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Macrocarpins A–D, New Cytotoxic Nor-Triterpenes from *Maytenus macrocarpa*

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Abstract—Macrocarpins A (**1**), B (**2**), C (**3**) and D (**4**), four new nor-triterpenes, have been isolated from the roots of *Maytenus macrocarpa*. The structures were established by spectroscopic examinations. Natural compounds **1**, **2**, **4** and the acetyl derivative **1a** are cytotoxic against four tumoral cell lines with IC_{50} values ranging between 0.4 and 5.2 μM.

Species of the genus *Maytenus* (Celastraceae) have been widely used in folk medicine.[1,2] Two examples are the Peruvian species *Maytenus amazonica* and *Maytenus macrocarpa*, which are used in the treatment of rheumatism, influenza, gastrointestinal diseases, and as an antitumor agent against skin cancer. In previous papers[3,4] we reported on the isolation and characterization of 14 triterpenes, from cytotoxic fractions of the roots of *M. amazonica*. Among them 2,3,22-trihydroxy-6,23-dioxo-tingenol[3] exhibited high cytotoxicity against A-549 human lung carcinoma with an IC_{50} value of 0.25 μg/mL (0.5 μM). Following this search for novel plant-derived cytotoxic agents in *Maytenus* spp. plants, we investigated the cytotoxic constituents of the roots of *M. macrocarpa*.[5,6] Fractionation of the extract (*n*-hexane:Et_2O 1:1) (40 g) using column chromatography on silica gel, Sephadex LH-20, and semi-preparative scale HPLC on Kromasil 100 Si 5μ, has led to the isolation of four new triterpenes, named macrocarpins A (**1**, 15 mg), B (**2**, 7 mg), C (**3**, 5 mg), and D (**4**, 12 mg).

Macrocarpin A (**1**)[7] was isolated as a pale yellow lacquer. The molecular formula $C_{30}H_{40}O_6$ was established by HREIMS. Its IR spectrum showed absorption bands for hydroxyl (3373 cm^{-1}) and carbonyl (1726, 1645 cm^{-1}) groups. Its ^1H NMR spectrum had signals for a CHO group, five angular methyls, an aromatic proton, and one methoxy group. These data, together with ^{13}C NMR data indicated that **1** was a 24-nor-D:A-friedelane. From the HMQC and HMBC spectra, we confirmed that the B, C, D and E rings were the same as in the previously reported blepharodol.[8]

With regard to ring A, the position of the CHO group on C-23 was established by HMBC experiments (see Fig. 1), which showed three-bond couplings between the aldehyde proton at δ 10.53 and two quaternary carbons at δ 148.65 and δ 124.41 (C-3 and C-5, respectively). A ROESY experiment showing the NOE effect between H-8 and Me-27 confirmed that the configuration of H-8 is α. Compound **1** formed a diacetate **1a**[9] when it was treated with Ac_2O/Py (δ 2.32 (3H, s), 2.26 (3H, s)). All these data allowed us to establish the structure of **1** as (20α)-2, 3-dihydroxy-6,23-dioxo-24-nor-friedela-1 (10), 3,5,7-tetraen-carboxylic acid-(29)-methylester, and we named it macrocarpin A.

Macrocarpin B (**2**)[10] showed the base peak at *m/z* 482, corresponding to the molecular formula $C_{28}H_{34}O_7$. The ^{13}C NMR, DEPT and HMBC spectra of **2** indicated the presence of three carbonyl carbons (213.09 s, 188.00 s, 173.69 s), five methyl groups (δ 14.73, 20.20, 20.41, 25.07, 37.96), two vinylic carbons conjugated to carbonyl

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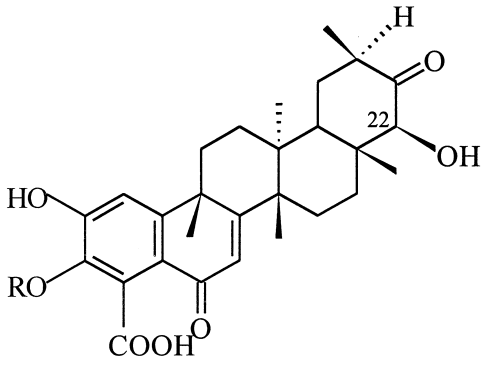

2 R=H
3 R=Me

group (δ 124.47 d, 178.29 s), five methylene carbons (δ 28.17, 29.39, 30.07, 31.92, 34.82), three methine carbons (δ 42.56, 44.72, 44.96), and six aromatic carbons. Among these aromatic carbons, five were quaternary (δ 111.36, 119.56, 153.03, 153.46, 155.48) and the remaining one was an unsubstituted aromatic carbon (δ 113.69). Its ^{1}H NMR spectrum revealed the presence of the typical doublet at δ 4.52 ($J = 3.2$ Hz) characteristic of the 22-β-hydroxy functionalization in the tingenone skeleton.[3] Two protons appeared in the low field region as singlets at δ 7.33 and 6.52. These signals were similar to the corresponding H-1, H-7 in the 2,3,22β-trihydroxy-24,29-dinor-1,3,5(10), 7-friedelatetraene-6,21-dione-23-al, recently isolated by us from the roots of *M. amazonica*.[3] The previously mentioned compound could be considered as the biogenetic precursor of **2**.

Macrocarpin C (**3**)[11] was isolated as a yellow lacquer with a molecular formula $C_{29}H_{36}O_7$. Its spectral data were very similar to those of **2**, except for the signal corresponding to a methoxy group (δ 3.94 s). The position of this group on C-3 was verified by a ROESY experiment, which did not show any NOE effect between the H-1 and the OMe group. The HMBC spectra showed two three bond couplings, between H-1 and C-3, and between the singlet corresponding to the OMe group and C-3. These data allowed us to establish the structure of Macrocarpin C as **2**, 22β-dihydroxy-3-methoxy-24, 29-dinor-1,3,5(10), 7-friedelatetraene-6, 21-dione-23-oic acid.

Macrocarpin D (**4**)[12] had the molecular formula $C_{28}H_{38}O_5$. Its ^{1}H NMR spectrum revealed the presence of five angular methyls (one of them on an aromatic ring), one doublet methyl and one doublet at δ 4.58 ($J = 3.3$ Hz). In addition, there were two singlets at δ 6.24 and δ 6.68, and a doublet at δ 4.77 ($J = 9.6$ Hz). These signals characterize H-1, H-6 and H-7 in 7-hydroxy-quinonemethide triterpenoids, which are rather

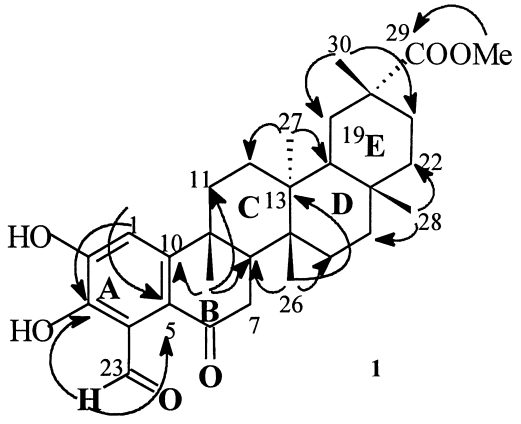

Figure 1. C–H long range correlations.

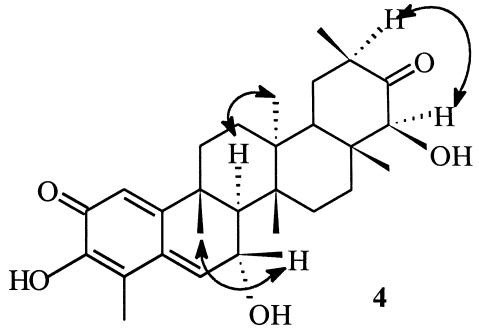

Figure 2. Selected NOEs observed.

Table 1. ^{13}C NMR ($CDCl_3$) (75 MHz) of **1**–**4**[a]

C	1	2	3	4	C	1	2	3	4
1	114.57 d	113.69 d	112.72 d	116.82 d	**15**	28.38 t	28.17 t	28.31 t	29.19 t
2	149.46 s	153.03 s	152.08 s	181.36 s	**16**	36.05 t	29.39 t	29.52 t	29.65 t
3	148.65 s	153.46 s	143.27 s	145.73 s	**17**	30.17 s	44.96 s	44.84 s	44.65 s
4	117.82 s	111.36 s	107.82 s	117.72 s	**18**	44.50 d	44.84 d	45.04 d	45.51 d
5	124.41 s	119.56 s	115.53 s	131.17 s	**19**	30.41 t	31.92 t	31.93 t	31.87 t
6	199.46 s	188.00 s	183.74 s	144.02 d	**20**	40.44 s	40.85 d	40.82 d	41.31 d
7	36.52 d	124.47 d	124.44 d	69.43 d	**21**	29.57 t	213.09 s	213.43 s	213.85 s
8	42.71 d	178.29 s	178.72 s	53.05 d	**22**	35.84 t	76.42 d	76.53 d	77.20 d
9	37.24 s	42.56 s	39.93 s	40.47 s	**23**	199.04 d	173.69 s	172.54 s	10.46 q
10	152.40 s	155.48 s	150.01 s	162.21 s	**25**	25.10 q	37.96 q	37.61 q	27.47 q
11	32.83 t	34.82 t	33.93 t	31.87 t	**26**	15.19 q	20.41 q	20.63 q	16.31 q
12	29.77 t	30.07 t	29.74 t	30.78 t	**27**	16.75 q	20.20 q	20.51 q	19.29 q
13	38.72 s	42.56 s	40.54 s	39.32 s	**28**	31.63 q	25.07 q	25.04 q	25.16 q
14	37.34 s	44.72 s	44.42 s	41.21 s	**30**	32.10 q	14.73 q	14.72 q	14.83 q

[a]Data based on HMBC, HMQC and DEPT experiments.

Table 2. Cytotoxic activity against cultured cell lines (IC_{50})[a]

Cell lines	1		1a		2		3		4	
	(μg/mL)	(μM)	(μg/mL)	(μM)	(μg/mL)	(μM)	(μg/mL)	(μM)	(μg/mL)	(μM)
P-388D1 (ATCC CCl-46)	0.5	1.0	0.25	0.4	2.5	5.2	10.0	20.7	0.5	1.0
A-549 (ATCC CCl-185)	0.5	1.0	0.25	0.4	2.5	5.2	10.0	20.7	1.0	2.0
HT-29 (ATCC HTB-38)	1	2.0	0.25	0.4	2.5	5.2	10.0	20.7	1.0	2.0
SK-MEL-28 (ATCC HTB-72)	0.5	1.0	0.25	0.4	2.5	5.2	10.0	20.7	1.0	2.0

[a]P-388: mouse lymphoma (ATCC:CCL 46); A-549: human lung carcinoma (ATCC:CL85); HT-28: human colon carcinoma (ATCC:HTB38); MEL-28: human melanoma (ATCC: HTB72).

uncommon (only two triterpenoids are known to belong to this category).[8] The C-7 hydroxyl was confirmed by ^1H–^1H COSY experiments, which showed the coupling between the singlet at δ 6.68 (H-6) and the doublet. The α-configuration of the hydroxyl group was deduced from the coupling constant between its geminal proton and H-8 ($J = 9.6$ Hz), which agreed with the predicted conformation from molecular mechanics calculations.[13] A ROESY experiment showing NOE effect between H-7 and Me-25 confirmed the relative configuration (Fig. 2).

Cytotoxic effects[14] of the pure isolates were evaluated with a battery of cultured tumor cell as summarized in Table 2. Since the A, B and E rings of the macrocarpins are significantly different, it is difficult to establish a clear relationship between structure and cytotoxicity. However, the addition of acetate groups increases the activity from **1** to **1a**, and we also note how the substitution of an OH group by an methoxy group in the carbon 3 drastically decreases the activity from **2** to **3**.

Acknowledgements

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7. Macrocarpin A (**1**): $[\alpha]_D^{20}$ −68 (*c* 0. 6, $CHCl_3$); UV (EtOH) λ_{max}: 204, 241, 294, 370; IR (film) V_{max} 3373, 2930, 2980, 1726, 1645, 1593, 1455, 1213, 882, 756 cm^{-1}; EIHRMS *m/z*: 496.2823 (calcd for $C_{30}H_{40}O_6$, $(M)^+$, 496.2825); ^1H NMR ($CDCl_3$, 400 MHz): δ 12.43 (1H, bs, OH-C3), 10.53 (1H, s, H-23), 7.14 (1H, s, H-1), 6.23 (1H, bs, OH-C-2), 3.62 (3H, s COOMe), 2.61 (1H, s, H-7 b), 2.60 (1H, s, H-7 a), 2.30 (1H, m, H-8), 1.62 (1H, m, H-18), 1.17 (3H, s, Me-30), 1.15 (3H, s, Me-25), 1.08 (3H, s, Me-28), 1.01 (3H, s, Me-26), 0.82 (3H, s, Me-27); ^{13}C NMR (CDCl3, 400 MHz): δ 51.50 (q, COOME), 179.03 (s, COOMe), for the rest of the signals, see Table 1.
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9. Acetate of Macrocarpin A (**1a**): It was obtained from the reaction of **1** with Ac_2O/py and DMAP. $[\alpha]_D^{20}$ −36° (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max}: 200, 219, 260, 300; IR (film) V_{max} 2926, 2920, 1760.6, 1636, 1700, 1458, 1120, 756 cm^{-1}; EIHRMS *m/z*: 580.3037 (calcd for $C_{34}H_{44}O_8$, $(M)^+$, 580.3036); ^1H NMR ($CDCl_3$, 400 MHz): δ 10.03 (1H, s, H-23), 7.30 (1H, s, H-1), 3.62 (3H, s, COOMe), 2.67 (2H, m, H-7), 2.32 (3H, s, MeCOO-), 2.26 (3H, s, MeCOO-), 1.24 (3H, s, Me-30), 1.19 (3H, s, Me-25), 1.10 (3H, s, Me-28), 1.04 (3H, s, Me-26), 0.83 (3H, s, Me-27); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 15.39 (q, Me-26), 16.97 (q, Me-27), 20.39 (q, COOMe), 20.63 (q, COOMe), 25.61 (q, Me-25), 28.49 (t, C-15), 29.54 (t, C-12), 29.81 (t, C-21), 30.24 (s, C-17), 30.53 (t, C-19), 31.77 (q, Me-28), 32.17 (q, Me-30), 32.72 (t, C-11), 35.65 (t, C-16), 35.87 (t, C-22), 36.22 (t, C-7), 37.69 (s, C-9), 38.87 (s, C-14), 39.57 (s, C-13), 40.55 (s, C-20), 43.15 (d, C-8), 44.58 (d, C-18), 51.66 (q, COOMe), 120.90 (d, C-1), 124.27 (s, C-4), 129.04 (s, C-5), 134.27 (s, C-3), 147.16 (s, C-2), 156.40 (s, C-10), 167.66 (s, -OCOMe), 168.18 (s, -OCOMe), 179.14 (s, COOMe), 189.93 (d, C-23), 198.96 (s, C-6).
10. Macrocarpin B (**2**): $[\alpha]_D^{20}$ −64 (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max}: 203, 250, 303, 340 nm; IR (film) V_{max}; 3455, 2926, 1710, 1621, 1461, 1304, 1104, 992, 880, 755; EIHRMS *m/z*: 482.2300 (calcd for $C_{28}H_{34}O_7$, $(M)^+$, 482.2304); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.33 (1H, s, H-1), 6.52 (1H, s, H-7), 4.52 (1H, d, $J = 3.2$ Hz, H-22), 3.67 (1H, bs, OH-22), 2.66 (1H, m, H-20), 1.67 (3H, s, Me-25), 1.44 (3H, s, Me-26), 1.07 (3H, d, $J = 6.3$ Hz), 0.99 (3H, s, Me-27), 0.89 (3H, s, Me-28).
11. Macrocarpin C (**3**): $[\alpha]_D^{20}$ −41 (*c* 0. 3, $CHCl_3$); UV (EtOH) λ_{max}: 204, 246, 260, 336 nm; IR (film) V_{max} 3417, 2929, 2980, 1710, 1641, 1592, 1456, 1380, 1311, 1119, 1043, 755 cm^{-1}; EIHRMS *m/z*: 496.2444 (calcd for $C_{29}H_{36}O_7$, $(M)^+$, 496.2461); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.09 (1H, s, H-1), 6.35 (1H, s, H-6), 4.54 (1H, bs, H-22), 3.94 (3H, s, OMe), 2.65 (1H, m, H-20), 1.58 (3H, s, Me-25), 1.39 (3H, s, Me-26), 1.06 (3H, d, $J = 6.1$ Hz, Me-30), 1.02 (3H, s, Me-27), 0.87 (H, s, Me-28).
12. Macrocarpin D (**4**): $[\alpha]_D^{20}$ −39 (*c* 0.7, $CHCl_3$); UV (EtOH) λ_{max}: 202, 230, 266, 309 nm; IR (film) V_{max} 3407, 2928, 1706, 1614, 1454, 1381, 1306, 1223, 999, 755 cm^{-1}; EIHRMS *m/z*: 454.2711 (calcd for $C_{28}H_{38}O_5$, $(M)^+$, 454.2719); ^1H NMR ($CDCl_3$, 400 MHz): 6.77 (1H, bs, OH-C3), 6.68 (1H, s, H-7), 6.24 (1H, s, H-1), 4.77 (1H, d, $J = 9.6$ Hz, H-7), 4.58 (1H, bs, H-22), 3.67 (1H, bs, OH-22), 2.72 (1H, m, H-20), 2.07 (s, Me-

23), 1.98 (1H, d, $J = 9.8$ Hz), 1.31 (6H, s, Me-26 + Me-27), 1.19 (3H, s, Me-25), 1.08 (3H, d, $J = 9.6$ Hz), 0.88 (3H, s, Me-28).
13. The Molecular Mechanics PC Model program (version 6.0) was used in the Molecular Mechanics Calculations.
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