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Novel 24-nor-, 24-nor-2,3-*seco*-, and 3,24-dinor-2,4-*seco*-ursane triterpenes from *Diospyros decandra*: evidences for ring A biosynthetic transformations

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Abstract—Novel 24-nor-, 24-nor-2,3-*seco*, and 3,24-dinor-2,4-*seco*-ursane type triterpenes have been isolated along with one known compound from the stem bark of *Diospyros decandra*. The structures of these highly oxidized metabolites were established on the basis of extensive NMR and MS studies. They possibly represent intermediates in the biosynthetic transformation of ring A in an ursane triterpene. Some isolates showed mild anti-mycobacterial activity against *Mycobacterium tuberculosis* with MIC values ranging from 25 to 200 μg/mL.

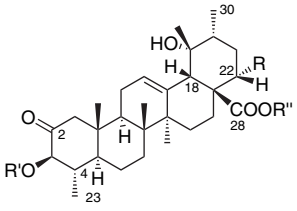
1. Introduction

Diospyros species are found in most part of tropics and subtropics and many of them have been used in traditional medicines. A considerable number of species has been investigated and the presence of quinones,[1–3] triterpenes,[1,4,5] flavonoids,[1] coumarin derivatives,[1,6] and phenolic glycosides[1,4] has been reported. In our ongoing search for bioactive compounds from Thai medicinal plants we have studied *Diospyros decandra* Lour. (syn. *Diospyros packmanii*) known in Thailand as 'Chan'. This plant is a tree in the Ebenaceae family, which grows to about 10–20 m in height. Its pale yellow flowers are small and fragrant. The round fruits are edible and fragrant when mature. The wood decoction is drunk as a blood tonic, anthelmintic, and also to relieve fever.[7]

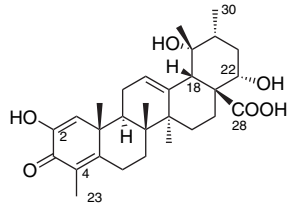
2. Results and discussion

Repeated column chromatography of the chloroform extract of the bark of *D. decandra* resulted in the isolation of five novel compounds, in addition to one known compound. The known compound was identified as betulinic acid.[8]

Compound **1** was obtained as colorless solid, mp 161–162 °C. The FTIR spectrum showed the presence of a carboxyl group at ν_{max} 3569 and 1709 cm^{-1} as well as the



1 R = R' = R'' = H
1a R = R'' = H, R' = $COCH_3$
1b R = H, R' = $COCH_3$, R'' = CH_3
2 R = OH, R' = R'' = H
2a R = $OCOCH_3$, R' = $COCH_3$, R'' = H
2b R = $OCOCH_3$, R' = $COCH_3$, R'' = CH_3



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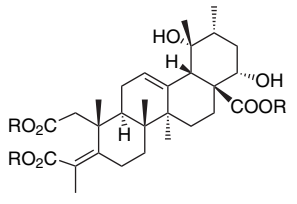
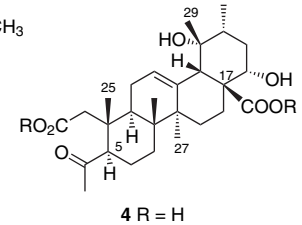


5 R = CH_3



4 R = H
4a R = CH_3

C=C stretching at ν_{max} 1686 cm^{-1}. The HRFABMS negative ionization mode displayed a $[M-H]^-$ ion at *m/z* 471.3118 corresponding to $C_{29}H_{43}O_5$ or to M^+ of

Keywords: *Diospyros decandra*; Ebenaceae; 19-Hydroxy ursane triterpenes.
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$C_{29}H_{44}O_5$. The ^{13}C NMR spectrum showed 29 carbon signals comprising of six methyls, eight methylenes, seven methines, and eight quaternary carbons including one carboxyl, one keto, and one olefinic quaternary carbon. The detection of a secondary methyl signal at δ_H 0.91 (d, 6.8), and a trisubstituted olefinic proton signal at δ_H 5.32 [δ_C 128.5 (d) and 138.4 (s)] provide the most useful indications for an urs-12-ene triterpene skeleton.[9] The presence of a carboxyl group at C-28 and an OH group at C-19 was established from long range ^1H–^{13}C correlations between H-18 (δ_H 2.52)/C-12 (δ_C 128.5), C-16 (δ_C 25.4), C-17 (δ_C 47.6), C-19 (δ_C 73.0), C-20 (δ_C 41.1), C-28 (δ_C 182.0), and C-29 (δ_C 27.3). The placement of a keto group at C-2 (δ_C 210.3), an additional secondary hydroxyl group at C-3 (δ_H 3.62, d, J=10.2 Hz, δ_C 80.5), and only one methyl group connected to C-4 (δ_H 1.14, d, J=6.2 Hz, δ_C 16.6, q, assigned for H_3-23), instead of two methyl groups as commonly found in an ursane triterpene, was proven by the ^1H–^{13}C correlations between H-5 (δ_H 1.25)/C-1 (δ_C 52.2, t), C-3, C-23, and C-25 (δ_C 14.7), and between H-1(δ_H 2.03 and 2.52)/C-2, C-3, and C-25. A 3-β-hydroxy-24-nor-ursane backbone was established by examination of the NOE effects from the NOESY spectrum of **1** (Fig. 1). The NOE correlations between H-5/H-3, and a methyl proton doublet signal at δ_H 1.14, in conjunction with NOE interaction of H_3-25/H-4 indicated that both H-3 and a methyl group bonded to C-4 are α-oriented. The α-oriented methyl group at C-4 corresponds to H_3-23 of the ursane skeleton. The 'nor' methyl group is thus the H_3-24. The NOE effects between H-12/H-18, and H_3-29 clearly established ring E as cis-fused ring.[10] The remaining ^1H and ^{13}C resonances could be unambiguously assigned by the use of ^1H–^1H COSY, HMQC, and HMBC correlations spectra (Tables 1 and 2). Compound **1**, for which the trivial name diospyric acid A was proposed, was identified as 2-oxo-3β,19α-dihydroxy-24-nor-urs-12-en-28-oic acid. Further confirmation of the presence of a secondary hydroxyl and a carboxylic functional group was made by acetylating **1** with acetic anhydride in pyridine to produce **1a** and further methylation of **1a** with CH_2N_2 to produce the methyl ester **1b**. The ^1H NMR spectrum of **1a** indicated the presence of an acetyl group at δ_H 2.16 (s) and a downfield shift of the oxymethine proton at δ_H 4.80 (d, J=11.2 Hz, H-3). An additional OCH_3 signal at δ_H 3.56 (s) was observed in the ^1H NMR spectrum of **1b**. Full assignments of the ^1H and ^{13}C NMR chemical shifts of **1**, **1a**, and **1b** are shown in Tables 1 and 2.

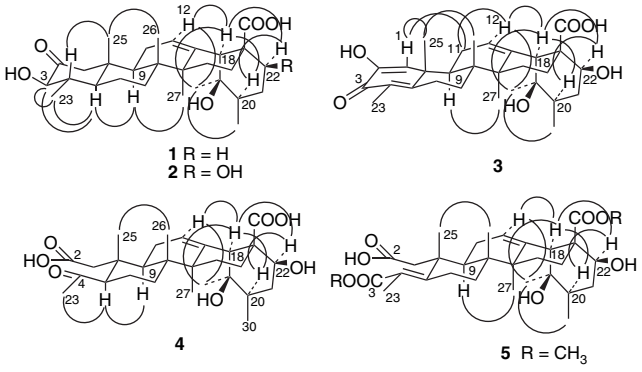


Figure 1. Important NOE effects in compounds **1**–**5**.

Table 1. ^1H NMR spectroscopic data of **1**–**2b** ($CDCl_3$)

Position	1	1a	1b	2	2a	2b
1	2.03 (d, 12.7) 2.52[a]	2.06 (d, 12.9) 2.47 (d, 12.9)	2.08 (α-H), 2.48	2.02, 2.48[e]	2.05 (d, 12.8) 2.50 (d, 12.8)	2.05 (d, 12.3) 2.46 (d, 12.8)
3	3.62 (d, 10.2)	4.80 (d, 11.2)	4.77 (d, 11.3)	3.61[f]	4.78 (d, 11.2)	4.78 (d, 11.2)
4	1.56	1.86[b]	1.90	1.48	1.85	1.86
5	1.25	1.34[c]	1.32	1.25	1.33	1.31
6	1.21, 1.70	1.18, 1.73	1.22, 1.72	1.21, 1.71	1.23, 1.74	nd
7	1.32, 1.53	1.36[c], 1.57	1.35, 1.57	1.32, 1.53	1.34, 1.56	1.34, 1.55
8	—	—	—	—	—	—
9	1.89	1.88[b]	1.88	1.87	1.92	1.90
10	—	—	—	—	—	—
11	1.95	1.97	1.95	1.84, 1.97	1.84, 1.96	1.85, 1.97
12	5.32 (br s)	5.33 (br s)	5.32 (br s)	5.30 (br s)	5.32 (br s)	5.31 (br s)
15	1.74, 2.09	1.04, 1.75	1.02, 1.60	1.23, 1.65	1.08, 1.83	1.06, 1.76
16	1.58, 2.52	1.55, 2.51[d]	1.58, 2.51	1.88, 2.22	1.96, 2.36 (ddd, 4.9, 8.4, 13.3)	1.96, 2.26 (ddd, 4.9, 8.5, 13.4)
18	2.52[a]	2.53[d]	2.57	2.46[e]	2.55 (br s)	2.61 (br s)
20	1.37	1.38	1.43	1.54	1.61	1.58
21	1.25, 1.72	1.26, 1.68	1.24	1.49, 1.75	1.44, 1.84	1.43, 1.84
22	1.63, 1.77	1.63, 1.78	1.59 (β-H), 1.72	3.64 (m)[f]	5.02 (dd, 4.4, 12.1)	5.00 (dd, 4.6, 12.3)
23	1.14 (d, 16.2)	1.04 (d, 6.3)	1.03 (d, 6.2)	1.13 (d, 5.7)	1.03 (d, 6.3)	1.03 (d, 6.3)
25	0.74 (s)	0.77 (s)	0.81	0.74[g]	0.76 (s)	0.78 (s)
26	0.72 (s)	0.70 (s)	0.68	0.74[g]	0.68 (s)	0.64 (s)
27	1.26 (s)	1.28 (s)	1.28	1.26	1.29 (s)	1.29 (s)
28	—	—	—	—	—	—
29	1.17 (s)	1.18 (s)	1.20 (s)	1.14	1.17 (s)	1.18 (s)
30	0.91 (d, 6.8)	0.92 (d, 6.7)	0.92 (d, 6.7)	0.93 (d, 6.5)	0.94 (d, 6.6)	0.95 (d, 6.7)
3-COCH_3	—	2.16 (s)	2.17 (s)	—	2.17 (s)	2.16 (s)
22-COCH_3	—	—	—	—	1.99 (s)	2.00 (s)
28-OCH_3	—	—	3.56 (s)	—	—	3.52 (s)

nd=not detected.
[a–g] Overlapping signals.

Table 2. ^{13}C NMR spectroscopic data of **1**–**2b** ($CDCl_3$)

Position	1	1a	1b	2	2a	2b
1	52.5 (t)	53.7 (t)	53.7 (t)	52.7 (t)	53.7 (t)	53.7 (t)
2	210.7 (s)	203.5 (s)	203.4 (s)	210.9 (s)	203.5 (s)	203.4 (s)
3	80.5 (d)	81.7 (d)	81.7 (d)	80.5 (d)	81.6 (d)	81.6 (d)
4	42.2 (d)[a]	38.2 (d)	38.2 (d)	38.9 (d)	38.1 (d)	38.2 (d)
5	50.4 (d)	51.1 (d)	51.2 (d)	50.4 (d)	51.1 (d)	51.1 (d)
6	20.8 (t)	21.0 (t)	21.0 (t)	20.7 (t)	21.0 (t)	21.0 (t)
7	31.6 (t)	31.6 (t)	31.6 (t)	31.6 (t)	31.4 (t)	31.4 (t)
8	40.0 (s)	40.0 (s)	39.9 (s)	39.9 (s)	40.1 (s)	40.1 (s)
9	44.6 (d)	44.5 (d)	44.4 (d)	44.5 (d)	44.3 (d)	44.3 (d)
10	42.2 (s)[a]	41.5 (s)[b]	41.4 (s)[d]	42.0 (s)[e]	41.4 (s)	41.4 (s)
11	24.0 (t)	24.0 (t)	24.0 (t)	24.0 (t)	24.0 (t)	24.0 (t)
12	128.5 (d)	128.6 (d)	128.4 (d)	128.5 (d)	129.3 (d)	129.2 (d)
13	138.4 (s)	138.3 (s)	138.5 (s)	138.0 (s)	137.4 (s)	137.7 (s)
14	41.5 (s)	41.5 (s)[b]	41.4 (s)[d]	42.0 (s)[e]	41.6 (s)	41.6 (s)
15	28.2 (t)	28.2 (t)	28.2 (t)	27.7 (t)	27.3 (t)	27.4 (t)
16	25.4 (t)	25.4 (t)	25.5 (t)	18.5 (t)	18.5 (t)	18.7 (t)
17	47.6 (s)	47.8 (s)	47.9 (s)	53.2 (s)	51.8 (s)	52.0 (s)
18	53.0 (d)	52.9 (d)	53.3 (d)	53.5 (d)	53.8 (d)	54.0 (d)
19	73.0 (s)	73.1 (s)	73.1 (s)	72.7 (s)	72.5 (s)	72.5 (s)
20	41.1 (d)	41.1 (d)	41.1 (d)	42.8 (d)	39.1 (d)	39.2 (d)
21	25.9 (t)	25.9 (t)	26.0 (t)	33.3 (t)	31.1 (t)	31.3 (t)
22	37.3 (t)	37.4 (t)	37.3 (t)	73.6 (d)	74.9 (d)	74.7 (d)
23	16.6 (q)	16.4 (q)[c]	16.4 (q)	16.5 (q)	16.4 (q)	16.4 (q)
25	14.7 (q)	14.5 (q)	14.5 (q)	14.7 (q)	14.5 (q)	14.2 (q)
26	16.3 (q)	16.4 (q)[c]	16.3 (q)	16.1 (q)	15.9 (q)	16.1 (q)
27	24.2 (q)	24.3 (q)	24.2 (q)	24.1 (q)	24.8 (q)	24.6 (q)
28	182.0 (s)	183.8 (s)	178.2 (s)	180.0 (s)	180.2 (s)	175.0 (s)
29	27.3 (q)	27.4 (q)	27.4 (q)	26.9 (q)	27.1 (q)	27.0 (q)
30	16.0 (q)	16.1 (q)	16.0 (q)	15.7 (q)	15.5 (q)	15.5 (q)
3-*CO*CH_3	—	170.4 (s)	170.4 (s)	—	170.4 (s)	170.5 (s)
3-CO*C*H_3	—	20.6 (q)	20.6 (q)	—	20.6 (q)	20.6 (q)[f]
22-CO*C*H_3	—	—	51.7 (q)	—	20.9 (q)	20.6 (q)[f]
22-*CO*CH_3	—	—	—	—	170.6 (s)	170.3 (s)
28-O*C*H_3	—	—	—	—	—	52.0 (q)

[a–f] Overlapping signals.

Compound **2** was obtained as a crystalline solid. The FTIR spectrum showed the presence of a carboxyl group at ν_{max} 3397 and 1711 cm^{-1}, and a C=C stretching at ν_{max} 1635 cm^{-1}. The HRFABMS negative ionization mode showed a $[M-H]^-$ ion at *m/z* 487.3067, which corresponded to $C_{29}H_{43}O_6$. The ^{13}C NMR spectrum displayed 29 carbon signals comprising six methyls, seven methylenes, eight methines, and eight quaternary carbons including one carboxyl, one keto and one olefinic quaternary carbon. The ^1H and ^{13}C NMR spectra showed a close resemblance to those of compound **1**, except for the presence of an additional secondary alcohol group (δ_H 3.64, m, and δ_C 73.6, d). The position of an extra hydroxyl group at C-22 was established from the ^1H–^{13}C long-range correlations between H-22/C-16 (δ_C 18.5), C-20 (δ_C 42.8), C-21 (δ_C 33.3), and C-28 (δ_C 180.0). Treatment of **2** with acetic anhydride-pyridine gave a di-acetate derivative (**2a**), which displayed two additional methyl singlet signals at δ_H 1.99 and 2.17 in the ^1H NMR spectrum, and as a consequence, acetylation also caused the downfield shift of the methine protons at C-3 and C-22 to δ_H 4.78 (d, *J*=11.2 Hz) and 5.02 (dd, *J*=12.1 and 4.4 Hz), respectively. The use of diazomethane to react with **2a** gave the corresponding mono-methyl ester (**2b**). The ^1H and ^{13}C NMR chemical shifts assignment of **2**, **2a**, and **2b** were achieved from ^1H–^1H COSY, HMQC, and HMBC NMR spectra (Tables 1 and 2). The relative stereochemistry of **2** in most of the molecule is identical to that of **1** as shown by NOESY spectra of **1**, **2**, and their derivatives. Additional NOE correlations between H-22/H-18 and H-20 indicated a relative stereochemistry of **2** as shown in Figure. 1. In the ^1H NMR spectrum of **2a** and **2b**, the signal at ~δ_H 5.00 (dd, *J*=12.1 and 4.4 Hz) was assigned to H-22, this is in accord with values reported for H-22 in methyl musangate A isolated from *Musanga cecropioides*,[10] and gave further support to the assignment of OH-22 of **2**, **2a**, and **2b** as α-equatorial with respect to ring E. Compound **2**, which was assigned a trivial name as diospyric acid B, and was identified as 2-oxo-3β,19α,22α-trihydroxy-24-nor-urs-12-en-28-oic acid.

Compound **3** was obtained as solid, mp 182–184 °C. The FTIR spectrum indicated the presence of a carboxyl (ν_{max} 3419 cm^{-1}) and a conjugated keto (ν_{max} 1697 cm^{-1}) function. The HREIMS negative ionization mode displayed a $[M-H]^-$ ion at *m/z* 483.2752 requiring $C_{29}H_{39}O_6$. The ^{13}C NMR spectrum showed 29 carbon signals comprising six methyls, six methylenes, six methines, and 11 quaternary carbons including one keto, one carboxyl, one oxygenated, and four olefinic carbons (Table 3). The ^1H NMR spectrum, which displayed a carbinolic proton at δ_H 3.64 (dd, *J*=11.6 and 4.2 Hz), an olefinic proton at δ_H 5.34 and a methyl signal at δ_H 0.91 (d, *J*=6.6 Hz) indicated that rings C–E part of the molecule is similar to compound **2**. The 2-hydroxy-$\Delta^{1,4}$-dien-3-one subunit was established from the key ^3J ^1H–^{13}C correlations of the less shielded olefinic proton at δ_H 6.24 (H-1)/C-9 (δ_C 44.7), the methyl carbon at δ_C 21.1(C-25), oxygenated C-2 (δ_C 144.3), and C-3 (δ_C 181.5). The long-range correlations from the vinyl methyl

Table 3. ^{1}H and ^{13}C NMR spectroscopic data of **3–5** ($CDCl_3$)

Position	δ_H **3**	δ_H **4**	δ_H **4a**	δ_H **5**	δ_C **3**	δ_C **4**	δ_C **4a**	δ_C **5**
1	6.24 (s)	2.18 (d, 13.6) 2.44 (d, 13.6)	2.27 (d, 14.5) 2.37 (d, 14.5)	2.54 (d, 16.3) 2.79 (d, 16.3)	125.4 (d)	44.4 (t)	43.5 (t)	44.8 (t)
2	—	—	—	—	144.3 (s)	173.4 (s)	172.2 (s)	172.0 (s)
3	—	—	—	—	181.5 (s)	—	—	174.0 (s)
4	—	—	—	—	126.0 (s)	216.9 (s)	212.8 (s)	120.3 (s)
5	—	2.66 (dd, 12.7, 2.7)	2.88 (dd, 12.2, 3.0)	—	167.5 (s)	56.7 (d)	56.1 (d)	145.3 (s)
6	2.29, 2.70	1.71, 1.83	1.64, 1.72	2.31	25.2 (t)	21.7 (t)	21.9 (t)	25.7 (t)
7	1.57	1.25, 1.54	1.30 (dt, 13.2, 3.3), 1.56	1.19, 1.97	34.7 (t)	29.7 (t)	31.1 (t)	29.8 (t)
8	—	—	—	—	40.1 (s)	39.8 (s)	40.0 (s)	38.8 (s)
9	1.85	2.14 (dd, 6.4, 4.4)	2.07 (dd, 16.1, 11.0)[a]	2.68 (dd, 8.1, 9.2)	44.7 (d)	39.1 (d)	39.0 (d)	37.9 (d)
10	—	—	—	—	43.7 (s)	40.2 (s)	39.5 (s)	41.3 (s)
11	2.24	2.05, 2.32	2.06[a]	2.04 (dd, 3.5, 7.2)	25.8 (t)	23.5 (t)	23.5 (t)	25.1 (t)
12	5.34 (br s)	5.35 (obs t, 3.7)	5.34 (obs dd, 3.3, 2.6)	5.43 (t, 3.4)	128.6 (d)	129.0 (d)	129.0 (d)	130.6 (d)
13	—	—	—	—	138.1 (s)	137.2 (s)	137.6 (s)	137.3 (s)
14	—	—	—	—	42.8 (s)	42.4 (s)	42.3 (s)	43.2 (s)
15	1.08, 1.65	1.54	1.52	1.29, 1.53	28.2 (t)	27.8 (t)	27.8 (t)	27.6 (t)
16	2.22, 1.86	1.91, 2.30	1.95, 2.26	1.96, 2.26 (dt, 3.8, 1.4)	18.6 (t)	18.4 (t)	18.5 (t)	18.6 (t)[c]
17	—	—	—	—	53.4 (s)	53.6 (s)	53.9 (s)	54.0 (s)
18	2.47 (br s)	2.48 (br s)	2.53 (br s)	2.55 (s)	53.6 (d)	53.4 (d)	53.5 (d)	54.4 (d)
19	—	—	—	—	72.5 (s)	72.7 (s)	72.9 (s)	72.4 (s)
20	1.41	1.49	1.54[b]	1.51 (m)	38.9 (d)	38.9 (d)	39.0 (d)	39.0 (d)
21	1.48, 1.75	1.53 1.80 (q, 12.4)	1.54,[b] 1.75 (dd, 12.0, 11.3)	1.56 (ddd, 12.6, 4.1, 2.9), 1.79 (q, 12.2)	33.3 (t)	33.7 (t)	33.6 (t)	33.2 (t)
22	3.64 (dd, 11.6, 4.2)	3.71 (dd, 11.4, 4.4)	3.61 (dd, 12.0, 4.4)	3.64 (dd, 11.9, 4.3)	73.5 (d)	73.7 (d)	73.7 (d)	73.5 (d)
23	1.94 (s)	2.24	2.21 (s)	1.85 (s)	10.5 (q)	30.3 (q)	31.3 (q)	18.7 (q)[c]
25	1.23 (s)	1.04 (s)	1.09 (s)	1.21 (s)	21.1 (q)	18.5 (q)	17.8 (q)	23.2 (q)
26	1.02 (s)	0.82 (s)	0.72 (s)	0.80 (s)	16.3 (q)	16.6 (q)	16.4 (q)	19.0 (q)
27	1.09 (s)	1.23 (s)	1.23 (s)	1.35 (s)	23.6 (q)	24.0 (q)	24.1 (q)	23.5 (q)
28	—	—	—	—	178.9 (s)	181.0 (s)	176.4 (s)	177.2 (s)
29	1.11 (s)	1.17	1.18 (s)	1.20 (s)	26.8 (q)	27.1 (q)	27.2 (q)	27.2 (q)
30	0.91 (d, 6.6)	0.97 (d, 6.8)	0.96 (d, 6.6)	0.99 (d, 6.4)	15.7 (q)	15.8 (q)	15.6 (q)	15.7 (q)
2-OCH_3	—	—	3.64 (s)	3.63 (s)	—	—	51.0 (q)	51.2 (q)
3-OCH_3	—	—	—	3.71 (s)	—	—	—	51.5 (q)
28-OCH_3	—	—	3.65 (s)	3.67 (s)	—	—	51.9 (q)	52.0 (q)

[a–c] Overlapping signals.

proton (δ_H 1.94) to C-3, C-4 (δ_C 126.0), and C-5 (δ_C 167.5) confirmed the connectivity of this methyl group to C-4. The NOESY spectrum showed NOE effects as found in compound **2**, with additional interactions between H-1/H-11 and H_3-25 (Fig. 1). Compound **3**, which was given a trivial name as diospyric acid C, could be identified as 3-oxo-2,19α,22α-trihydroxy-24-nor-urs-1,4,12-trien-28-oic acid.

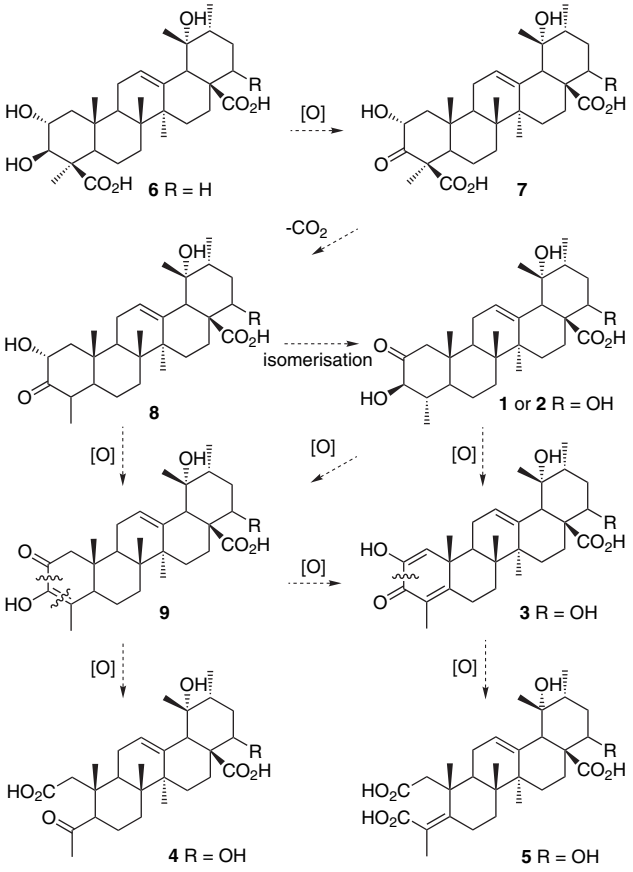
Compound **4** was obtained as solid. The FTIR spectrum showed the presence of a carboxyl group at ν_{max} 3554 and 1690 cm^{-1}. The HRFABMS negative ionization mode displayed a $[M-H]^-$ ion at *m/z* 489.2857 corresponding to the elemental formula of $C_{28}H_{41}O_7$. The ^{13}C NMR spectrum showed 28 carbon signals comprising six methyls, seven methylenes, six methines, and nine quaternary carbons including one keto and two carboxyl carbons. A singlet signal at δ_H 2.24, and a keto ^{13}C signal at δ_C 216.9, implied the presence of a CH_3CO moiety. The absence of the secondary methyl group doublet signal at ~δ_H 1.1 and a less shielded oxymethine proton doublet at ~δ_H 3.6, which were assigned as H_3-23 and H-3 of compounds **1** and **2**, respectively, indicated further loss of one more carbon atom of ring A. The ^{1}H–^{13}C correlations between H-5 (δ_H 2.66)/C-4 (δ_C 216.9), C-6 (δ_C 21.7), C-10 (δ_C 40.2), and C-25 (δ_C 18.5) requires the placement of a keto group at C-4 and a rupture of bond joining C-3 and C-4. The location of a carboxylic acid as well as its relationship to the B ring was established by ^{1}H–^{13}C correlations between H-1 (δ_H 2.18 and 2.44)/C-2 (δ_C 173.4), C-5 (δ_C 56.7), C-9 (δ_C 39.1), C-10 (δ_C 40.2), and C-25 (δ_C 18.5). The relative stereochemistry of **4** (Fig. 1) was deduced by NOESY and was very similar to the already described compounds **1** and **2**. Compound **4**, which was given a trivial name of diospyric acid D, was identified as 4-oxo-19α,22α-dihydroxy-3,24-dinor-2,4-*seco*-urs-12-en-2,28-dioic acid. Compound **4a**, which is the dimethyl ester of **4**, was obtained after reacting **4** with ethereal solution of diazomethane. The ^{1}H and ^{13}C NMR spectroscopic data of **4a** are also included in Table 3.

Compound **5** was obtained in a small quantity after methylation of the crude fraction containing **4** using diazomethane and purification by HPLC. The HREIMS of **5** showed a $[M]^+$ ion at *m/z* 560.3441 corresponding to $C_{32}H_{48}O_8$. The ^{1}H NMR spectrum revealed three methyl ester singlet signals at δ_H 3.63, 3.67, and 3.71, indicating the presence of three carboxyl groups in the parent compound. Comparison of the 1D and 2D NMR spectra of **5** with the respective data of **2** and **4** results the same configurations of rings B–E in **5**. The absence of a doublet signal at approximately δ_H 1.04–1.14 (assigned to H_3-23 in **2**), together with the presence of a low-field methyl signal at δ_H 1.84, assignable to CH_3–C═C, indicated differences in ring A compared to **2** and **4**. Long-range ^{1}H–^{13}C correlations of the signal at δ_H 1.84 (H_3-23) to ^{13}C NMR signals at δ_C 174.0 (s), 120.1

(s), and 145.0 (s) in particular, indicated a carboxyl group at C-3 and a double bond between C-4 and C-5. Heteronuclear long-range coupling between H-25/C-1, C-5, and C-10 as well as between the diastereotopic methylene proton signals at δ_H 2.54 and 2.78 (H_2-1) to the ^{13}C NMR signals at δ_C 171.9 (C-2, s), 145.0 (C-5, s), and 23.0 (C-25, q) indicated the presence of the second carboxyl group at C-2. Rupture of bond joining C-2 to C-3 was therefore envisaged. The third carboxyl group at C-28 could be detected from the HMBC correlations of signals at δ_H 2.55 (H-18) and 2.27 (H-16) to a carboxyl carbon signal at δ_C 177.2 (C-28, s). The ROESY spectrum indicated similar spatial arrangements of **5** to those of **4** (Fig. 1). All ^1H and ^{13}C chemical shifts derived from HMBC, HMQC, and dqf COSY NMR spectra are listed in Table 3. Compound **5** could thus be proposed as the trimethyl ester of the naturally occurring 19α,22α-dihydroxy-24-nor-2,3-*seco*-urs-12-en-2,3,28-trioic acid, which has been trivially named as diospyric acid E.

To the best of our knowledge, these are among the first examples of the occurrence of a 24-nor-ursane, as well as, a 24-nor-2,3-*seco*-, and 3, 24-dinor-2,4-*seco*-ursane type triterpenes in nature.

One plausible biogenetic pathway of these compounds could therefore be proposed (Scheme 1). Vismiaefolic acid, 2α,3β,19α,-trihydroxyurs-12-ene-24,28-dioic acid (**6**)[11] precipitated out of the MeOH extract,[12] was proposed as a precursor of 24-nor-ursane compounds (**1** and **2**). Oxidation of **6** at C-3-OH gave a corresponding 3-keto acid (**7**), from which further decarboxylation led to **8** and double bond isomerization in **8** led to compounds **1** or **2**. A 2,3-dicarbonyl compound (**9**) could be formed as a result of oxidation of **1** or **2** and **8** at ring A. Upon further oxidation of **9** with a loss of one molecule of CO_2 caused cleavage of bonds joining C-2/C-3 and C-3/C-4, could lead to **4**. Formation of compound **3** from **1** or **2** or **9** is probable through oxidation. Oxidative cleavage of bond joining C-2/C-3 in **3** lead to compound **5**. Although a compound of type **9** was not isolated in the present study, there has been a report of the isolation of an oleanane triterpene with 3-hydroxy-3,4-en-2-one subunit in ring A, as proposed for **9** from *Physena madagascariensis*.[13]



Scheme 1. Biogenetic pathways to the formation of compounds **1**–**5**.

Compounds **1**–**4** and betulinic acid were tested for their antitubercular, antifungal, and antimalarial activities. Betulinic acid and **1** showed moderate to weak anti-TB activity with MIC values of 25 and 200 μg/mL, respectively. Betulinic acid and **2** showed inhibition activity against *Candida albicans* with IC_{50} values of 27.2 and 42.6 μg/mL, respectively. All five compounds were inactive in an antimalarial assay. Due to the scarcity of pure compounds, only **1**–**3** were further tested for their cytotoxicity against the breast cancer (BC), nasopharyngeal carcinoma (KB) and human small cell lung NCI-H187 cell lines, and **3** exhibited very mild activity against NCI-H187 cell line with IC_{50} of 12.6 μg/mL, while **1** and **2** were inactive with all cell lines at 20 μg/mL.

3. Experimental

3.1. General

The specific rotations were measured by a JASCO DIP 1020 polarimeter. The IR spectra were obtained on a Perkin Elmer Spectrum One FTIR spectrometer with universal ATR accessory. EIMS and HREIMS spectra were recorded on a Finnigan MAT 90 instrument. ^1H and ^{13}C spectra were obtained with a Bruker AVANCE 400 MHz and a Varian 500 MHz spectrometer with solvent signal as internal reference.

3.2. Plant material

The bark of *D. decandra* was collected from Suratthani Province, in 1995. The botanical identification was kindly made by Assoc. Professor Dr. Nijsiri Ruangrungsi, Department of Pharmacognosy, Faculty of Pharmaceutical Science, Chulalongkorn University. A voucher specimen (SSDD/1997) is deposited at the Chemistry Department, Faculty of Science, Ramkhamhaeng University, Bangkok.

3.3. Extraction and isolation

The pulverized dry bark (868.5 g) was defatted with hexane, then extracted using Soxhlet extractor with $CHCl_3$ and MeOH to yield $CHCl_3$ (18.92 g) and MeOH (176.02 g) extracts. The $CHCl_3$ extract was subjected to gradient column chromatography (silica gel, hexane to $CHCl_3$–MeOH 40:60) to obtain three major fractions. The most polar fraction, fraction 3 was purified using silica gel column chromatography (hexane–$CHCl_3$ 50:50 to $CHCl_3$–MeOH 80:20) to obtain three subfractions (3.1–3.3), further purification of subfraction 3.2 (2×, silica gel, hexane–$CHCl_3$ 65:35 to

$CHCl_3$–MeOH 10:90 then $CHCl_3$ to $CHCl_3$–MeOH 90:10) gave subfractions 3.2.1–3.2.4. Subfraction 3.2.3 was rechromatographed (silica gel, hexane–$CHCl_3$ 20:80 to $CHCl_3$–MeOH 10:90), and gave compound **1** (50 mg). Acetylation of **1** (20 mg) using acetic anhydride–pyridine gave **1a** (18 mg). Methylation of **1a** (10 mg) with CH_2N_2 gave **1b** (10 mg). Fraction 3.2.1 after purification using silica gel column chromatography (2×, hexane–EtOAc 80:20 to 50:50 then hexane–EtOAc 85:15) gave betulinic acid (8.8 mg). Column chromatography of subfraction 3.3 (silica gel, $CHCl_3$ to $CHCl_3$–MeOH 80:20) gave four subfractions (3.3.1–3.3.4). Subfraction 3.3.3 after purification (silica gel, hexane–EtOAc 50:50 to EtOAc–MeOH 80:20) gave subfractions 3.3.3.1–3.3.3.4. Subfraction 3.3.3.3 contained compound **2** (48 mg). Compound **2a** (18 mg) was obtained from **2** (20 mg) after acetylation. Methylation of **2a** (10 mg) gave the methyl ester **2b** (9.5 mg). Purification of subfraction 3.3.2 using column chromatography (silica gel, hexane–EtOAc 60:40 to EtOAc–MeOH 90:10) gave four subfractions (3.3.2.1–3.3.2.4). Compound **3** (12.7 mg) was obtained from subfraction 3.3.2.3 after chromatographic separation ($C_{18,}$ MeOH–H_2O 50:50 to MeOH then silica gel, hexane–EtOAc 70:30). Subfraction 3.3.2.2 after reversed phase column chromatography (C_{18}, MeOH–H_2O 50:50 to MeOH) gave four subfractions (3.3.2.2.1–3.3.2.2.4). Compound **4** (7.7 mg) was obtained after subfraction 3.3.2.2.2 was further purified (silica gel, CH_2Cl_2–MeOH 98:2). Methylation of subfraction 3.3.2.2.3 using CH_2N_2 and further purification using HPLC (RP-18 Lichrosphere 100×5 mm, MeOH–H_2O 70:30, flow rate 0.7 mL/min) gave **4a** (t_R=20.9 min, 1.5 mg) and **5** (t_R=30.2 min, 0.4 mg).

3.3.1. 2-Oxo-3β,19α-dihydroxy-24-nor-urs-12-en-28-oic acid (1). Colorless needles, mp 161–162 °C; R_f=0.45 (silica gel, hexane–EtOAc 60:40): $[\alpha]_D^{27}$ 14.4 (*c* 0.290, MeOH); IR (film) ν_{max}: 3569, 2925, 2860, 1709, 1686, 1623, 1461, 1385, 1375, 1266, 1218, 1158, 1107, 1044, 1018, 968, 935, 756 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC; H-1/C-2, C-3, C-4, C-5, C-9, C-10, C-25; H-3/C-2, C-4, C-5, C-23; H-4/C-3; H-5/C-1, C-3, C-9, C-23, C-25; H-6/C-8; H-7/C-5, C-26; H-9/C-1, C-5, C-8, C-11, C-14, C-25, C-26; H-11/C-9, C-12, C-13; H-12/C-9, C-11, C-14, C-18; H-15/C-16, C-17, C-27; H-16/C-15; H-18/C-12, C-13, C-16, C-17, C-19, C-20, C-28, C-29; H-21/C-17, C-19; H-22/H-17, C-20; H-23/C-3, C-4, C-5; H-25/C-1, C-4, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21, C-29; HRFABMS negative ionization mode *m/z* 471.3118 $[M-H]^-$ (calcd for $C_{29}H_{43}O_5$, 471.3111).

3.3.2. 2-Oxo-3β-*O*-acetyl-19α-hydroxy-24-nor-urs-12-en-28-oic acid (1a). Colorless needles, mp 260–261 °C: R_f=0.51 (silica gel, hexane–EtOAc 60:40, double runs): $[\alpha]_D^{27}$ 105.0 (*c* 0.14, MeOH); IR (film) ν_{max}:3504, 2936, 1726, 1676, 1453, 1247, 1227 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-9, C-10, C-25; H-3/C-2, *CO*CH_3-3, C-4, C-23; H-4/C-3, C-5, C-8, C-10, C-23; H-5/C-4, C-6, C-9, C-10, C-23, C-25; H-6/C-3, C-7, C-8; H-7/C-6, C-8, C-26; H-9/C-1, C-5, C-8, 11, C-25; H-11/C-8, C-9, C-12, C-13; H-12/C-9, C-11, C-14, C-18; H-15/C-8, C-14, C-16, C-17, C-27; H-16/C-14, C-15, C-17, C-18; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-20, C-28, C-29; H-20/C-18, C-21, C-29, C-30; H-21/C-17; H-22/C-16, C-17, C-18, C-20, C-21; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-C-18, C-19, C-20; H-30/C-19, C-20, C-21; CO*CH*$_3$-3/C-3, *CO*CH_3-3 HREIMS *m/z* 514.3294 $[M]^+$ (calcd for $C_{31}H_{46}O_6$, 514.3292).

3.3.3. 2-Oxo-3β-*O*-acetyl-19α-hydroxy-24-nor-urs-12-en-28-oic acid methyl ester (1b). R_f=0.55 (silica gel, hexane–EtOAc 80:20): $[\alpha]_D^{27}$ 57.2 (*c* 0.185, MeOH); IR (film) ν_{max}: 3516, 2930, 1720, 1452, 1375, 1283, 1251, 1234, 1192, 1154, 1096, 1074, 1050, 1030, 1013, 962, 769 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-6, C-9, C-10, C-25; H-3/C-2, CH_3*CO*-3, C-4, C-23; H-4/C-C-1, C-3, C-5, C-23; H-5/C-C-6, C-6, C-23, C-25; H-7/C-5, C-6, C-8, C-26; H-9/C-1, C-8, C-10, C-11, C-14, C-25, C-26; H-11/C-8, C-9, C-12, C-13, C-19; H-12/C-9, C-11, C-18, C-19; H-15/C-13, C-14, C-16, C-17, C-27; H-16/C-14, C-15, C-17, C-18, C-28; H-18/C-12, C-13, C-16, C-17, C-19, C-28, C-29; H-20/C-22; H-21/C-17; H-22/C-16, C-17, C-18, C-28; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; *CH*$_3$CO-3/C-3, CH_3*CO*-3; O*CH*$_3$-28/C-28; HRFABMS positive ionization mode *m/z* 529.3532 $[M+H]^+$ (calcd for $C_{32}H_{49}O_6$, 529.3529).

3.3.4. 2-Oxo-3β,19α,22α-trihydroxy-24-nor-urs-12-en-28-oic acid (2). R_f=0.43 (silica gel, hexane–EtOAc 50:50): $[\alpha]_D^{27}$ 19.5 (*c* 0.060, MeOH); IR (film) ν_{max}: 3397, 2916, 2850, 1711, 1635, 1451, 1378, 1259, 1216, 1165, 1087, 1051, 1011, 930 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-6, C-9, C-10, C-25; H-3/C-2, C-4, C-23; H-4/C-3, C-5, C-23; H-7/C-5, C-6, C-8, C-11, C-14; H-9/C-1, C-8, C-11, C-14, C-25, C-26; H-11/C-8, C-9, C-12, C-13, C-25; H-12/C-9, C-11, C-14, C-18; H-15/C-12, C-16, C-27; H-16/C-14, C-15, C-17, C-18, C-28; H-18/C-12, C-13, C-16, C-17, C-19, C-28, C-29; H-21/C-17, C-19, C-20, C-22, C-30; H-22/C-16, C-20, C-21, C-28; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; HRFABMS negative ionization mode *m/z* 487.3067 $[M-H]^-$ (calcd for $C_{29}H_{43}O_6$, 487.3060).

3.3.5. 2-Oxo-3β,22α-di-*O*-acetyl-19α-hydroxy-24-nor-urs-12-en-28-oic acid (2a). R_f=0.35 (silica gel, hexane–EtOAc 70:30, double runs): $[\alpha]_D^{27}$ 72.1 (*c* 0.55, MeOH); IR (film) ν_{max}: 3498, 2934, 2875, 1721, 1453, 1371, 1240, 1226, 1155, 1087, 1049, 1027, 969, 933, 885, 804, 760 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-6, C-9, C-10, C-19, C-25; H-3/C-2, C-4, C-23, *CO*CH_3-3; H-4/C-5, C-25; H-5/C-4, C-25; H-6/C-9; H-7/C-5, C-6, C-8, C-26; H-9/C-1, C-8; H-11/C-3, C-9, C-10, C-12, C-13; H-12/C-9, C-11, C-14, C-16, C-18, C-19; H-15/C-14, C-16, C-17, C-27; H-16/C-14, C-15, C-17, C-18, C-22, C-28; H-18/C-12, C-13, C-14, C-16, C-17, C-22, C-28, C-29; H-20/C-30; H-21/C-17, C-19, C-20, C-22, C-30; H-22/C-16, C-17, C-20, C-21, C-28, *CO*CH_3-22; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21,

C-22; *CH*$_3$CO-3/C-3, CH$_3$*CO*-3; *CH*$_3$CO-22/C-22, CH$_3$*CO*-22; HRFABMS positive ionization mode *m/z* 573.3429 [M+H]$^+$ (calcd for $C_{33}H_{49}O_8$, 573.3428).

3.3.6. 2-Oxo-3β,22α-di-*O*-acetyl-19α-hydroxy-24-nor-urs-12-en-28-oic acid methyl ester (2b). R_f=0.52 (silica gel, hexane–EtOAc 70:30): $[\alpha]_D^{27}$ 72.8 (*c* 0.15, MeOH); IR (film) ν_{max}: 3539, 2949, 2875, 2156, 1722, 1456, 1370, 1240, 1228, 1154, 1087, 1049, 1025, 970, 930, 886, 806, 770, 692 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-9, C-10, C-25; H-3/C-2, CH$_3$*CO*-3, C-4, C-23; H-4/C-1, C-3, C-5, C-25; H-5/C-4, C-6, C-25; H-9/C-5, C-8, C-10, C-11, C-25, C-26; H-11/C-9, C-10, C-12, C-13; H-12/C-9, C-11, C-18; H-15/C-8, C-14, C-16, C-27; H-16/C-15, C-17, C-18, C-22, C-28; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28, C-29; H-20/C-30; H-21/C-17, C-20; H-22/C-16, C-17, CH$_3$*CO*-22, C-28; H-23, C-3, C-4, C-5; H-25/C-7, C-8, C-9, C-14; H-27/C-13, C-14; *CH*$_3$CO-28/CH$_3$*CO*-28; H-29/C-19, C-20, C-21; *CH*$_3$CO-3/C-3, *CO*CH$_3$-3; *CH*$_3$CO-22/CH$_3$*CO*-22; *CH*$_3$CO-28/C-28; HRFABMS positive ionization mode *m/z* 587.3582 [M+H]$^+$ (calcd for $C_{34}H_{51}O_8$, 587.3584).

3.3.7. 3-Oxo-2,19α,22α-trihydroxy-24-nor-urs-1,4,12-trien-28-oic acid (3). Colorless solid, mp 182–184 °C; R_f=0.46 (silica gel, CH_2Cl_2/MeOH 92:8): $[\alpha]_D^{23}$ 45.9 (*c* 0.26, MeOH); IR (film) ν_{max}: 3419, 2930, 2881, 1697, 1623, 1456, 1429, 1376, 1239, 1167, 1058, 1018, 756, 517 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 3; HMBC: H-1/C-2, C-3, C-5, C-9, C-10, C-25; H-6/C-4, C-7; H-9/C-1, C-5, C-7, C-8, C-10, C-11, C-25, C-26; H-11/C-8; H-12/C-9; H-15/C-8, C-14, C-16, C-27; H-16/C-15, C-18; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28; H-21/C-17, C-19, C-20, C-22; H-22/C-16, C-18, C-28; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; HREIMS negative ionization mode *m/z* 483.2752 [M−H]$^-$ (calcd for $C_{29}H_{39}O_6$, 483.2749)

3.3.8. 4-Oxo-19α,22α-dihydroxy-3,24-dinor-2,4-*seco*-urs-12-en-2,28-dioic acid (4). R_f=0.31 (silica gel, CH_2Cl_2/MeOH 95:5, double runs): $[\alpha]_D^{27}$ 28.7 (*c* 0.135, MeOH); IR (film) ν_{max}: 3554, 2951, 2922, 2850, 2653, 1690, 1456, 1375, 1362, 1259, 1158, 1082, 1029, 930, 890, 804 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 3; HMBC: H-1/C-2, C-5, C-9, C-10, C-25; H-5/C-4, C-6, C-10, C-25; H-9/C-1, C-10, C-11, C-14, C-21, C-25, C-26; H-12/C-9, C-11, C-14; H-15/C-8, C-27; H-16/C-15, C-17; H-17, C-14; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28, C-29; H-20/C-18; H-21/C-17, C-20, C-22, C-30; H-22/C-16, C-18, C-21; H-23/C-4, C-5; H-25/C-1, C-2, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; HRFABMS negative ionization mode *m/z* 489.2857 [M−H]$^-$ (calcd for $C_{28}H_{41}O_7$, 489.2853).

3.3.9. 4-Oxo-19α,22α-dihydroxy-3,24-dinor-2,4-*seco*-urs-12-en-2,28-dioic acid dimethyl ester (4a). R_f=0.40 (silica gel, CH_2Cl_2/MeOH 98:2): $[\alpha]_D^{24}$ 65.6 (*c* 0.08, MeOH); ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 3; HMBC: H-1/C-2, C-5, C-9, C-25; H-5/C-4, C-9, C-10, C-25; H-7/C-6, C-8, C-14, C-26; H-9/C-1, C-8, C-11, C-14, C-25, C-26; H-12/C-9, C-11, C-14; H-15/C-16, C-27; H-16/C-15, C-28; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28; H-21/C-18, C-20, C-22, C-30; H-22/C-16; H-23/C-4; H-25/C-1, C-2, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-9, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; *CH*$_3$CO-2/C-2; *CH*$_3$CO-28/C-28; HREIMS *m/z* 518.3307 [M]$^+$ (calcd for $C_{30}H_{46}O_7$, 518.3243).

3.3.10. 19α,22α-Dihydroxy-24-nor-2,3-*seco*-urs-12-en-2,3,28-trioic acid trimethyl ester (5). R_f=0.41 (silica gel, CH_2Cl_2/MeOH 98:2): IR (film) ν_{max}: 3508, 2941, 2918, 1721, 1456, 1433, 1373, 1350, 1266, 1216, 1193, 1158, 1102, 1089, 1014, 935, 735 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 3; HMBC: H-1/C-2, C-5, C-9, C-10, C-25; H-6/C-4, C-5, C-7, C-8; H-7/C-5, C-9, C-14, C-26; H-9/C-1, C-8, C-10, C-11, C-14, C-25, C-26; H-11/C-8, C-9, C-12, C-13; H-12/C-9, C-14, C-18; H-15/C-8; H-16/C-14, C-15, C-17, C-28; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28; H-20/C-30; H-21/C-17, C-19, C-20, C-22, C-30; H-22/C-16, C-21; H-23/C-1, C-3, C-4, C-5, C-10, C-25; H-25/C-1, C-2, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19; H-30, C-19, C-20, C-21; O*CH*$_3$-2/C-2; O*CH*$_3$-3/C-3; O*CH*$_3$-28/C-28; HREIMS *m/z* 560.3441 [M]$^+$ (calcd for $C_{32}H_{48}O_8$, 560.3349).

3.4. Bioassays

Antimalarial activity was evaluated against *Plasmodium falciparum* (K1 multidrug-resistant strain) cultured continuously according to Trager and Jensen.[14] Quantitative determination of antimalarial activity in vitro was achieved by means of the microculture radioisotope technique based on Desjardins et al. described method.[14] The anti-mycobacterial activity (anti-TB) assay was performed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay.[15] Antifungal test was undertaken against *Candida albicans* (ATCC 90028) using tetrazolium/formazan assay method.[16] Cytotoxicity assays were evaluated using the colorimetric method.[17]

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