New Phenolic and Quinone−Methide Triterpenes from *Maytenus amazonica*

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Received September 21, 1998

The new nortriterpene methylene quinones amazoquinone (**1**) and (*7S*,*8S*)-7-hydroxy-7,8-dihydro-tingenone (**2**), and the new norphenolic triterpenes 7,8-dihydro-6-oxo-tingenol (**3**), 23-*nor*-6-oxo-tingenol (**4**), and 23-oxo-iso-tingenone (**5**) were isolated from *Maytenus amazonica*. Their structures were elucidated by spectroscopic methods. Compounds **1**, **2**, **3**, and **5** showed low antitumor activity against four cancer cell lines.

Maytenus amazonica C. Martius (Celastraceae)[1] is a large tree found throughout the Amazonian region of Peru. The genus *Maytenus* is rich in triterpenes,[2,3] and some members of this genus are extensively used in traditional Peruvian medicine[4] in the treatment of rheumatism, influenza, gastrointestinal diseases, and as an antitumor agent for skin cancer. This, together with the absence of any previous phytochemical work on *M. amazonica*, encouraged us to examine this species. Its roots have turned out to be extremely rich in secondary metabolites. Here we report the isolation and structure elucidation of five new nortriterpenoids (**1**−**5**) related to tingenone. We have also isolated the known compounds pristimerin, tingenone; celastrol; netzahualcoyene; blepharodol; 6-oxo-pristimerol; 6-oxo-tingenol; 22-α-hydroxy-tingenone; 3-*O*-methyl-23-hydroxy-6-oxo-tingenol; 3-*O*-methyl-23-hydroxy-22-α-hydroxy-tingenone; 7,8-dihydro-escutionin αA; 7,8-dihydro escutidin A; escutidin; and scutionin.[5−9]

Compound **1** was isolated as an orange lacquer with a molecular formula $C_{28}H_{36}O_4$. Its IR spectrum revealed the presence of hydroxyl (3550 cm^{-1}) and carbonyl groups (1720, 1660 cm^{-1}). Its ^1H NMR spectrum showed five angular methyls (one of them on an aromatic ring; δ 2.13) and one doublet methyl (δ 1.09). The spectrum also contained two doublets at δ 6.39 and 6.44, and two singlets at δ 3.02 and 7.02 (interchangeable with D_2O). These four signals characterize protons H-1, H-6, H-8, and the OH proton on C-3 in 7-oxo-quinonemethide nortriterpenoids.[10] Analysis of ^{13}C NMR, HMQC, and HMBC spectra (Figure 1) allowed the unequivocal assignment of all carbons. The absolute stereochemistry at C-8 was determined as *8S* after analysis of the CD spectrum. It showed a positive Cotton effect at 315 nm, in agreement with the rule for the n → π^* transitions in conjugated cyclohexanones.[11] All data mentioned above indicate that the structure of compound **1** is (8*S*)-7,8-dihydro-7-oxo-tingenone, for which we propose the name amazoquinone.

Compound **2**, with molecular formula $C_{28}H_{38}O_4$, showed NMR data similar to those of compound **1**. The main difference was the presence of a broad triplet at δ 4.75, produced by a proton geminal to a secondary alcohol. The C-7 hydroxyl group was established by $^1H-^1H$ COSY experiments, which showed the coupling between the singlet at δ 6.67 (H-6) and the broad triplet. The α stereochemistry of the hydroxyl group was deduced from the coupling constant between its geminal proton and H-8

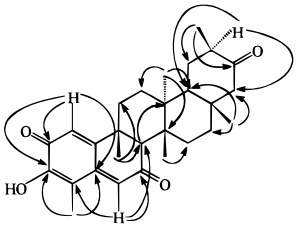

Figure 1. C−H long-range correlations for **1**.

(J = 9.7 Hz), which agreed with the predicted conformation from molecular mechanics calculations.[12] A ROESY experiment showing an NOE effect between H-7 and Me-25 confirmed the relative stereochemistry. The carbons on the A and B rings of compound **2** also had chemical shifts very close to those of a triterpene quinone−methide with this functionalization at C-7 reported in the literature.[6] Compound **2** can be considered the biogenetic precursor of **1**, so both compounds are assumed to share the same absolute stereochemistry *8S*. Consequently, we propose that the absolute stereochemistry of C-7 is *7S*. All data mentioned above allowed us to propose the structure of **2** as (*7S*,*8S*)-7-hydroxy-7,8-dihydro-tingenone.

Compound **3** showed IR bands for carbonyl (1700, 1650 cm^{-1}) and hydroxyl groups (3550 cm^{-1}) and the molecular formula $C_{28}H_{38}O_4$. The ^1H NMR spectrum contained signals for six methyl groups, which included four angular methyls, one doublet methyl at δ 1.02, and one methyl on an aromatic ring at δ 2.52. The latter must be coplanar with a carbonyl because of its chemical shift.[6] In the lowfield region, just one proton appeared as a broad singlet (δ 6.76). These data, together with the ^{13}C NMR spectra, indicated that **3** was a 6-oxo-phenolic triterpene related to blepharodol.[6] The structure of **3** was assigned as 7,8-dihydro-6-oxo-tingenol.

Compound **4**, with the molecular formula $C_{27}H_{34}O_4$, was obtained as a very minor component. Only five methyl signals were present in its ^1H NMR spectrum, and none of them corresponded to an aromatic methyl. The ^1H NMR spectrum also showed three aromatic protons as singlets at δ 7.70, 7.03, and 6.37. These signals were similar to the corresponding H-4, H-1, and H-6 signals in 23-*nor*-6-oxo-pristimerol.[13] All data mentioned above indicate that **4** is 23-*nor*-6-oxo-tingenol.

Compound **5** had the molecular formula $C_{28}H_{34}O_4$. Its IR spectrum showed absorption bands for hydroxyl (3381 cm^{-1}) and carbonyl (1708, 1639 cm^{-1}) groups. The ^1H NMR spectrum included signals for an aldehyde group; four angular methyls; a secondary methyl; and three olefinic

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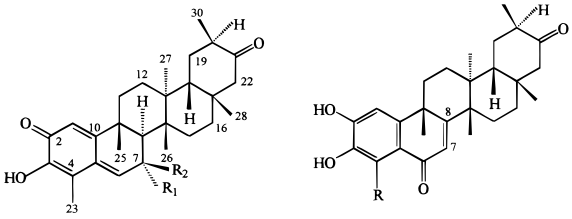
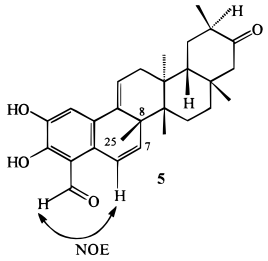
10.1021/np980412+ CCC: $18.00
Published on Web 02/11/1999

protons at δ 5.68 (d, J = 5.5 Hz), 6.70 (d, J = 10.5 Hz), and 6.76 (d, J = 10.5 Hz). These three vinyl hydrogens are characteristic of the H-11, H-7, and H-6 in triterpenes with the isotingenone skeleton.[14] The position of the CHO group on C-23 was established by the absence of an aromatic methyl and by ROESY experiments, which showed an NOE effect between the aldehyde proton (δ 10.38 s) and the doublet corresponding to H-6. These data indicate that **5** is 23-oxo-isotingenone III.





Compounds **1**, **2**, **3**, and **5** were tested for antitumor[15] and aldose reductase[16] inhibitory activities. Only compound **1** showed moderate antitumor activity (see Experimental Section) against four cell lines. None of the compounds showed significant inhibitory activity in the aldose reductase assay (IC_{50} >25 μg/mL).

Experimental Section

General Experimental Procedures. IR spectra were taken on a PE 681 spectrophotometer. ^{1}H and ^{13}C NMR spectra were gathered using a Bruker W-200SY at 400 and 100 MHz, respectively, with TMS as internal reference. The HMBC, HMQC, and ROESY were run on a Bruker at 400 MHz. Optical rotations were measured with a Perkin−Elmer 241 automatic polarimeter; $[\alpha]^{20}_D$ are given in 10^{-1} deg cm^{2}g^{-1}. UV spectra were collected with a Perkin−Elmer model 550-SE. MS were recorded on a VG Micromass ZAB-2F and a Hewlett−Packard 5995. HRMS were recorded on a VG Autospec spectrometer. Schleicher−Schüll F-100/LS 254 and preparative TLC 1510/LS 254 foils were used for TLC, while Si gel (0.2−0.63 mm) and Sephadex LH-20 were used for column chromatography. CD spectra were run on a JASCO J-600 spectropolarimeter.

Plant Material. The plant was collected in Loreto Region (Perú), in November 1996, and was identified by the botanist J. Ruiz. A voucher specimen is on file with the Herbarium of the Departamento de Botánica, Universidad Nacional de la Amazonía (Iquitos, Peru).

Extraction and Isolation. Root bark of *M. amazonica* (0.3 kg) was extracted with *n*-hexane−Et_2O (1:1) (2 L) in a Soxhlet apparatus. The extract (70 g) was repeatedly chromatographed on Sephadex LH-20 and Si gel using as solvents mixtures of *n*-hexane−$CHCl_3$−MeOH (2:1:1) and of *n*-hexane−EtOAc, respectively. The chromatographed extract yielded **1** (13 mg), **2** (3.3 mg), **3** (6 mg), **4** (1.5 mg), and **5** (3.5 mg).

(8*S*)-7,8-Dihydro-7-oxo-tingenone (1): orange lacquer; $[\alpha]^{20}_D$ −129° (*c* 0.3, $CHCl_3$); CD λ_{max} (EtOH) nm ($\Delta\epsilon$) 315.0 (+1.7); UV (EtOH) λ_{max} 201, 219, 318, 339, 340 nm; IR ($CHCl_3$) ν_{max} 3550, 2960, 2800, 1720, 1660, 1620, 1460, 1380, 1190, 850 cm^{-1}; EIMS *m/z* (rel. int) 436 (M^+) (79), 286 (12), 245 (11), 231 (22), 217 (100), 204 (12), 149 (57), 135 (35); HREIMS calcd for $C_{28}H_{36}O_4$ 436.2250, found 436.2247; ^{1}H NMR ($CDCl_3$,400 MHz) δ 7.07 s (OH-3), 6.44 (1H, d, J = 1.3 Hz, H-6), 6.39 (1H, d, J = 1.3 Hz, H-1), 3.02 s (1H, H-8), 2.13 s (3H, Me-23), 1.39 s (3H, Me-25), 1.38 s (3H, Me-26),1.29 s (3H, Me-27), 1.01 s (3H, Me-28), 1.09 d (3H, J = 6.1 Hz, Me-30); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 213.90 (s, C-21), 200.15 (s, C-7), 181.14 (s, C-2), 161.77 (s, C-10), 146.63 (s, C-3), 141.08 (s, C-5), 131.69 (d, C-6), 119.65 (d, C-1), 117.26 (s, C-4), 57.58 (d, C-8), 53.52 (t, C-22), 42.98 (d, C-18), 42.18 (d, C-20), 41.73 (s, C-9), 40.04 (s, C-17), 39.51 (s, C-13), 38.16 (s, C-14), 35.16 (t, C-16), 32.63 (q, C-30), 31.71 (t, C-15), 31.67 (t, C-19), 29.96 (q, C-25), 28.73 (t, C-11), 27.14 (t, C-12), 18.20 (q, C-27), 15.17 (q, C-30), 14.86 (q, C-26), 10.40 (q, C-23).

(7*S*,8*S*)-7-Hydroxy-7,8-dihydro-tingenone (2): yellow lacquer; $[\alpha]^{20}_D$ −200° (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} 230, 309, 340 nm; IR (film) ν_{max} 3391, 2959, 2925, 2854, 1707, 1616, 1601, 1456, 1379, 1261, 1094, 1023, 861 cm^{-1}; EIMS *m/z* (rel. int) 438 (M^+) (8), 432 (2), 230 (2), 217 (3), 203 (5), 188 (5), 145 (4), 124 (70), 107 (100); HREIMS calcd for $C_{28}H_{38}O_4$ 438.2770, found 438.2772; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.67 (1H, brs, H-6), 6.25 (1H, brs, H-1), 4.75 (1H, brt, H-7), 2.15 (3H, Me-23), 1.97 (1H, d, J = 9.7 Hz, H-8), 1.31 (3H, s, Me-25), 1.30 (3H, s, Me-27), 1.20 (3H, s, Me-26), 0.88 (3H, d, J = 6.4 Hz, Me-30), 0.86 (3H, s, Me-28); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 214.02 (s, C-21), 181.33 (s, C-2), 162.17 (s, C-10), 145.71 (s, C-3), 143.68 (d, C-6), 131.24 (s, C-5), 117.56 (s, C-4), 116.85 (d, C-1), 69.50 (d, C-7), 53.32 (d, C-8), 53.18 (t, C-22), 44.02 (d, C-18), 42.24 (d, C-20), 41.55 (s, C-14), 40.49 (s, C-9), 39.39 (s, C-13), 37.96 (s, C-17), 35.57 (t, C-16), 31.89 (t, C-19), 32.75 (q, C-28), 31.62 (t, C-11), 30.93 (t, C-12), 29.28 (t, C-15),27.37 (q, C-25), 18.49 (q, C-27), 16.16 (q, C-26), 15.18 (q, C-30), 10.44 (q, C-23).

(8*S*)-7,8-Dihydro-6-oxo-tingenol (3): orange lacquer; $[\alpha]^{20}_D$ −9.3° (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} 203, 225, 256, 285, 339 nm; IR ($CHCl_3$) ν_{max} 3550, 2960, 2800, 1700, 1650, 1600, 1450, 1290, 1190, 850 cm^{-1}; EIMS *m/z* (rel. int) 438 (M^+) (100), 423 (27), 259 (5), 245 (9), 217 (25), 203 (35), 191 (16), 151 (8), 55 (4); HREIMS calcd for $C_{28}H_{38}O_4$ 438.2770, found 438.2767; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.76 (1H, brs, H-1), 2.55 (1H, m, H-7), 2.52 (3H, s, Me-23), 1.28 (3H, s, Me-25), 1.26 (3H, s, Me-26), 1.09 (3H, s, Me-27), 1.02 (3H, d, J = 6.0 Hz, Me-30), 1.00 (3H, s, Me-28), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 214.37 (s, C-21), 200.60 (s, C-6), 147.95 (s, C-2), 152.73 (s, C-10), 140.24 (s, C-3), 126.67 (s, C-4), 125.00 (s, C-5), 107.00 (d, C-1), 53.58 (t, C-22), 43.91 (d, C-18), 42.00 (d, C-20), 42.31 (d, C-8), 39.93 (s, C-13), 39.42 (s, C-14), 38.29 (s, C-17), 37.34 (t, C-7), 37.12 (s, C-9), 35.30 (t, C-16), 32.99 (t, C-11), 32.74 (q, C-28), 32.18 (t, C-19), 31.80 (t, C-12), 27.89 (t, C-15), 26.30 (q, C-25), 18.09 (q, C-27), 15.20 (q, C-26), 14.97 (q, C-30), 13.58 (q, C-23).

23-*nor*-6-Oxo-tingenol (4): Orange lacquer; $[\alpha]^{20}_D$ +2.5° (*c* 0.4, $CHCl_3$); UV (EtOH) λ_{max} nm 202, 230, 250, 286, 340; IR (film) ν_{max} 3392, 2961, 2926, 1707, 1638, 1586, 1456, 1379, 1261, 1093, 1020, 799 cm^{-1}; EIMS *m/z* (rel. int) 422 (M^+) (27), 407 (23), 227 (12), 217 (18), 204 (30), 167 (25), 149 (100); HREIMS calcd for $C_{27}H_{34}O_4$ 422.2457, found 422.2466; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (1H, s, H-4), 7.03 (1H, s, H-1), 6.37 (1H, s, H-7), 1.61 (3H, s, Me-25), 1.41 (3H, s, Me-26), 1.02 (6H, s, Me-27 + Me-28), 1.01 (3H, d, J = 6.0 Hz, Me-30).

23-Oxo-isotingenone (5): pale yellow lacquer; $[\alpha]^{20}_D$ −9° (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} 238, 294, 340, 392 nm; IR ($CHCl_3$) ν_{max} 3381, 2959, 2924, 2854, 1708, 1639, 1456, 1378, 1262, 1094 cm^{-1}; EIMS *m/z* (rel. int) 434 (M^+) (72), 419 (23), 281 (10), 267 (22), 255 (100), 241 (25), 228 (91), 210 (18), 165 (8), 95 (28), 57 (60); HREIMS calcd for $C_{28}H_{34}O_4$ 434.2457, found 434.2447; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.38 (1H, s, H-23), δ 7.20 (1H, s, H-1), δ 6.76 (1H, d, J = 10.5 Hz, H-7), δ 6.70 (1H, d, J = 10.5 Hz, H-6),δ 5.68 (1H, d, J = 5.5 Hz, H-11), δ 1.31 (3H, s, Me-27), δ 1.11 (3H, s, Me-25), δ 1.06 (3H, s, Me-26), δ 1.02 (3H, d, J = 6.6 Hz, Me-30), δ 0.98 (3H, s, Me-28); ^{13}C NMR ($CDCl_3$, 100 MHz) 214.40 (s, C-21), 196.11 (d, C-23), 148.30 (s, C-3), 143.83 (s, C-2), 142.95 (d, C-6), 140.79 (s, C-5), 129.32 (s, C-9), 126.80 (s, C-10), 124.74 (d, C-11), 118.22 (d, C-1), 115.59 (d, C-7), 114.18 (s, C-4), 51.05 (t, C-22), 45.66 (d,

C-20), 44.03 (s, C-8), 42.32 (d, C-18),40.76 (s, C-14), 39.99 (s, C-13),39.13 (s, C-17), 37.41 (t, C-19), 35.74 (t, C-16), 32.37 (t,C-12), 31.41 (q, C-28), 23.98 (t, C-15), 22.67 (q, C-25), 20.42 (q, C-26), 19.51 (q, C-27), 15.33 (q, C-30).

Cytotoxic Assays. Compounds **1**, **2**, **3**, and **5** were tested for cytotoxic activity[15] against the following cell lines: P-388 (ATCC CCL-46), suspension culture of a lymphoid neoplasm from a DBA/2 mouse; A-549 (ATCC CCL-185), monolayer culture of a human lung carcinoma; HT-29 (ATCC HTB-38), monolayer culture of a human colon carcinoma; MEL-28 (ATCC HTB-72), monolayer culture of a human melanoma. Cells were maintained, in logarithmic growth in EMEM/neaa, supplemented with 5% fetal calf serum, 10^{-2} M sodium bicarbonate, and 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate. The compounds assayed were dissolved in DMSO−MeOH (1:9) and tested following the method described previously.[15] Compound **1** [IC_{50} (μg/mL) = 2.5 (P-388); 5 (A-549); 5 (HT-28); 5 (MEL-28)], Compound **2** [IC_{50} (μg/mL) = 5 (P-388); 5 (A-549); 5 (HT-28); 5 (MEL-28)], Compound **3** [IC_{50} μg/mL = 5 (P-388); 5 (A-549); 10 (HT-28); 10 (MEL-28)], Compound **5** [IC_{50} μg/mL = 10 (P-388); 10 (A-549); 10 (HT-28); 10 (MEL-28)].

Assay of Aldose Reductase Activity. The purification of the recombinant human aldose reductase used in the bioassay is based on the method described by Nishimura et al.[16] The aldose reductase inhibitory activity in vitro was determined following a modification of the method reported above. Compounds **1**,**2**, **3**, and **5** showed an IC_{50} = 25μg/mL.

Acknowledgment. This work has been partly funded by the Spanish DGES (projects PB 96-1039 and PB96-1033), by the Gobierno Autónomo de Canarias (project CAC 246/125/96) and by the Instituto Tecnologico Canarias. BIOMAR S.A. (Dr. D. Grávalos and Dr. I. Raymundo) carried out the antitumor and the aldose reductase assays. H. Chávez thanks Agencia Española de Cooperación Internacional for a MUTIS grant.Thanks are also due to Prof. L. Ruíz for gathering the plant.

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NP980412+