SYNTHESIS OF TRINORDITERPENOID DERIVATIVES AND EVALUATION OF ANTIMICROBIAL AND CYTOTOXIC ACTIVITY

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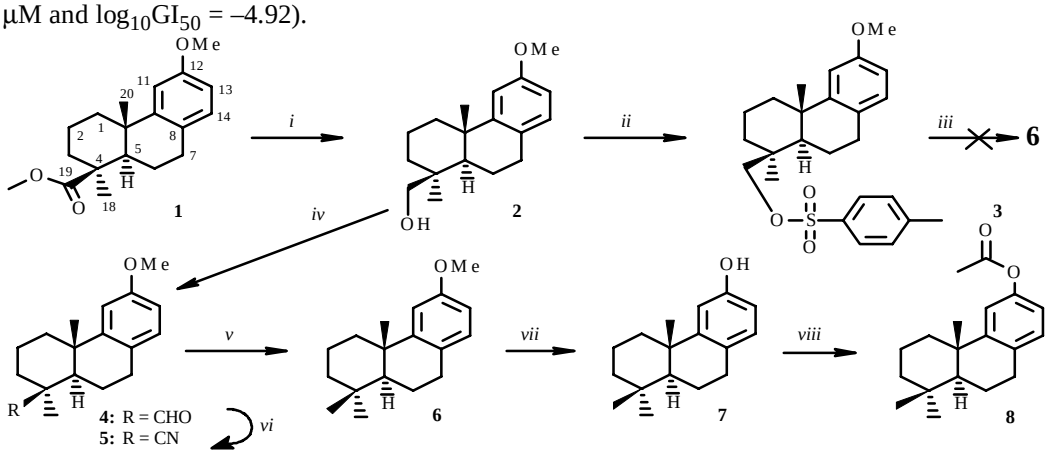
*The synthesis of optically active trinorditerpenes was carried out, and their antimicrobial and antitumor activity was tested. The synthetic derivative 12-hydroxypodocarpa-8,11,13-triene (**7**) showed GI_{50} at 6.6 μM against breast cancer MDA-MB-435 (LC_{50} = 50.9 μM and log10 GI_{50} = –5.18). The 12-acetyloxypodocarpa-8,11,13-triene (**8**) showed GI_{50} at 12.1 μM against leukemia RPMI-8226 (LC_{50} = 76.1 μM and log_{10} GI_{50} = –4.92).*

Key words: trinorditerpenoid derivatives, antimicrobial and cytotoxic activity.

The trinorditerpenes have attracted the interest of natural product chemists during the past decades. In this context, simple and sequential modifications were performed on the methyl O-methylpodocarpane molecule (**1**) because it is a chiral starting material for the synthesis of diterpenoids with biological activity [1–3]. Our goal was to make this class of trinorditerpenoids readily available for the investigation of the basic structural requirements to explore the antimicrobial and antitumor activity.

The compounds synthesized by us were evaluated for their antimicrobial activity in a preliminary screening. We observed that the compound **7** showed activity against Gram-positive bacteria, Gram-negative bacteria, yeast, and mold (Table 1). This particular antimicrobial ability attracted our attention to the compound **7** since it has a more simple structure than the other compounds reported here. Of the eight compounds reported here, four were accepted and tested at the National Institute of Health (NIH), Developmental Therapeutics Program (DTP), to evaluate the antitumor activity, where the 12-hydroxypodocarpa-8,11,13-triene (**7**) showed GI_{50} at 11.3 μM against melanoma SK-MEL5 (LC_{50} = 48.3 μM and $log_{10}GI_{50}$= –4.95). The 12-acetyloxypodocarpa-8,11,13-triene (**8**) showed GI_{50} at 12.1 μM against leukemia RPMI-8226 (LC_{50} = 76.1 μM and $log_{10}GI_{50}$ = –4.92).



i. $LiAlH_4$, THF, 89%; *ii.* Ts-Cl, Py, THF, 90%; *iii.* NaI/Zn, DMF; *iv.* TPAP, NMO, CH_2Cl_2, 87%;
v. NH_2NH_2, KOH, 48%; *vi.* NH_2NH_2, KOH, Microwave, 25%; *vii.* $AlCl_3$, CH_2Cl_2, 63%; *viii.* Py, acetic anhydride, 61%

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TABLE 1. Antimicrobial Activity of Compounds **2-8**

Microorganisms	**2**	**3**	**4**	**5**	**6**	**7**	**8**	(C)
Pseudomonas aeruginosa	-	10	-	-	-	12	7	20
Escherichia coli	-	-	-	9	-	10	9	22
Staphylococcus aureus	13	-	-	-	8	17	-	20
Bacillus subtilis	12	-	-	-	15	20	-	30
Candida tropicalis	13	-	-	10	11	14	-	31
Aspergillus niger	12	-	-	-	-	16	-	25

Inhibition diameters in mm at 2 mg/mL, **–** = no activity, paper discs (⌀ = 5 mm, 10 μL), (C) = antibiotic control: amikacine, nalidilixic acid, vancomicine, and nistatine. Note: In the case of the inhibition zone around of the paper filter of 7–8 mm, positive disc of inhibition is considered very small, which indicates that the different compounds are not very interesting. The inhibition zone of 9–14 mm is interesting; nevertheless the compounds are still not important. Important inhibition zones appear from 15 mm, and it is important to mention that those compounds can be considered as an alternative when problems of resistance appear on the test.

TABLE 2. Primary Anticancer Assay of 3-Cell Line Panel

Compound	Concentration	Growth percentage		
		Breast MCF7	N-S Cell Lung NCI-H460	CNS SF-268
2	1.00 E-04 M	178	145	115
5	1.00 E-04 M	195	176	115
7	1.00 E- 04 M	4	0	1
8	1.00 E-04 M	0	5	1

All these synthetic compounds **2**, **4**, **6**, and **7** had physical and spectroscopic data identical to those reported by R. Cambie and co-worker [1, 3, 4].

The starting material for preparation of compound **8** was methyl O-methyl podocarpane (**1**) (Scheme 1) which was reduced with lithium aluminium hydride in THF to afford the alcohol compound **2** in 89% yield; then the protection of the alcohol group using *p*-toluenesulfonyl chloride produced the compound **3** in 90% yield. In 1960, Matsumoto and co-workers [5] reported the deoxygenation of the alcohol using *p*-toluenesulfonyl ether and the NaI/Zn system, but this method failed to produce the compound **6**. The aldehyde **4** was obtained in 87% yield by oxidation of the alcohol compound **2** using TPAP reagents with NMO [6]. Wolff-Kishner reduction of the aldehyde **4** with partial demethylation affored the phenol **7** in 27.2% yield [10] and compound **6** in 48% yield. R. Cambie and co-worker [7] reported identical spectroscopic data for compound **6**; we used here a Huang Minlon [8] modification of the Wolff-Kishner reduction process to increas the yield of compound **6** up to 60%. Anpai Li and co-workers [9] reported similar spectroscopy data for compound **7** synthesized by us. We search another method to reduce the C-19 aldehyde, and finally we used microwave-induced synthesis of hydrazones and Wolff-Kishner reduction of compound **4**, as reported by Jagir S. Sandhu and co-workers [10], but this method failed; perhaps we obtained the compound **5** in 25% yield. Therefore, cleavage of the 12-methoxy group of compound **6** using $AlCl_3$ conditions affords the compound **7** in 63% yield. Acetylation of compound **7** produced a stable compound **8** in 61% yield.

Antimicrobial. These synthetic trinorditerpenoids **2**, **6**, and **7** were significant to continue exploring the chemotherapeutic potential as an antitumor agent.

Antitumor. The evaluation of potential chemotherapeutic activity was performed on compounds **2**, **5**, **7**, and **8** at DTP against a panel of 3 cell lines MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS) according to the standard protocol [11] (Table 1). Both compounds **7** and **8** that have passed DTP criteria for activity in this assay were scheduled automatically for evaluation against the full panel of 60 tumor cells derived from nine cancer types (leukaemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast) according to the standard protocol [12] (Table 2).

TABLE 3. *In Vitro* Antitumor Screening for Compounds **1** and **2**

Cell line	Compound							
	7				**8**			
	G, %	GI_{50}	LC_{50}	Log_{10}; GI_{50}	G, %	GI_{50}	LC_{50}	Log_{10}; GI_{50}
				Leukemia				
CCRF-CEM					46	87.4	>100	-4.06
HL-60(TB)	-33	16.0	>100	-4.80	1	34.7	>100	-4.46
K-562	-61	10.9	79.9	-4.96	14	40.0	>100	-4.40
MOLT-4	-11	11.7	>100	-4.93	21	30.9	>100	-4.51
RPMI-8226	-65	12.3	75.5	-4.91	-65	12.1	76.1	-4.92
SR	6	8.72	>100	-5.06	61	>100	>100	>-4.00
				N-SC Lung				
A549/ATCC	-77	19.0	70.4	-4.72	-22	24.7	>100	-
EKVX	-94	21.5	62.7	-4.67	-27	26.0	>100	4.61
HOP-62					-93	15.9	57.7	-4.59
HOP-92					-66	21.7	80.9	-4.80
NCI-H226	-78	18.2	68.7	-4.74	-70	18.5	75.7	-4.6
NCI-H23	-78	17.3	68.0	-4.76	-76	19.8	71.7	-4.73
NCI-H322M	-92	18.3	60.8	-4.74	-84	13.5	59.9	-4.70
NCI-H460	-57	18.6	89.9	-4.73	-4	27.5	>100	-4.87
NCI-H522	47	21.5	>100	-4.67	-18	24.2	>100	-4.56
				Colon				
COLO 205	-48	20.4	>100	-4.69	-35	21.9	>100	-4.66
HCT-116	24	42.2	>100	-4.37	-96	18.4	58.7	-4.74
HCT-15	-72	17.1	72.6	-4.77	-76	19.5	71.2	-4.71
HT29	-65	2.32	82.2	-4.63	-1	31.7	>100	-4.50
KM-12	-73	14.1	69.0	-4.85	-47	19.2	>100	-4.72
SW-620	-83	13.8	60.9	-4.86	8	28.2	>100	-4.55
				CNS cancer				
SF-268	-77	16.4	67.9	-4.79	-44	20.9	>100	-4.68
SF-295	-52	20.6	96.8	-4.69	-26	19.1	>100	-4.72
SF-539	-93	18.3	59.8	-4.74				
SNB-19	-78	17.0	67.7	-4.77	-66	19.6	79.5	-4.71
SNB-75	-81	12.4	61.0	-4.91				
U251	-91	14.6	58.0	-4.84	-92	19.9	61.8	-4.70
				Melanoma				
LOX IMVI	-78	16.9	67.5	-4.77	-95	17.7	58.3	-4.75
M14	6	28.2	>100	-4.55	-88	18.2	62.6	-4.74
SK-MEL-2	-56	20.1	91.8	-4.70	4	29.8	>100	-4.53
SK-MEL-28	-78	19.6	70.0	-4.71	-37	19.3	>100	-4.71
SK-MEL-5	-100	11.3	48.3	-4.95	-18	30.9	>100	-4.51
UACC-257	-84	19.6	66.0	-4.71	-48	18.2	>100	-4.74
UACC-62	-100	13.1	50.8	-4.88	-89	17.6	61.2	-4.75

TABLE 3. (continued)

Cell line	Compound							
	7				**8**			
	G, %	GI_{50}	LC_{50}	Log_{10}; GI_{50}	G, %	GI_{50}	LC_{50}	Log_{10}; GI_{50}
				Ovarian				
IGROV1	-74	20.4	73.5	-4.69	27	49.4	>100	-4.31
OVCAR-3	-76	17.0	69.2	-4.77	-47	15.8	>100	-4.80
OVCAR-4	-77	16.6	68.1	-4.78				
OVCAR-5	-93	14.0	55.5	-4.85	-75	22.3	73.8	-4.65
OVCAR-8	-70	21.7	77.8	-4.66	-55	18.8	91.9	-4.73
				Renal cancer				
786-0	-11	27.3	>100	-4.56	-94	18.1	59.3	-4.74
A 478	-100	16.2	54.5	-4.79				
ACHN	-88	18.1	62.2	-4.74	-97	19.1	58.8	-4.72
CAKI-1	-84	19.5	66.0	-4.71	7	28.0	>100	-4.55
RXF 393	-67	16.0	76.6	-4.80	-10	41.2	>100	-4.39
SN 12C	-100	17.5	55.9	-4.76	-96	18.6	58.8	-4.73
TK-10	-88	18.2	62.6	-4.74	-78		63.5	
UO-31	-65	19.9	81.4	-4.70	-12	31.3	>100	-4.50
				Prostata cancer				
PC-3	-92	15.8	57.9	-4.80	-71	20.1	75.7	-4.70
DU-145	-95	16.4	57.0	-4.79				
				Breast cancer				
MCF7	-48	13.3	>100	-4.88		34.6	>100	-4.46
NCI/ADR/RES	-83	16.5	63.9	-4.78	-53	26.0	95.7	-4.59
MDA-MB 231	-95	16.2	57.0	-4.79	-82	17.8	65.8	-4.75
HS 578T	-83	15.6	62.9	-4.81	-34	24.7	>100	-4.61
MDA-MB-435	-88	6.65	50.9	-5.18	-53	16.8	95.5	-4.77
BT-549	-100	19.4	58.0	-4.71	1	36.5	>100	-4.44
T-47D					10	37.5	>100	-4.43

G, % = Growth percentage at 1.00 E - 4.0 molar concentration, GI_{50} at μM concentration, LC_{50} at μM concentration, means $\log_{10} GI_{50}$, and empty space = ND or not determined.

Compound **2** showed activity against Gram-positive bacteria, yeast, and mold (Table 1) but failed the primary anticancer assay at DTP (Table 2).

The results of cytostatic and cytotoxic activity of both **7** and **8** are shown in Table 3. The synthetic trinorditerpene compound **7** inhibited cell proliferation at 11.3 μM against melanoma SK-MEL5, and was active against all cell lines, with mean $\log_{10} GI_{50}$ values ranging from –4.55 (melanoma M14) to –5.06 (leukemia SR). Cytotoxic effects, evaluated by the LC_{50} parameter, became visible at somewhat higher concentrations, i.e., 70.4 μM for the 60 cell line panels. The compound **8** is an acetyl derivative of compound **7** and inhibited cell proliferations with GI_{50} at 12.1 μM against leukemia RPMI-8226. It was active against all cell lines, with mean $\log_{10} GI_{50}$ values ranging from –4.31 (ovarian cancer IGROVI) to –4.92 (leukemia RPMI-8226) (Table 3).

In fact, this work was an initial biological evaluation of these synthetic trinorditerpenoids. Both **7** and **8** had promising activity against human cancer cell lines and are targets for future investigation in vivo in our laboratory. Further studies will be required to elucidate the cell targets as antitumor.

EXPERIMENTAL

Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded with a Nicolet 5DXC FT-IR spectrometer. NMR spectra were obtained for solutions in $CDCl_3$ on a Bruker Avance-300 and 500 spectrometer. The assignments of carbon signals were made by means of 2D NMR ^1H and ^{13}C single bond and multiple bond correlation studies. Mass spectra were determined on a Kratos MS 25 RFA spectrometer at 70 eV using a direct inlet system. Rotations were measured in chloroform solutions at 25°C with a Perkin-Elmer instrument 341 polarimeter. Elemental analysis was performed at Atlantis Microlab. For column chromatography, silica gel 60 (Merck, 70–230 mesh) was used, thin layer chromatograms were prepared on silica gel G or silica gel GF254 60 (Merck) and the spots were observed by exposure to iodine vapor or UV light. All organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure below 60°C. Ether refers to diethyl ether. Tetrahydrofuran, dichloromethane, and pyridine were freshly distilled before used. All other solvents and reagents were obtained from commercial suppliers and were used without further purification.

19-Hydroxymethyl-12-methoxypodocarpa-8,11,13-triene (2). A solution of lithium aluminium hydride (1.88 g, 49.0 mmol) was added to compound **1** (5 g, 16.5 mmol) in tetrahydrofuran (15 mL) and the contents were stirred under nitrogen at 5°C for 24 hours. The excess reagent was decomposed with water and after addition of 5% aqueous H_2SO_4 the product was extracted with ether. Evaporation of the ether extract and subsequent purification by column chromatography (hexane–ether 10:1) gave 4.5 g (89%) of compound **2** as a white crystal (hexane), mp 105°C, lit., [13] 90–91.5°C; ν_{max} (liquid film)/cm^{-1} 3373 (OH); ^1H NMR (500 MHz, δ, J/Hz, $CDCl_3$): 1.03 (3H, s, 20-CH_3), 1.16 (3H, s, 18-CH_3), 1.37–1.48 (2H, m, 3-H), 1.62 (1H, m, 1α-H), 1.61–1.65 (2H, m, 2-H), 1.72–1.83 (2H, m, 6-H), 1.89 (1H, m, 5-H), 2.26 (1H, m, 1β-H), 2.69–2.90 (2H, m, 7-H), 3.70 (2H, dd, J = 81, J = 9, 19-CH_2), 3.75 (3H, s, O-CH_3), 6.64 (1H, dd J = 9, J = 3, 13-H), 6.85 (1H, d, J = 2.4, 11-H), 6.93 (1H, d, J = 9, 14-H); ^{13}C NMP (75.45 MHz, δ, $CDCl_3$): 19.76, 20.03, 22.84, 27.80, 27.98, 35.72, 37.60, 37.90, 39.13, 50.04 (C-5), 55.26 (Ar-O$\underline{C}H_3$), 71.70 (C-19), 110.00, 111.20, 125.20, 130.08, 150.20, 157.85; *anals*. C 73.96%, H 9.60%, calcd for $C_{18}H_{26}O_2$, C 73.93%, H 9.65%.

12-Methoxy-19-tosyloxypodocarpa-8,11,13-triene (3). A solution of *p*-toluenesulfonyl chloride (3.6 mL, 0.0184 mol) in 20 mL dry THF was added to a solution of compound **2** (3.9 g, 0.142 mol) and pyridine (6.61 g 0.184 mol) in the same solvent (20 mL) under nitrogen atmosphere over 20 min with cooling. After another hour, the mixture was warmed to room temperature during 3 hours. The solution was then washed with 10% HCl (150 mL) and water (300 mL), then extracted with chloroform (2 × 200 mL), dried (Na_2SO_4), and evaporated in vacuum to give a yellow solid. This crude was purified further by column chromatography on silica gel (hexane–ether, 10:2) to afford 5.26 g (90%) of a yellow solid (methanol–hexane) mp 74°C; $[\alpha]_D^{20}$ +1° (*c* 1.08, $CHCl_3$); ^1H NMR (500 MHz, δ, J/Hz, $CDCl_3$): 0.99 (3H, s, 18-CH_3), 1.01 (3H, s, 20-CH_3), 1.90–2.19 (2H, m, 1-H), 1.43–1.58 (2H, m, 2-H), 1.32–1.40 (2H, m, 3-H), 1.90 (1H, m, 5-H), 1.60–1.75 (2H, m, 6-H), 2.69–2.75 (2H, m, 7-H), 3.73 (3H, s, ArO-CH_3), 4.10 (2H, dd J = 12, J = 9, 19-CH_2), 6.71 (1H, d J = 3, 11-H), 6.63 (1H, dd J = 9, J = 3, 13-H), 6.90 (1H, d, J = 9, 14-H), 7.53–7.93 (4H, m, SO_2–$C_6\underline{H}_4$–CH_3); ^{13}C NMP (75.45 MHz, δ, $CDCl_3$): 18.67, 18.80, 24.90, 26.44, 29.39, 34.90, 37.07, 37.31, 38.59, 50.39 (Ar-O$\underline{C}H_3$), 50.59 (C-5), 78.88, 109.80, 110.68, 126.33, 127.44, 128.74, 128.74, 129.35, 133.19, 133.19, 135.69, 149.85, 157.38; *anals*. C 70.74%, H 7.61%, calcd for $C_{25}H_{32}O_4S$, C 70.06%, H 7.53%.

12-Methoxy-19-carbaldehyde-podocarpa-8,11,13-triene (4). Compound **2** (0.123 g, 0.40 mmol) was dissolved in dichloromethane (5 mL) containing both 4 Å molecular sieves (0.279 g, 0.55 mmol) and N-methylmorpholine N-oxide (0.070 g, 0.60 mmol). After stirring the mixture for 5 min, tetra-*n*-propylammonium perrutherate (0.007 g, 0.02 mmol) was added and the reaction followed by TLC until complete. The reaction mixture was filtered through silica gel (hexane–ether, 10:2) and afforded 0.096 g (87%) compound **4** as a white solid (methanol–hexane) mp 137°C, lit., [7] 130–132°C; $[\alpha]_D^{20}$ +54° (*c* 2.25, $CHCl_3$); ^1H NMR (500 MHz, δ, J/Hz, $CDCl_3$): 1.09 (3H, s, 18-CH_3), 1.12 (3H, s, 20-CH_3), 1.69 (1H, m, 1α-H), 2.02 (1H, m,1β-H), 1.42–1.47 (2H, m, 2-H), 1.23–1.28 (2H, m, 3-H), 1.67–1.81 (2H, m, 6-H), 2.26 (1H, m, 5-H), 2.85 (1H, m, 7-H), 2.96 (1H, dd J = 17.5, J = 10, 7-H), 3.80 (3H, s, O-CH_3), 6.71 (1H, d, J = 8.5, 14-H), 6.83 (1H, bs, 11-H), 7.01 (1H, d J = 8.5, 13-H) and 9.86 (1H, s, CHO); ^{13}C NMP (75.45 MHz, δ, $CDCl_3$): 18.91, 19.18, 24.00, 24.17, 30.35, 33.80, 38.23, 38.30, 48.61, 51.87 (C-5), 55.22 (Ar-O$\underline{C}H_3$), 110.64, 111.26, 126.88, 129.91, 148.78, 157.79, 205.66 (C-19); *anals*. C 79.83%, H 9.08%, calcd for $C_{18}H_{24}O_2$, C 79.37%, H 8.88%.

12-Hydroxypodocarpa-8,11,13-triene (7). A solution of compound **6** (0.150 g, 0.47 mmol) in anhydrous dichloromethane (7 mL) and aluminum trichloride (0.183 g, 1.78 mmol) was stirred at –10°C for 15 min. After the reaction was stirred overnight at room temperature, the mixture was poured into ice-water and extracted with dichloromethane. The combined organic layer was successively washed with saturated $NaHCO_3$ and brine solution, and then with dried (Na_2SO_4),

filtered, and evaporated under reduced pressure to give crude. The crude was purified by column chromatography (hexane–ether 10:1) to afford 0.088 g (63%) of compound **7** as a white crystal (hexane–methanol) m.p. 140°C, $[\alpha]_D^{20}$ +66.8° (*c* 3.76, $CHCl_3$); lit., [3] 139–142°C, $[\alpha]_D^{20}$ +72° (*c* 0.86, $CHCl_3$); ^{1}H NMR (500 MHz; J/Hz, $CDCl_3$): δ_H = 0.90 (3H, s, 19-CH_3), 0.92 (3H, s, 18-CH_3), 1.15 (3H, s, 20-CH_3), 1.20 (1H, dd J = 12, J = 4, 3α-H), 1.29 (1H, dd, J = 13, J = 2.3, 3β-H), 1.37 (1H, m, 2-H), 1.45 (1H, bd, J = 15, 1β-H), 1.58 (1H, m, 2-H), 1.61 (1H, m, 6-H), 1.68 (1H, m, 6β-H), 1.82 (1H, m, 5-H), 2.17 (1H, bd, J = 10, 1α-H), 2.76 (1H, m, 7-H), 2.84 (1H, dd, J = 6, J = 3, 7-H), 4.47 (1H, bs, OH), 6.53 (1H, dd, J = 7.5, J = 5, 13-H), 6.70 (1H, d, J = 5, 11-H) and 6.88 (1H, d J = 8, 14-H); ^{13}C NMP (75.45 MHz, δ, $CDCl_3$): 19.10, 19.27, 21.63, 24.71, 29.56, 33.28, 33.46, 37.88, 38.78, 41.63, 50.22 (C-5), 110.94, 112.52, 127.49, 129.95, 151.70, 153.54; GC/MS *m/z* 244 (100, M^+), 228 (74), 147 (75), 91 (10), 69 (20), 41 (12); *anals*. C 77.82%, H 9.99%, calcd for $C_{17}H_{24}O$, C 77.78%, H 9.98%.

12-Methoxypodocarpa-8,11,13-triene (6). A mixture of compound **4** (6.6 g, 24.2 mmol), 8.9 mL of 90% hydrazine hydrate (13.2 g, 410 mmol), diethylenglycol (50 mL), and potassium hydroxide pellets (8.14 g, 145 mmol) in a 250 mL two-necked round-bottomed flask was put under reflux conditions until most of the potassium hydroxide had dissolved, then heated at a 112°C under reflux conditions for 1 hour. After that we removed the reflux condenser and fitted a still-head and condenser on it for downward distillation. We distilled it until the temperature of the liquid rose to 216°C and kept it at that temperature for 3.5 hours. After that the mixture was cooled down, the solution was then washed with 5% HCl (150 mL) and water (300 mL), extracted with ether (3 × 200 mL), dried (Na_2SO_4), and evaporated in vacuum to give a yellow oil as a crude product; it was then purified by column chromatography (hexane–ether, 10:1) to afford 1.7 g (27.2%) of compound **7** and the desired compound **6** as a white solid (hexane–methanol): 3 g (48%), mp 34°C, lit., [3] 31–32°C, [14] 30–31°C; $[\alpha]_D^{20}$ +65.5° (*c* 6.6, $CHCl_3$); ^{1}H NMR (500 MHz, δ, J/Hz, $CDCl_3$): 0.90 (3H, s, 19-CH_3), 0.92 (3H, s, 20-CH_3), 1.16 (3H, s, 18-CH_3), 1.28 (1H, bd J = 13.2, 3β-H), 1.38 (1H, td, J = 12.9, J = 3.8, 1α-H), 1.40 (1H, td, J = 13.2, J = 4.2, 3α-H), 1.55 (1H, m, 2-H), 1.66 (1H, m, 6-H), 1.78 (1H, m, 2-H), 1.82 (1H, m, 6-H), 1.85 (1H, dd, J = 12.3, J = 2.2, 5-H), 2.23 (1H, br d, J = 12.9, 1β-H), 2.77 (1H, m, 7-H), 2.87 (1H, dd, J = 17, J = 1.6, 7-H), 3.76 (3H, s, O-CH_3), 6.78 (1H, d, J = 2.5, 11-H), 6.63 (1H, dd, J = 8.3, J = 2.3, 13-H), 6.93 (1H, d, J = 8.5, 14-H); ^{13}C NMP (75.45 MHz, δ, $CDCl_3$): 19.18, 19.34, 21.69, 24.74, 29.44, 33.03, 33.35, 38.00, 38.8, 41.70, 50.34 (C-5), 55.23 (O-CH_3), 110.15 (C-11), 110.71 (C-13), 127.47, 129.76 (C-14), 151.45, 157.66; GC/MS *m/z* 258 (100, M^+), 243 (61), 161 (58), 115 (7), 91 (3), 69 (7), 41 (6); *anals*. C 83.62%, H10.13%, calcd for $C_{18}H_{26}O$, C 83.67%, H 10.14%.

12-Methoxy-19-carbonitrile-podocarpa-8,11,13-triene (5). A mixture of compound **4** (4.7 g, 17 mmol) and 90% hydrazine hydrate (9.29 g, 290 mmol) in diethyleneglycol (50 mL) was taken in an Erlenmeyer flask and placed in a commercial microwave oven operating at 2450 MHz frequency. After irradiation of the mixture for 20 min (monitored by TLC), it was cooled to room temperature, extracted with chloroform, and dried over anhydrous Na_2SO_4. Removal of the solvent gave the respective hydrazone derivative. This hydrazone derivative compound was used without purification in the next steps. For the Wolff-Kishner reduction, a mixture of hydrazone and potassium hydroxide pellets (5.7 g, 102 mmol) was taken in an Erlenmeyer flask and placed in a microwave oven. After irradiation for 30 min and the usual work up, we obtained a yellow oil as a crude product, which was then purified by column chromatography (hexane–ether, 10:2) to afford 1.7 g (27.2%) of nitrile compound **5** as a white solid (hexane–methanol), mp 99°C; ν_{max} (liquid film)/cm^{-1} 3607.82, 3582.89, 2919.16, 2845.23, 2300.27 (CN), 1605.97; ^{1}H NMR (500 MHz, δ, J/Hz, $CDCl_3$): 1.37 (3H, s, 20-CH_3), 1.46 (3H, s, 18-CH_3), 1.52 (1H, m, 1-H), 1.82 (1H, d, J = 9 Hz, 5-H), 1.90–2.00 (2H, m, 6-H), 2.09–2.18 (2H, m, 2-H), 2.11–2.19 (2H, m, 3H), 2.36 (1H, d, J = 9, 1-H), 2.88 (1H, m, 7-H), 2.99 (1H, dd, J = 9.6, J = 3, 7-H), 3.81 (3H, s, O-CH_3), 6.82 (1H, br s, 11-H), 6.72 (1H, d, J = 10, 13-H) and 7.02 (1H, d, J = 10, 14-H); ^{13}C NMP (75.45 MHz, δ, $CDCl_3$): 19.76, 19.96, 22.75, 27.79, 27.98, 35.32, 37.47, 37.84, 39.08, 49.12, 55.25 (O-CH_3), 109.85, 111.12, 124.52, 126.61 (C-19), 129.86, 149.08, 157.86; GC/MS *m/z* 269 (60 M^+), 254 (100), 237 (23), 171 (8), 134 (9), 115 (8), 670 (11), 55 (3), 39 (5); *anals*. C 80.66%, H 8.63%, calcd for $C_{18}H_{13}NO$, C 80.26%, H 8.61%.

12-Acetylhydroxypodocarpa-8,11,13-triene (8). A white crystal (hexane–methanol); mp 170°C, GC/MS *m/z* 286 (6, M^+), 244 (100), 147 (75), 229 (30), 147 (31), 115 (5), 69 (2); anals. C 79.78%, H 9.10%, calcd for $C_{19}H_{26}O_2$, C 79.68%, H 9.15%.

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