H and ^{13}C NMR data with those reported.[14] The MS of **1** determined under a variety of conditions failed to show the expected molecular ion peak. However, the peak with the highest

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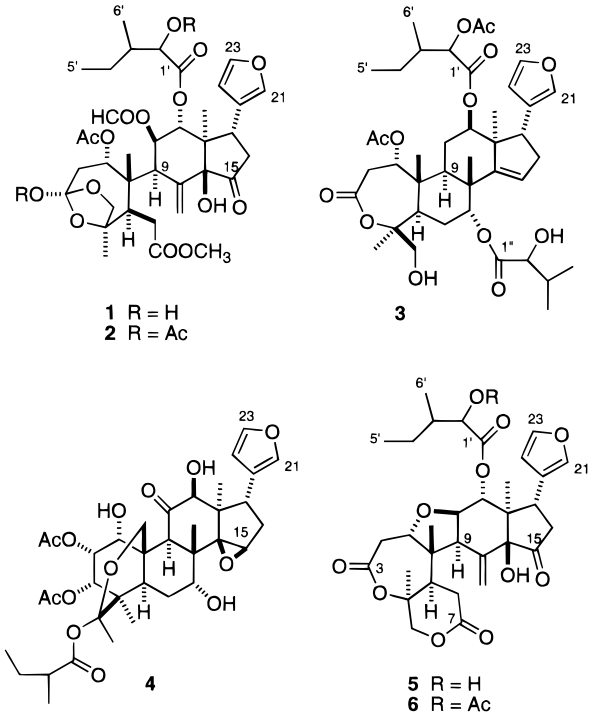
Table 1. Bioactivity Data of Crude Extract, Fractions, and Pure Compounds from *Trichilia emetica*

	DNA-damaging activity		
fractions/compds	RS 322 YK (*rad 52Y*)[a]	RS 188 N (*RAD*$^+$)[a]	cytotoxicity (IC_{50}, μg/mL)
crude extract	40	161	NT[d]
80% aq MeOH fraction	11	NT[d]	NT[d]
hexane fraction	>1000	NT[d]	NT[d]
$CHCl_3$ fraction	8.5	NT[d]	NT[d]
60% aq MeOH fraction	>1000	NT[d]	NT[d]
nymania 1 (**1**)	0.9 (0.7, 1.1)	100 (25, 177)	12.0[b]
Tr-B (**7**)	13 (16, 10)	>100	>20[c]
camptothecin[e]	0.6	110	0.054[b]

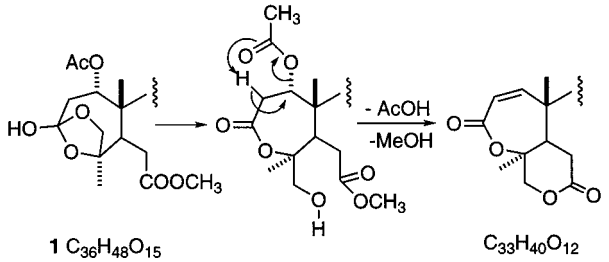
[a] Results are expressed as IC_{12} values (μg/mL). Data for active fractions were obtained twice, and both results together with the average value are reported. [b] Vero monkey cell line. [c] CHO-AUX-B1 cell line. [d] Not tested. [e] Standard reference compound.



m/*z* was found to be at *m*/*z* 628 due to the fragment ion **a** $[C_{33}H_{40}O_{12}]^+$ formed as a result of the loss of molecules of HOAc and MeOH from the molecular ion as depicted in Scheme 1. Spectroscopic data (MS, ^{1}H and ^{13}C NMR) for the diacetate **2** of nymania 1 were consistent with those reported for nymania 1 diacetate.[15]

Compound **3** had ^{1}H and ^{13}C NMR spectral characteristics consistent with those reported for drageana 4, previously isolated from *Trichilia drageana*.[16] Since the ^{13}C NMR spectrum for **3**[16] has not been assigned, we

Scheme 1. MS Fragmentation of **1**



assigned it with the aid of a ^{1}H−^{13}C HETCOR spectrum and by comparison with ^{13}C NMR data reported for related compounds. These assignments are given in Table 3.

The HRFABMS of limonoid **4** indicated the molecular formula $C_{35}H_{46}O_{13}$ for this compound. The ^{1}H NMR spectrum (Table 2) revealed the presence of a furan ring [δ 6.53 br s, 7.22 br s, 7.33 br s], two acetyl groups [δ 2.02 s, 2.12 s], three methyl singlets [δ 0.83, 1.14, 1.15], a methine of a hemiacetal [δ 5.74 s], a methylene bearing an oxygen and attached to a tertiary carbon [δ 4.33 dd (J = 12.8)], and an α-methyl butyrate moiety [δ 0.91 d (J = 7.0)], 1.18 d (J = 6.5), 3.00 m]; these signals are similar to those found in the trichilins.[6,9] Comparison of ^{1}H and ^{13}C NMR data of **4** with those reported for trichilin A[6] suggested that **4** was identical with trichilin A, but the presence of some discrepancies in the ^{13}C NMR data reported for trichilin A[6] led us to undertake a complete analysis of the ^{13}C NMR spectrum by application of DEPT, ^{1}H−^{13}C HETCOR, HMQC, and HMBC techniques. Our ^{13}C NMR spectral assignments for trichilin A (**4**) are listed in Table 3.

Limonoid **5**, $C_{32}H_{40}O_{11}$ (HREIMS), had some features similar to those of nymania 1 (**1**) in its ^{1}H and ^{13}C NMR spectra. A close examination revealed that the C,D rings in the two compounds were identically substituted except that the formate group at C-11 of nymania 1 was absent in **5**. A comparison of ^{1}H NMR data with those of limonoids having C,D rings similar to that of nymania 1 suggested that **5** was identical with rohituka 3, which has previously been isolated from *Aphanamixis polystacha* (Meliaceae) but which resisted purification and was therefore characterized as its monoacetate (**6**).[17] Complete assignments of the ^{1}H and ^{13}C NMR spectra of rohituka 3 have now been made by application of 2D NMR techniques and by comparison with our data for related compounds. These assignments are given in Tables 2 and 3, respectively.

The ^{1}H NMR spectrum of the bioactive limonoid **7** revealed some features similar to nymania 1 (**1**) and rohituka 3 (**5**). Comparison of ^{1}H NMR data for **7** with those of known prieurianin-type limonoids suggested that **7** was identical with the limonoid designated *Trichilia* substance Tr-B encountered previously in *Trichilia roka*.[8] The absence of ^{13}C NMR data for Tr-B in the literature prompted us to undertake a complete analysis of its ^{13}C NMR spectrum, and this was achieved by the application of DEPT and ^{1}H−^{13}C HETCOR techniques and by comparison with our data for nymania 1 (**1**) and rohituka 3 (**5**) (Table 3).

Compound **8**, $C_{33}H_{52}O_9$ (HRFABMS), had ^{1}H and ^{13}C NMR spectral features that differed significantly from

Table 2. ^{1}H NMR Spectral Data of the Limonoids **4**, **5**, and **7**

proton	**4**[a]	**5**[a]	**7**[a]
H−1	4.57 d (4.0)	3.19 dd (10.0, 6.6)	5.44 dd (6.8, 5.9)
H−2	5.90 t (4.57)	2.65 dd (10.0, 6.6)	2.05 dd (11.8, 9.0)
H−3	5.52 d (4.74)		
H−5	2.84 dd (14.3, 4.27)	2.70 m	3.18 dd (7.7, 3.8)
H−6a		2.85 dd (8.0, 3.8)	2.48 dd (9.0, 7.7)
b			2.63 dd (7.7, 3.8)
H−7	4.57 br s		
H−9	4.55 s	3.18 d (7.1)	3.78 d (7.4)
H−11		3.71 dd (9.9, 7.1)	5.46 dd (7.4, 10.3)
H−12	4.10 s	5.89 d (9.9)	6.16 d (10.3)
H−15	3.77 s		
H−16a	2.33 ddd (0.9, 13.9, 7.30)	2.86 dd (8.7, 9.0)	2.34 dd (8.5, 9.0)
b		2.38 dd (10.8)	2.82 dd (8.8, 9.0)
H−17	2.50 dd (13.9, 7.3)	3.85 t (8.6)	3.95 t (8.5)
H−18	1.14 s	0.82 s	0.97 s
H−19	4.33 dd (12.8)	1.13 s	1.62 s
H−21	7.22 s br	7.24 d (1.8)	7.20 s br
H−22	6.53 s br	6.21 d (2.7)	6.23 s br
H−23	7.33 s br	7.38 dd (2.7, 1.8)	7.38 t
H−28	0.83 s	1.86 s	1.81 s
H−29A	5.74 s	4.22 d (11.9)	4.22 dd (12.0)
B		4.02 d (11.9)	
H−30	1.15 s	5.39 s br; 5.38 s br	5.89 s br; 5.49 s br
H−2′	3.00 m	3.45 d (3.80)	3.30 d br.
H−3′		1.59 m	1.45 m
H−4′			1.15 m
Me−2′	1.18 d (6.5)		
Me−3′	0.91 d (7.0)	0.88 d (6.7)	0.82 d (6.5)
Me−4′		0.90 t (7.0)	0.84 t (7.6)
C*H*O			7.74 s
CH_3CO	2.12 s		1.99 s
CH_3CO	2.02 s		
CO_2CH_3			3.74 s

[a] Assignments based on DQCOSY and HETCOR.

those of other limonoids. In its ^{1}H NMR spectrum, **8** showed a total of eight methyl singlets due to five methyl groups on tertiary carbons (δ 0.87, 0.93, 1.07, 1.27 and 1.42), one vinylic methyl group (δ 1.79 br s), one acetyl group [δ 1.99 and 2.01 together integrating for 3H, probably due to a diastereoisomeric mixture], and one methoxyl (δ 3.81) group. The ^{1}H NMR spectrum also showed the presence of three olefinic protons (δ 4.85 s, 4.99 s, and 5.51 dd) and two methine protons attached to carbons bearing hydroxy functions (δ 3.83 d, and 3.86 dd). The ^{13}C NMR spectrum was found to be quite complex, having 44 signals instead of the expected 33; it was subsequently interpreted as being due to an inseparable mixture of two diastereoisomers in solution. In particular, a pair of singlets at δ 97.6 and 96.0 suggested the presence of a hemiketal as in spicatin[18] and recently encountered *seco*-A ring protolimonoids (e.g., **9**) of *Trichilia elegans* ssp. *elegans*.[19] The above ^{1}H and ^{13}C NMR features suggested that **8** is closely related to the *seco*-A ring protolimonoid **9**.[19] The MS of **8**, in addition to having ions due to stepwise loss of two molecules of H_2O and a molecule of HOAc, showed a significant fragment ion at *m*/*z* 371 (55%) due to loss of the side chain and a molecule of H_2O (Scheme 2). The structure **8** proposed for this compound was further supported by DEPT, HMQC, and HMBC spectra, which were also useful in assigning its ^{1}H and ^{13}C NMR spectra (Table 3). HMBC correlations observed for **8** are depicted in Figure 1.

The biological activity data for nymania 1 (**1**) and Tr-B (**7**) in our mechanism-based yeast mutant bioassays are given in Table 1. Nymania 1 showed reproducible, significant, and selective activity against the DNA repair-deficient RS 322YK (*rad52Y*) yeast strain, whereas Tr-B exhibited moderate but selective activity, suggesting that they might have cytotoxic activity mediated by a DNA-damaging mechanism. Nymania 1 and Tr-B were thus tested for cytotoxicity, but they only showed

Scheme 2. MS Fragmentation of **8**

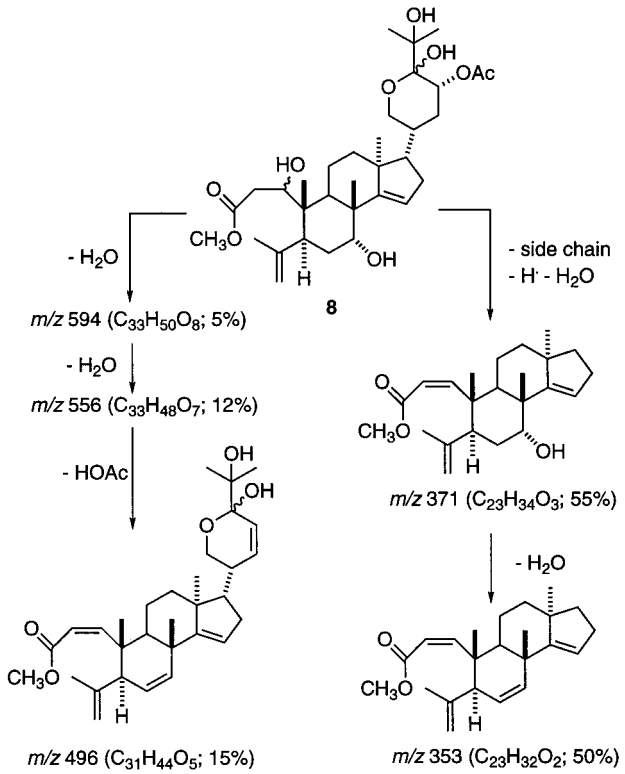


Table 3. ^{13}C NMR Spectral Data (100.57 MHz; $CDCl_3$; δ ppm) for Limonoids **3**−**5** and **7**[a]

carbon	3	4	5	7
1	70.4 d	72.8 d	77.2 d	73.1 d
2	37.1 t	68.5 d	38.7 t	41.9 t
3	168.7 s	72.9 d	173.4 s	169.7 s
4	85.1 s	40.6 s	79.4 s	79.3 s
5	44.3 d	41.1 d	41.1 d	38.0 d
6	26.4 t	22.6 t	41.1 t	42.2 t
7	74.9 d	71.6 d	168.1 s	173.7 s
8	41.4 s	42.3 s	136.6 s	138.2 s
9	38.9 d	47.2 d	56.3 d	51.3 d
10	44.1 s	43.3 s	50.7 s	45.8 s
11	25.3 t	213.1 s	74.6 d	78.1 d
12	77.2 d	78.8 d	79.5 d	71.2 d
13	51.2 s	46.1 s	48.9 s	49.6 s
14	155.0 s	72.8 s	87.3 s	80.8 s
15	122.4 d	59.2 d	205.5 s	206.3 s
16	34.9 t	33.1 t	32.5 t	35.0 t
17	49.77d	38.5 d	37.1 d	37.9d
18	15.4 q	14.3 q	12.6 q	12.9 q
19	15.3 q	64.1 t	14.1 q	22.9 q
20	124.1 s	123.5 s	122.1 s	125.2 s
21	140.2 d	140.6 d	140.5 d	140.6 d
22	111.5 d	112.7 d	110.4 d	110.3 d
23	142.2 d	142.3 d	143.2 d	143.3 d
28	29.1 q	18.7 q	28.1 q	28.5 q
29	65.5 t	93.4 d	74.0 t	75.5 t
30	28.1 q	22.8 q	118.0 t	122.5 t
OCHO	160.3 d			
1′	174.7 s	175.3 s	175.6 s	174.9 s
2′	75.4 d	41.1 d	74.9 d	74.6 d
3′	36.7 d	26.5 t	38.7 d	37.9 d
4′	23.6 t	11.4 q	22.6 t	22.7 t
Me−2′		16.3 q		
Me−3′	15.7 q		15.1 q	15.1 q
Me−4′	11.7 q		11.8 q	11.4 q
1″	173.9 s			
2″	76.5 d			
3″	31.8 d			
4″	19.3 q			
5″	14.6 q			
Ac−1	169.5 s			
	21.3 q			
Ac−2′	170.8 s			
	20.7 q			

[a] Assignments by HETCOR, HMQC, and HMBC; type of carbon (q = CH_3, t = CH_2, d = CH, s = C) determined by DEPT experiments.

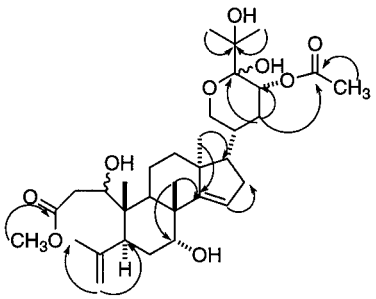


Figure 1.

modest activity; nymania 1 had an IC_{50} value of 12 μg/mL against the Vero monkey cell line, while Tr-B had an IC_{50} value greater than 20 μg/mL against the CHO-AUX-B1 cell line.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter in $CHCl_3$ solutions. NMR spectra (δ in ppm, J in Hz) were obtained on a Varian Unity 400 spectrometer in $CDCl_3$ using residual $CHCl_3$ as internal standard (δ_H 7.24 ppm; δ_C 77.0 ppm). DQCOSY, DEPT, ^1H-^{13}C HETCOR, HMQC, and HMBC NMR experiments were performed on the same spectrometer using standard Varian pulse sequences. HRMS were obtained from the Nebraska Center for Mass Spectrometry. Column chromatography employed Si gel 60 (230−400 mesh) for normal phase, LRP-2 for reversed phase and Sephadex LH-20 (25−100 μM) for gel filtration. Analytical and preparative TLC were performed by using precoated Si gel 60 F_{254} (Merck, analytical) and Si gel 500 or 1000 μM (Analtech, preparative) plates, and detection for analytical TLC was accomplished by UV absorption and by spraying with vanillin/H_2SO_4 reagent followed by heating. HPLC separations were carried out using a Waters C_{18} RP radial-pak 5 μm cartridge column (10 cm) on an apparatus consisting of a Waters M-6000A pump connected to a Waters 990 Series photodiode array detector, with detection at 205 nm.

Plant Material. The stem bark of *T. emetica* was collected in November 1988 near Negelle Borana, Ethiopia, and identified by Mr. Gilbert of Kew Botanic Gardens, Kew, England. A voucher specimen (No. S 019) has been deposited in the National Herbarium, Addis Ababa University, Ethiopia.

Biological Assays. The procedures utilized in the mechanism-based yeast bioassay were performed as previously described.[3] The IC_{12} values refer to the concentration in μg/mL required to produce a zone of inhibition of 12 mm diameter around a 100 μL well during a 48 h incubation period at 32 °C.

Extraction and Isolation. The dried and powdered stem bark of *T. emetica* (480 g) was sequentially extracted with cold MeCOEt and MeOH. Evaporation gave MeCOEt (18.4 g) and MeOH (40.2 g) extracts. A portion of the bioactive MeCOEt extract was partitioned between hexane and 80% aqueous MeOH, giving a hexane fraction (6.14 g) that was found to be inactive. Water was then added to the bioactive 80% aqueous MeOH fraction to give a 60% aqueous MeOH fraction, and this fraction was extracted thoroughly with $CHCl_3$ to afford a bioactive $CHCl_3$ fraction (3.49 g). A portion of the $CHCl_3$ fraction (2.5 g) was chromatographed on a Si gel column made up in CH_2Cl_2 and eluted with increasing amounts of *i*-PrOH in CH_2Cl_2. One hundred fractions of 50 mL each were collected and combined on the basis of on their TLC behavior to obtain 15 combined fractions. Of these only the fractions 5−9 were found to be bioactive.

Further purification of fraction 5 (45.0 mg) by reversed-phase column chromatography on LRP-2 and elution with 40−0% H_2O in MeOH afforded nymania 1 (**1**) (3.2 mg) and drageana 4 (**3**) (8.4 mg). Fraction 7 (30.0 mg) from the above Si gel column on further fractionation by repeated RP-HPLC using 40% aqueous MeOH furnished trichilin C (**4**) (3.8 mg) and an unidentified limonoid (4.1 mg). Fraction 8 (75.0 mg) on repeated RP-HPLC using 40% aqueous MeOH as an eluant gave rohituka 3 (**6**) (6.4 mg), Tr-B (**7**) (6.6 mg), and a further quantity of trichilin C (**4**) (2.7 mg). RP-CC of fraction 9 (190.0 mg) on LRP-2 (10.0 g) and elution with a gradient solvent system ranging from 50% aqueous MeOH to 100% MeOH afforded **8** (8.8 mg). Fraction 11 (130 mg)

Table 4. ^{1}H and ^{13}C NMR Data (δ ppm) for Compound **8** in $CDCl_3$[a]

position	^{1}H[b]	^{13}C[c]	position	^{1}H[b]	^{13}C[c]
1	3.83 d (5.4)	67.6 d	18	0.87 s	15.0 q/14.6 q
2	2.10 m, 1.65 m	35.4 t	19	0.93 s	19.6 q/19.4 q
3		170.1 s	20	2.10 m	32.86 d/32.85 d
4		144.8 s	21	3.92 dd (11.2, 2.6)	
				3.58 d (11.2)	65.4 t/62.2 t
5	2.64 br d (13.0)	42.8 d	22	2.39 dd (11.0, 7.0)	
				2.87 dd (1.5, 7.0)	34.9 t
6	2.15 m	29.9 t	23	5.43 dd (1.5, 11.0)	77.5 d
7	3.86 dd (8.9, 2.9)	71.6 d/71.4 d	24		97.6 s/96.0 s
8		44.5 s	25		76.4 s
9	2.30 m	35.6 d	26	1.42 s	26.0 q
10		44.6 s	27	1.27 s	24.4 q
11		18.36 t/18.43 t	28	4.99 s, 4.85 s	116.0 t
12	2.10 m, 1.32 m	34.4 t	29	1.79 br s	23.2 q
13		47.0 s/46.5 s	30	1.07 s	27.2 q
14		161.5 s	CO_2CH_3	3.81 s	51.95 q/51.92 q
15	5.51 dd (4.5, 2.7)	120.0 d/119.8 d	$OCOCH_3$		172.1 s
16	2.80 m	34.0 t/33.9 t	$OCOCH_3$	2.01 s/1.99 s	21.2 q
17	2.64 m	57.3 d			

[a] Signal pairs are given together separated by a slant. [b] Measured at 400 MHz; coupling constants J (in Hz) in parentheses. [c] Measured at 100.57 MHz; type of carbon (q = CH_3, t = CH_2, d = CH, and s = C) determined by DEPT experiments.

from the first Si gel column was further separated by Si gel CC made up in 5% *i*-PrOH in CH_2Cl_2. Elution with 10−15% *i*-PrOH in CH_2Cl_2 afforded a white crystalline solid (22.8 mg), mp > 300 °C dec, which was identified as sitosterol 3β-glucoside from its ^{1}H and ^{13}C NMR spectral data.

Nymania 1 (1): white amorphous solid; EIMS (70 eV) *m/z* 628 (18), 582 [628-HCO_2H] (7), 497 (8), 450 (10), 409 (13), 341 (12), 279 (10), 225 (28), 94 (42), 43 (100); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3. ^{1}H and ^{13}C NMR spectral data identical with those reported recently.[14] HREIMS [M − AcOH − MeOH]$^+$ *m/z* 628.2521 (calcd for $C_{33}H_{40}O_{12}$, 628.2520).

Diacetyl Nymania 1 (2). Acetylation of nymania 1 (**1**) (4.0 mg) with Ac_2O (50 μL) in pyridine (100 μL) at 0 °C for 14 h afforded **2** (3.2 mg) as a pale yellow gum: $[\alpha]^{25}_D$ −40° (*c* 0.10, $CHCl_3$) (lit.[14] $[\alpha]^{25}_D$ −42°); EIMS (70 eV) *m/z* 744 [M − AcOH]$^+$ (2), 716 [744 − CO]$^+$ (3), 702 [716 − CH_2CO]$^+$ (3), 702 (3), 343 (25), 305 (35), 283 (40), 241 (9), 223 (70), 199 (90), 181 (8), 167 (85), 157 (100), 129 (100); ^{1}H NMR δ 7.95 (1H, s, OCHO), 7.40 (1H, d, J = 1.0, H-23), 7.38 (1H, br s, H-21), 6.25 (1H, d, J = 1.0, H-22), 6.07 (1H, br s, H-30A), 6.03 (1H, br s, H-30B), 5.97 (1H, br s, H-12), 5.28 (1H, m, H-11), 5.20 (1H, dd, J = 10.0 and 4.0, H-1), 4.80 (1H, d, J = 4, H-2′), 4.35 (1H, d, J = 8, H-29a), 3.95 (1H, t, J = 8.5, H-17), 3.80 (1H, d, J = 8.0, H-29b), 3.72 (3H, s, CO_2CH_3), 2.10 (3H, s, OAc), 2.12 (3H, s, OAc), 2.18 (3H, s, OAc), 1.00 (3H, s, H_3-18), 1.28 (3H, s, H_3-19), 1.42 (3H, s, H_3-28), 0.82 (3H, d, J = 7.0, H_3-6′), 0.80 (3H, t, J = 7.0, H_3-5′); ^{13}C NMR δ 207.1 (C-15), 175.6 (1-$OCOCH_3$), 170.2, 169.8, 168.9, 167.6 (each OCOR), 161.6 (OCHO), 143.1 (C-23), 141.2 (C-21), 138.4 (C-8), 126.4 (C-30), 123.3 (C-20), 121.0 (C-3), 110.7 (C-22), 85.6 (C-4), 81.2 (C-14), 76.0 (C-12), 74.2 (C-2′), 73.9 (C-29), 72.2 (C-1/C-11), 53.1 (CO_2CH_3), 50.7 (C-9), 49.57 (C-9/C-13), 49.55 (C-9/C-13), 49.2 (C-5), 41.8 (C-6/C-16), 39.2 (C-6/C-16), 36.2 (C-3′), 35.4 (C-17), 34.2 (C-2), 26.8 (C-28), 24.5 (C-4′), 22.2, 21.3, 20.7 (each $OCOCH_3$), 16.7 (C-19), 15.5 (C-6′), 13.3 (C-18), 11.6 (C-5′).

Dregeana 4 (3): white amorphous solid; $[\alpha]^{25}_D$ −20.5° (*c* 0.7, $CHCl_3$); ^{1}H NMR δ 7.32 (1H, dd, J = 2.7, 1.8, H-23), 7.18 (1H, d, J = 1.8, H-21), 6.22 (1H, d, J = 2.7, H-22), 5.50 (1H, m, H-15), 5.28 (1H, m, H-7), 5.05 (1H, br t, J = 4.0, H-12), 4.90 (1H, d, J = 4.5, H-2″), 4.75 (1H, m, H-1), 4.16 (1H, d, J = 3.0, H-29A), 4.02 (1H, d, J = 3.0, H-29B), 4.03 (1H, br s, H-2′), 3.22 (2H, m, H-2), 2.12 and 1.92 (3H each, s, 2 × OAc), 1.42, 1.25, and 1.20 (3H each, s, H_3-19, H_3-28, and H_3-30), 1.10 and 1.00 (3H each, d, J = 7.0, H_3-4″ and H_3-5″), 0.90 (3H, s, H-18), 0.86 (3H, t, J = 7.0, H_3-5′), 0.84 (3H, d, J = 7.0, H_3-6′); ^{13}C NMR data, see Table 3; HRFABMS [M + Li]$^+$ *m/z* 765.4031 (calcd for $C_{41}H_{58}O_{13}Li$, 765.4037).

Trichilin A (4): colorless amorphous solid; $[\alpha]^{25}_D$ −36.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3.

Rohituka 3 (5): white amorphous solid; $[\alpha]^{25}_D$ −54.3° (*c* 0.5, $CHCl_3$); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3.

Trichilia substance Tr-B (7): white amorphous solid; $[\alpha]^{25}_D$ −18.3° (*c* 0.15, $CHCl_3$); EIMS (70 eV) 688 [M]$^+$ (2), 628 [M − AcOH]$^+$ (10), 600 [628 − CO]$^+$ (2), 582 [600 − H_2O]$^+$ (13), 533 (5), 497 (10), 468 (12), 450 (20), 434 (15), 400 (15), 368 (10), 341 (15), 326 (25), 236 (5), 210 (70), 147 (50), 121 (40), 102 (50), 76 (70), 57 (100); ^{1}H NMR data, see Table 2; ^{13}C NMR data, see Table 3.

Methyl 1(*S*),23(*R*)-diacetoxy-7(*R*),24ξ,25-trihydroxy-(20*S*)-21,24-epoxy-3,4-*seco*-apotirucalla-4(28),14(15)-dien-3-oate (8): colorless gum; $[\alpha]^{25}_D$ −34.0° (*c* 0.8, $CHCl_3$); EIMS (70 eV) *m/z* 574 [M − H_2O]$^+$ (4), 556 [574 − H_2O]$^+$ (12), 516 (5), 496 [556 − HOAc]$^+$ (15), 487 (20), 425 (35), 371 [M − side chain − H_2O]$^+$ (55), 353 [371-H_2O]$^+$ (50), 311 (20), 246 (18), 145 (60), 107 (95), 55 (100); ^{1}H and ^{13}C NMR data, see Table 4; HREIMS [M + Na]$^+$ *m/z* 615.3494 (calcd for $C_{33}H_{52}O_9Na$, 615.3509).

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