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Antioxidant Xanthones from *Garcinia vieillardii*

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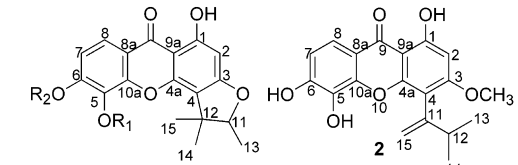
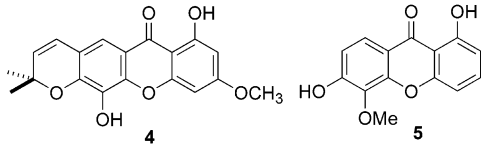
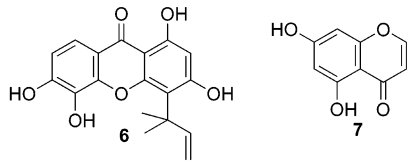
Two new xanthones, 6-*O*-methyl-2-deprenylrheediaxanthone B (**1**) and vieillardixanthone (**2**), were isolated from the stem bark of *Garcinia vieillardii*, as were four known compounds (**4**−**7**). The structures of **1** and **2** were determined by means of spectroscopic analysis and chemical derivatization. Each isolate was tested for its antioxidant properties based on a scavenging activity study using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical.

Natural antioxidants can be used both for food quality preservation, by preventing oxidative deterioration of lipids, and for medicinal purposes. Most of the antioxidant capability of vegetables is generally associated with their polyphenol content, such as flavonoids[1−3] or xanthones.[4−6] As part of an ongoing study on species from the Clusiaceae,[7−8] we report herein the first phytochemical investigation on *Garcinia vieillardii* P. (Clusiaceae) collected in New-Caledonia. A CH_2Cl_2-soluble extract of the stem bark of *G. vieillardii* was found to exhibit significant antioxidant effects, based on the scavenging activity study of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Fractionation of this extract led to the isolation of two new xanthones (**1**, **2**) and four known compounds (**4**−**7**).







The UV spectra of **1** and **2** were typical of 1,3,5,6-tetraoxygenated xanthones with λ_{max} absorption at ca. 255, 290, and 330 nm.[9] The HRESI$^-$ mass spectrum of **1** gave the pseudomolecular $[M - H]^-$ ion at *m*/*z* 341.1031, corresponding to $C_{19}H_{17}O_6$. The ^{1}H NMR spectrum of **1** was very similar to the one reported for 5-*O*-methyl-2-deprenylrheediaxanthone B (**3**), previously isolated from *Hypericum roeperanum*.[10] However, reciprocal NOE recorded between the 6-OMe (δ 4.06, s, 3H) and one of the *ortho*-coupled aromatic protons (δ 6.98, d, J = 8.9 Hz, 1H) revealed that the methoxy group of **1** was located at C-6. The complete assignments of all proton and carbon signals of **1**, for which we thus propose the name 6-*O*-methyl-2-deprenylrheediaxanthone B, are listed in Table 1.

Table 1. ^{13}C and ^{1}H NMR Data (δ) for Compounds **1** and **2** in $CDCl_3$ [a]

	1		**2**	
position	^{1}H	^{13}C	^{1}H	^{13}C
1		165.2		163.9
2	6.25 s	93.7	6.45 s	94.8
3		163.4		164.6
4		113.4		111.8
4a		150.2		154.2
5		132.9		133.6
6		152.1		147.9
7	6.98 d (8.9)	113.0	6.97 d (8.6)	113.2
8	7.81 d (8.9)	116.7	7.61 d (8.6)	117.4
8a		115.2		119.1
9		179.4		180.1
9a		106.8		103.2
10a		144.0		144.0
11	4.56 q (6.4)	90.9		147.1
12		77.2	2.63 sep (7.0)	34.6
		14.9	1.09 d (7.0)	21.6
14	1.63 s[b]	26.1[b]		
15	1.35 s[b]	21.7[b]	5.36 s 4.93 s	114.5
1-OH	13.23 s		13.38 s	
3-OCH_3			3.90 s	56.3
5-OH	5.62 s			
6-OMe	4.06 s	56.8		

[a] J values (Hz) are shown in parentheses. [b] Assignments with the same superscript in one column are exchangeable.

Compound **2** was isolated as a yellow oil, and its molecular formula was determined as $C_{19}H_{18}O_6$ through HRESI$^-$ MS analysis (pseudomolecular ion $[M - H]^-$ at *m*/*z* 341.1025). In the ^{1}H NMR spectrum of **2**, the presence of only three signals for aromatic protons indicated that the xanthone nucleus was pentasubstituted. The UV

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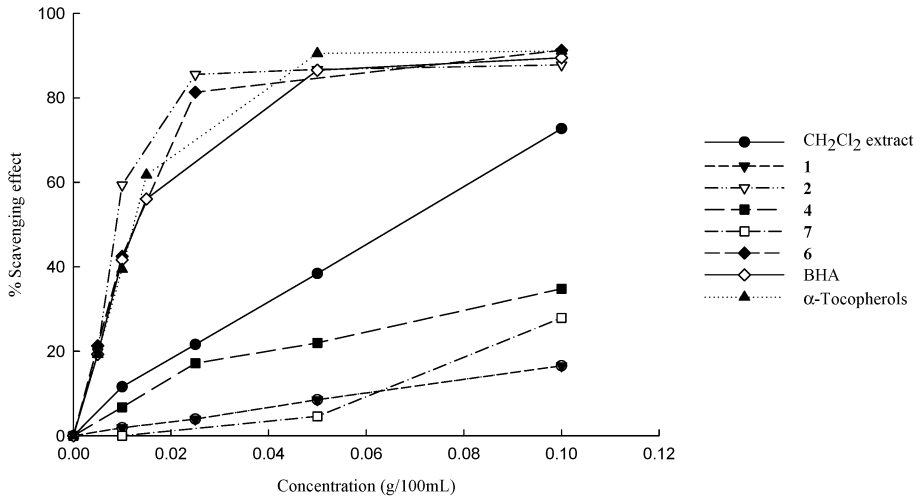

Figure 1. Antioxidant activities of CH_2Cl_2-soluble extract and compounds **1**, **2**, **4**, **6**, and **7** in the autoxidation assay. For protocols used, see Experimental Section. BHA = 2,6-di-*tert*-butyl-4-hydroxyanisol and α-tocopherols: standard control substances.

absorption band at λ_{max} 324 nm was shifted after addition of $AlCl_3$ (λ_{max} 398 nm) and $AlCl_3$ + HCl (λ_{max} 347 nm), pointing to the presence of C_1-OH, C_5-OH, and C_6-OH. The ^{1}H NMR spectrum exhibited one singlet (δ 3.90, s, 3H) for a methoxyl group, located at C-3 on the basis of a NOESY experiment. The HMBC spectrum of **2** showed that the OH (δ 13.38) was long-range-correlated with C-2 (δ 94.8). The remaining substituent was then deduced to be at C-4 and was characterized with NMR resonances for one terminal methylene (δ 4.93 and 5.36, s, 1H each) and for one isopropyl group (δ 1.09, d, J = 7.0 Hz, 6H, and δ 2.63, sep, J = 7.0 Hz, 1H). The HMBC experiment showed that both the methylene and the methyl protons correlated with the same methine carbon of the isopropyl unit. Furthermore, methyl protons were long-range-coupled with the quaternary sp^2 carbon at δ 147.1. The C-4 substituent was then identified as an unusual 1-isopropylethenyl unit, which has also been described for an isoflavan isolated from *Maackia tenuifolia*.[11] ^{1}H and ^{13}C NMR data of **2**, which we have named vieillardixanthone, are presented in Table 1.

Three known xanthones were also isolated during this study and identified as forbexanthone (**4**),[12] buchanaxanthone (**5**),[13] isocudraniaxanthone A (**6**),[14] and 5,7-dihydroxychromone (**7**).[15] The antioxidant activities, except for buchanaxanthone (**5**), for which insufficient material was available, were evaluated using the DPPH free-radical-scavenging test (Figure 1). Isocudraniaxanthone A (**6**) and compound **2** showed the best values, with activities similar to that of BHA. This result is in accordance with the former observation that free-radical-scavenging activity increases with the presence of hydroxyl groups or catechol moieties in related molecules.[16]

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Helios α V1.08 UNICAM spectrophotometer. ^{1}H, ^{13}C, and 2D NMR experiments (COSY, NOE, HMBC, HMQC) spectra were recorded on a Bruker Avance DRX 500 MHz. Mass spectrometry analyses were performed on a JMS-700 (JEOL LTD, Akishima, Tokyo, Japan) double-focusing mass spectrometer with reversed geometry, equipped with a pneumatically assisted electrospray ionization (ESI) source. Silica gel 60 (Macherey-Nagel, 0.04−0.063 mm) was used for column chromatography, and precoated silica gel (Macherey-Nagel, SIL G25 UV254, 0.25 mm) was used for analytical TLC. DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Sigma Chemical Co.

Plant Material. The stem bark of *G. vieillardii* P. was collected in January 2001 in the Froin Forest, on the Mandjélia's massif located in the North of New-Caledonia. A specimen (LIT 1298) is maintained at the Laboratoire des Plantes Médicinales in Noumea. This sample was successively extracted with hexane, CH_2Cl_2, and ethyl acetate. The CH_2Cl_2 extract was first subjected to column chromatography and then to semipreparative HPLC, to give two new xanthones (**1**, **2**) and known compounds **4**−**7**.

Extraction and Isolation. The powdered stem bark (1.4 kg) of *G. vieillardii* was extracted successively with cyclohexane (6 L), CH_2Cl_2 (6 L), and EtOAc (6 L) in a Soxhlet apparatus. Concentration under reduced pressure gave 62 g (4.4%) of cyclohexane extract, 9.2 g (0.6%) of CH_2Cl_2 extract, and 20 g (1.4%) of an EtOAc extract. The CH_2Cl_2 extract was subjected to MPLC over Si gel using 100% cyclohexane to 20% EtOAc/80% cyclohexane in 5% stepwise elutions and then 10% MeOH/90% EtOAc to 30% MeOH/70% EtOAc in 5% stepwise elutions and afforded 56 fractions. Fractions 12−13 (92 mg) were subjected to semipreparative HPLC using a normal-phase column (Kromasil, 5ù 60A, 250 × 10) with a EtOAc/$CHCl_3$ gradient, yielding **1** (2.3 mg, 0.025%, t_R 28 min), forbexanthone (**4**)[12] (3.4 mg, 0.037%, t_R 33 min), and buchanaxanthone (**5**)[13] (0.5 mg, 0.005%, t_R 35 min). Separation of the 18th (74 mg) fraction using the same method afforded 5,7-dihydroxychromone (**7**)[15] (9 mg, 0.1%, t_R 56 min), **2** (1.5 mg, 0.016%, t_R 46 min), and isocudraniaxanthone A (**6**)[14] (2 mg, 0.022%, t_R 50 min).

6-*O*-Methyl-2-deprenylrheediaxanthone B (1): white amorphous solid, $[\alpha]^{25}_D$ 0° (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 252 (4.53), 288 (3.92) 324 (4.21) MeOH+$AlCl_3$ 232 (4.24), 269 (4.38), 290 (3.91), 360 (4.17) MeOH+$AlCl_3$+HCl 234 (4.18), 267 (4.30), 290 (3.85) 360 (4.12) nm; ^{1}H and ^{13}C NMR, see Table 1; HRESI$^-$ *m/z* 341.1031 (calcd for $C_{19}H_{17}O_6$ [M − H]$^-$, 341.1025).

Vieillardixanthone (2): yellow oil; UV (MeOH) λ_{max} (log ϵ) 253 (4.18), 286 (3.61), 324 (3.80), 341 (3.71) MeOH +$AlCl_3$ 233 (3.93), 268 (4.06), 294 (3.75), 398 (3.81) MeOH+$AlCl_3$+HCl 231 (3.93), 265 (4.07), 298 (3.75) 347 (3.82) nm; ^{1}H and ^{13}C NMR, see Table 1; HRESI$^-$ *m/z* 341.1025 (calcd for $C_{19}H_{17}O_6$ [M − H]$^-$, 341.1025).

Scavenging Activity of DPPH Radicals. The free-radical-scavenging activity was tested as bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH).[17] In its radical form, DPPH$^•$ has an absorption band at 520 nm, which disappears upon reduction by an antiradical compound. The reaction mixture (3.15 mL) contained 3 mL of daily prepared DPPH solution (0.025 g/L) and various concentrations of tested compound or of standard reference dissolved in MeOH. After 30 min in the dark at room temperature, the absorbance was recorded at 520 nm. The percentage of disappeared (%$_{disp}$

DPPH) DPPH was calculated as follows:

$$\%_{disp}\,\mathrm{DPPH} = 100 - (([\mathrm{DO}]_X/[\mathrm{DO}]_T) \times 100)$$

where $[DO]_X$ is the absorbance measured for the tested compound, and $[DO]_T$ is that for the reference without any antioxidant.

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