COMMUNICATION

Chrysochlamic acid, a new diterpenoid-substituted quinol from *Chrysochlamys ulei* that inhibits DNA polymerase β

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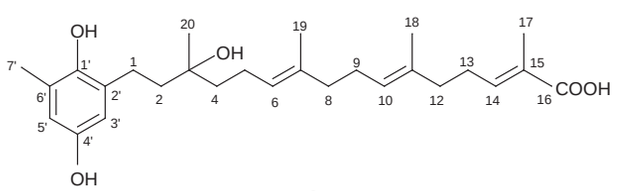
Chrysochlamic acid (1), a new DNA polymerase β inhibitor having an IC_{50} of 4.3 μM, has been isolated from *Chrysochlamys ulei* through bioassay-guided fractionation; it is the first example of a prenyl *m*-methylhydroquinone occurring in a higher terrestrial plant.

DNA damaging agents, such as bleomycin and cisplatin, are used clinically as antitumor agents.[1] Their potency is significantly reduced, however, through the action of DNA repair enzymes such as DNA polymerase β.[2–5] Because of its apparent role in the development of resistance to chemotherapeutic agents,[6] DNA polymerase β has become a target for the identification of specific inhibitors. Agents identified to date that inhibit this enzyme include dideoxythymidine triphosphate (ddTTP),[7] triterpenoids,[8,9] flavonoids,[10] fatty acids[11] and their derivatives,[12,13] phospholipids,[14,15] and sulfate or sialic acid-containing glycolipids.[16] Inhibition of DNA polymerase β could plausibly potentiate chemotherapeutic treatment and permit lower doses of antitumor agents to be administered. In the course of our search for naturally occurring DNA polymerase β inhibitors, we found that a methyl ethyl ketone extract prepared from dried stem bark of *Chrysochlamys ulei* strongly inhibited rat liver DNA polymerase β. Bioassay-guided fractionation led to the isolation of chrysochlamic acid (**1**), a potent



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new DNA polymerase β inhibitor. Compound **1** also represents the first example of a diterpenoid-substituted *m*-methylquinol occurring in a higher terrestrial plant. This inhibitor had an IC_{50} of 4.3 μM in the DNA polymerase β inhibition assay in the presence of 0.1 mg cm^{-3} bovine serum albumin (BSA), and an IC_{50} of 6.5 μM in the absence of BSA.

Chrysochlamic acid (**1**), obtained as viscous light yellow oil, had $[\alpha]_D^{20}$ + 6.7 (*c* 0.06, MeOH). Its molecular formula $C_{27}H_{40}O_5$ was deduced from the exact mass measurement of $[M - H_2O]^+$ in the HRFAB mass spectrum (experimental *m/z* 426.2763; calc. for $C_{27}H_{38}O_4$, *m/z* 426.2770; error 1.7 ppm). The UV absorptions at 220 nm (log ε/dm^3 mol^{-1} cm^{-1} 4.20) and 298 nm (log ε/dm^3 mol^{-1} cm^{-1} 3.56) in the UV spectrum of **1** suggested the presence of a hydroquinol chromophore.[17] This was further supported by the ^{13}C NMR spectrum which had two resonances corresponding to aromatic carbons bearing an oxygen function [δ 147.7 (s) and 145.9 (s)]. The ^1H NMR spectrum had resonances corresponding to two *meta*-coupled protons [δ 6.47 (d, *J* = 2.8 Hz) and 6.37 (d, *J* = 2.8 Hz)], one methyl group attached to an aromatic ring [δ 2.19 (br s)], and a methylene group attached to an aromatic ring [δ 2.66 (dt, *J* = 6.7, 2.4 Hz)].

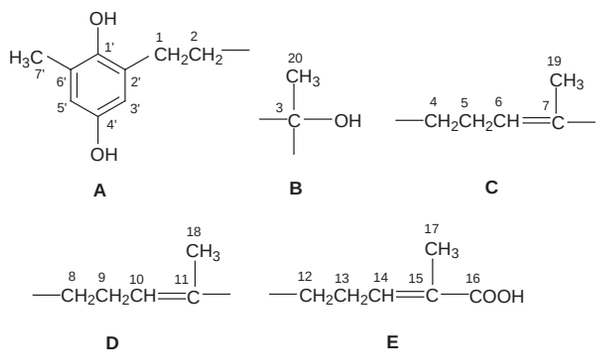

Fig. 1 Substructures **A**–**E** for **1**.

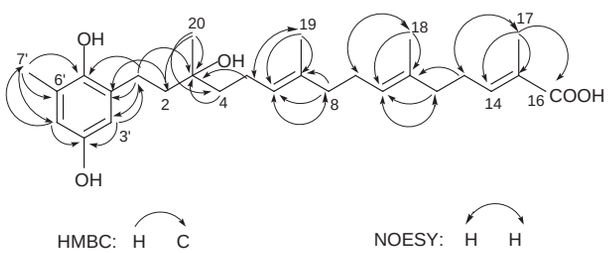

Fig. 2 Key HMBC and NOE correlations for **1**.

Further, the UV and ^{13}C NMR spectra indicated the presence of two *para* phenolic hydroxy groups, which together with the ^1H NMR spectrum permitted the elucidation of substructure **A** (Fig. 1). This assignment was further confirmed from the HMBC experiment (Fig. 2). In addition to the substituted phenol ring, the ^{13}C NMR and DEPT spectra of **1** indicated twenty carbons comprised of three olefinic methyls, one tertiary methyl, eight methylenes, three olefinic methines, three olefinic quaternary carbons, one oxygen-substituted quaternary carbon [δ 75.3 (s)], and one carboxy group [δ 172.9 (s)] (Table 1). The ^1H NMR spectrum of **1** also exhibited three olefinic methyl groups at δ 1.81 (br s), 1.60 (br s) and 1.59 (br s), one tertiary methyl group at δ 1.25 (s), and three olefinic protons at δ 6.86 (dt, *J* = 7.3, 1.2 Hz), 5.13 (br t, *J* = 7.0 Hz), and 5.12 (br t, *J* = 7.2 Hz). These observations, and analysis of the ^1H–^1H COSY and HMQC spectra, indicated substructures **B**–**E** (Fig. 1). The linkage of substructures **A**–**E** was determined from the HMBC experiment (Fig. 2). The HMBC spectrum showed the following key correlations: H_2-1→C-3; CH_3-20→C-2, C-3 and C-4; H_2-4→C-3; H_2-5→C-3; H_2-8→C-7 and C-6; H_2-12→C-10; and H_2-13→C-11. The NOEs between H_2-5 and CH_3-19, H-6 and H_2-8, H_2-9 and CH_3-18, H-10 and H_2-12, and H_2-13 and CH_3-17 in the NOESY spectrum indicated that the double bonds in the diterpenoid side chain had *trans* relationships (Fig. 2). Accordingly, the structure was established as **1**. However, the absolute configuration of the hydroxy group at C-3 of **1** remains unknown.

Table 1 ^{1}H and ^{13}C NMR data for chrysochlamic acid (**1**) in $CDCl_3$[a]

Position	δ_C	δ_H
1	22.5 (t)[b]	2.66 dt (6.7, 2.4)[c]
2	31.4 (t)	1.72 m, 1.81 m
3	75.3 (s)	
4	39.5 (t)	1.57 m, 1.65 m
5	26.5 (t)	2.07 m
6	124.5 (d)	5.13 br t (7.0)
7	134.9 (s)	
8	22.1 (t)	1.95 m
9	29.7 (t)	2.08
10	125.1 (d)	5.12 br t (7.2)
11	133.7 (s)	
12	38.0 (t)	2.05 m
13	27.5 (t)	2.26 q (7.3)
14	144.9 (d)	6.86 dt (7.3, 1.2)
15	126.8 (s)	
16	172.9 (s)	
17	12.0 (q)	1.81 br s
18	15.8 (q)	1.59 br s
19	15.9 (q)	1.60 br s
20	24.1 (q)	1.25 s
1′	147.7 (s)	
2′	121.2 (s)	
3′	115.6 (d)	6.47 d (2.8)
4′	145.9 (s)	
5′	112.6 (d)	6.37 d (2.8)
6′	127.3 (s)	
7′	16.0 (q)	2.19 br s

[a] The ^{1}H NMR spectrum was obtained at 500 MHz; the ^{13}C NMR spectrum at 125 MHz. [b] Letters s, d, t and q indicate, respectively, singlet, doublet, triplet and quartet, as determined from the DEPT spectrum. [c] *J* Values in Hz.

All prenyl *m*-methylhydroquinones, prenyl *m*-methylbenzoquinones, and other prenyl *m*-methylaryl derivatives have thus far been isolated only from marine sources, especially from those members of the brown algae families, Sargassaceae and Dictyotaceae.[17–22] Chrysochlamic acid (**1**) is the first example of a prenyl *m*-methylhydroquinone isolated from a higher terrestrial plant. It is interesting that some prenyl *m*-methylaryl derivatives have exhibited endothelin antagonistic activity[22] and cytotoxicity against cultured P388 lymphocytic leukemia cells.[21] This is the first report that a diterpenoid-substituted *m*-methylhydroquinone inhibits DNA polymerase β.

In addition to having good potency as an inhibitor of DNA polymerase β, the inhibitory potential of chrysochlamic acid was found to be unaffected by the presence of serum albumin, suggesting that this inhibitor may be of utility *in vivo*. In addition to having this advantage relative to the anacardic acids identified previously,[12] the structural rigidity imparted by the ring and double bonds should be of advantage in identifying those structural features required for polymerase β inhibitory activity.

Experimental

Extraction and isolation

Chrysochlamys ulei (Clusiaceae) was collected in Peru. The dried plant material (400 g) (stem bark) was soaked successively with hexanes, methyl ethyl ketone, MeOH, and water. The methyl ethyl ketone crude extract (5.7 g) contained a DNA polymerase β inhibitor (92% inhibition at 100 μg cm^{-3}, and 84% inhibition at 50 μg cm^{-3}); therefore, 1.52 g of extract was fractionated initially using a 35-g polyamide 6S column. The column was washed successively with H_2O, 1:1 MeOH–H_2O, 4:1 MeOH–CH_2Cl_2, 1:1 MeOH–CH_2Cl_2, and 9:1 MeOH–NH_4OH. The 4:1 MeOH-CH_2Cl_2 fraction (488 mg) was fractionated further using a 20-g Sephadex LH-20 column, which was washed successively with H_2O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, MeOH and acetone. The active 40% MeOH fraction (100 mg) was fractionated further using an 8-g C-8 reversed phase column which was washed successively with 40% MeOH, 60% MeOH, 80% MeOH, MeOH, and then acetone. The 80% MeOH fraction (39 mg) from the C-8 column was the most active and was purified further by reversed phase C-18 HPLC to obtain 19 mg of **1** as a light yellow oil-like gel.

DNA Polymerase β inhibition assay

After dissolving the samples in 1:1 DMSO–MeOH, 6×10^{-3} cm^3 of the sample and 4×10^{-3} cm^3 of rat liver DNA polymerase β[23] (6.9 units, 48 000 units mg^{-1}) were added to 50×10^{-3} cm^3 of 62.5 mM 2-amino-2-methylpropane-1,3-diol buffer (pH 8.6) containing 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.1 mg cm^{-3} of bovine serum albumin, 6.25 μM deoxynucleoside triphosphates (dNTPs), 0.04 Ci mmol^{-1} [^{3}H]TTP, and 0.25 mg cm^{-3} of activated calf thymus DNA. After incubation at 37 °C for 1 h, the radioactive DNA product was collected on diethylaminoethyl (DEAE)-cellulose filters (DE-81) and dried. The filters were washed successively in 0.4 M K_2HPO_4, pH 9.4, and 95% ethanol and then used for radioactivity determination.

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