

Antitumor Agents. 282. 2'-(R)-O-Acetylglauucarubinone, a Quassinoid from *Odyendyea gabonensis* As a Potential Anti-Breast and Anti-Ovarian Cancer Agent

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A new quassinoid, designated 2'-(R)-O-acetylglauucarubinone (**1**), and seven known quassinoids (**2**–**8**) were isolated, using bioactivity-guided separation, from the bark of *Odyendyea gabonensis* (Pierre) Engler [syn. *Quassia gabonensis* Pierre]. The structure of **1** was determined by spectroscopic analysis and by semisynthesis from glauucarubolone. Complete ¹H and ¹³C NMR assignments of compounds **1**–**8** were also established from detailed analysis of two-dimensional NMR spectra, and the reported configurations in odyendene (**7**) and odyendane (**8**) were corrected. Compound **1** showed potent cytotoxicity against multiple cancer cell lines. Further investigation using various types of breast and ovarian cancer cell lines suggested that **1** does not target the estrogen receptor or progesterone receptor. When tested against mammary epithelial proliferation in vivo using a *Brca1/p53*-deficient mice model, **1** also caused significant reduction in mammary duct branching.

Quassinoids are known as the bitter principles of Simaroubaceae plants.^{1–3} They are highly oxygenated triterpenes and possess a wide spectrum of in vitro and in vivo biological activities, including antitumor, antimalarial, antiviral, anti-inflammatory, antifeedant, insecticidal, amoebicidal, antiulcer, and herbicidal effects.¹ In particular, the quassinoid bruceantin was brought to a phase II clinical trial as an anticancer drug candidate;⁴ however, lack of significant efficacy in treating human cancer led to termination of its clinical development in the early 1980s.⁵ From the initial studies on various quassinoids, the mechanism of action was attributed to the inhibition of site-specific protein synthesis by prevention of ribosomal peptidyl transferase activity, leading to termination of chain elongation.⁶ However, other postulated mechanisms for inhibition of cancer cell growth include, but are not limited to, inhibition of plasma membrane NADH oxidase activity,⁷ down-regulation of *c-myc* oncogene,⁸ and mitochondrial membrane depolarization with caspase-3 activation.⁹

The stem bark of *Odyendyea gabonensis* (Pierre) Engler [syn. *Quassia gabonensis* Pierre (Simaroubaceae)] is a source of quassinoids, and seven quassinoids, 2'-(S)-O-acetylglauucarubinone (**2**), glauucarubinone (**3**), ailanthinone (**4**), 2'-(R)-O-acetylglauucarubin (**5**), excelsin (**6**), odyendene (**7**), and odyendane (**8**), were previously

isolated.^{10–12} We have reinvestigated the stem bark of this plant due to its potent selective cytotoxicity against breast cancer cell lines in our prior studies aimed at discovering antitumor agents from higher plants. Bioactivity-directed fractionation of this plant extract led to the isolation and characterization of a new quassinoid, 2'-(R)-O-acetylglauucarubinone (**1**), as an active principle, along with **2**–**8**. We describe herein the isolation and structure determination of **1** by semisynthesis. The isolated quassinoids were evaluated in vitro against human tumor cell replication (DU145 prostate cancer, A549 human lung carcinoma, KB human epidermoid carcinoma of the nasopharynx, and KB-V multi-drug-resistant expressing P-glycoprotein). Compound **1** was also further investigated for in vitro cytotoxic activity against multiple breast cancer cell lines. We also describe the effect of **1** on mammary epithelial proliferation in vivo using a *Brca1/p53*-deficient mice model.

During this investigation, detailed analyses of ¹H and ¹³C NMR spectra of quassinoids **1**–**8** were conducted and led to the revision of some previously reported data of **2**–**6**, as well as the correction of configurations in odyendene (**7**) and odyendane (**8**).¹² For the convenience of comparison and discussion, 2'-acetylglauucarubinone (**2**) is referred to as 2'-(S)-O-acetylglauucarubinone and its 2'-epimer (**1**) as 2'-(R)-O-acetylglauucarubinone.

Results and Discussion

O. gabonensis was collected in Gabon in 1991 by NCI. The active MeOH/CH₂Cl₂ (1:1) extract of the bark was fractionated into hexane- and EtOAc-soluble fractions, as well as an insoluble residue. The active EtOAc-soluble fraction was subjected to silica gel column chromatography (CC), (hexane/EtOAc gradient, then MeOH), followed by reversed-phase HPLC, to give the new compound **1** and the known quassinoids **2**–**8**.

Compound **1** was isolated as a colorless, amorphous solid, and its HREIMS indicated a molecular formula of C₂₇H₃₆O₁₁, which was identical with that of **2**. The close similarity in the ¹H and ¹³C NMR spectra (Tables 1 and 2, respectively) of **1** and **2**, including

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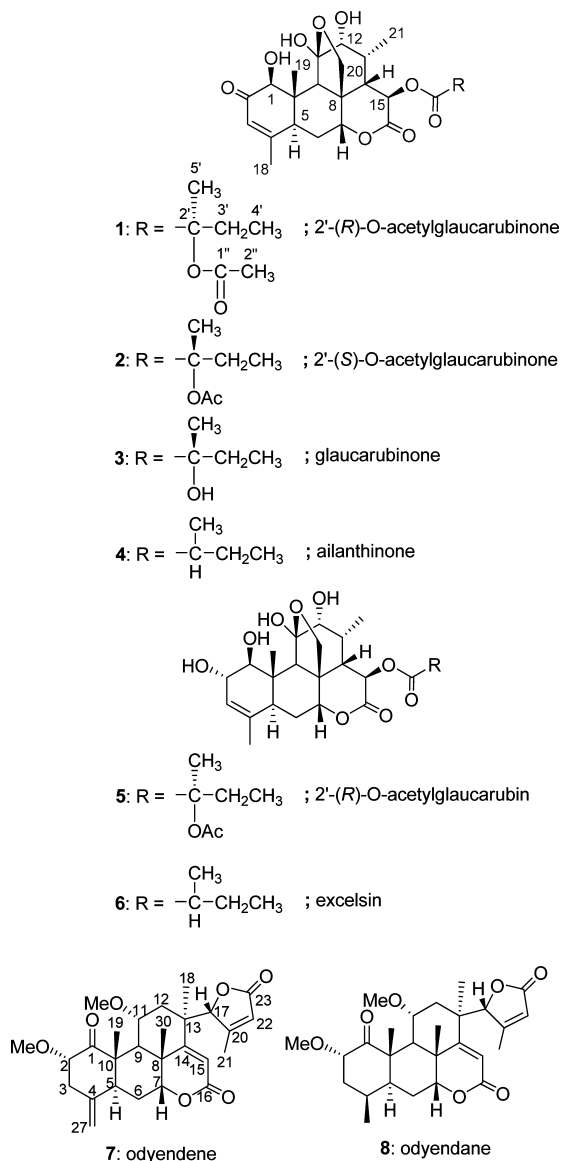
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coupling patterns, implied that **1** was an epimer of **2**. The only spectroscopic differences were in the chemical shifts of signals relative to the side chain at C-15, which indicated that **1** could be 2'-*epi*-acetoxyglaucaurbinone.

Due to the small amount of **1** isolated from the plant, as well as the limited supply of the original plant material, the C-2' epimer of **2** was synthesized from glaucaurbinone (**10**) to identify the structure and configuration of **1**. According to Valeriote's procedure,¹³ all four hydroxy groups of **10** were protected as trimethylsilyl (TMS) ethers (Scheme 1). The resulting fully protected tetra-*O*-trimethylsilylglaucaurbinone was carefully treated with tetra-*n*-butylammonium fluoride to selectively remove the C-12 and C-15 TMS groups and provide **11** in 86% yield. Condensation of **11** with **13**, which was derived from acetylation of (*R*)-2-hydroxy-2-methylbutyric acid with acetyl chloride, provided **14** in 77% yield, based on the recovery of **11**. The remaining TMS groups were removed with citric acid to afford **1** in 76% yield.

The equivalence of synthetic **1** and the natural product was confirmed by comparing their ¹H and ¹³C NMR spectra (Tables 1 and 2). Additional HPLC analysis also supported the structure of naturally occurring **1** as 2'-(*R*)-*O*-acetylglaucaurbinone. Both natural and synthetic **1** had a retention time of 15.6 min, while 2'-(*S*)-*O*-acetylglaucaurbinone (**2**) had a retention time of 14.2 min.¹⁴

Compounds **2** and **3** were identified as 2'-acetylglaucaurbinone [2'-(*S*)-*O*-acetylglaucaurbinone] and glaucaurbinone, respectively, by comparing their physical and spectroscopic data (IR, MS, ¹H and ¹³C NMR) with those reported previously in the literature.¹¹ The configuration of the asymmetric carbon atom at C-2' in the side chain of glaucaurbinone (**3**) has been assigned as *S*, on the basis of an enantioselective total synthesis.¹⁵ In addition, it has been reported that complete acetylation of **3** afforded **9**.¹⁶ Thus, **2** was acetylated to yield its tetra-acetate (Scheme 2), which was identified as tetracetylglaucaurbinone (**9**) by comparison with the spectroscopic data described in the literature. Thus, the absolute configuration of **2** at C-2' was established as *S*.

Because ¹H and ¹³C NMR spectroscopic data of **4**–**6** in the literature are incomplete or absent, we also established the complete assignment of ¹H and ¹³C NMR resonances of these three compounds using a combination of two-dimensional (2D) NMR techniques. Our spectroscopic data are shown in Tables 1 and 2.

Quassinoids **7** and **8** were identified as odyendene and odyendane, respectively;¹² however, detailed NMR spectroscopic analyses

Table 1. ¹H NMR Spectroscopic Data of **1**–**6**

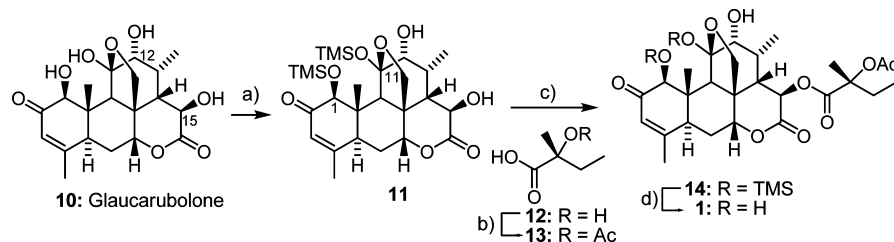
position	δ_{H} (J in Hz)					
	1 ^a	2 ^a	3 ^b	4 ^a	5 ^b	6 ^b
1	4.06, s	4.05, s	4.22, s	4.08, s	3.92, d (7.8)	3.86, d (7.9)
2					4.59, br d (7.8)	4.58, m
3	6.15, m	6.15, br s	6.09, br s	6.16, m	5.75, br s	5.74, br s
5	3.03, br d (12.7)	3.07, br d (12.5)	3.09, br d (11.5)	2.99, br d (12.1)	2.75, br d (13.2)	2.62 ^c , br d (~14.5)
6	2.29, ddd (14.5, 3.4, 2.5)	2.27, br d (14.6)	~2.15 ^c , m	2.30, ddd (13.5, 2.7, 2.5)	1.98, br d (13.2)	2.00, br d (~14.5)
	2.01 ^c , ddd (14.5, 12.7, 2.1)	1.99, br t (14.6)	~2.0 ^c , m	2.03, td (13.5, 1.9)	1.90, br t (13.2)	1.91, br t (~14.5)
7	4.70, dd (3.4, 2.1)	4.76, br s	4.82, br s	4.65, m	4.78, m	~4.7, m
9	2.72, br s	2.64, s	3.39, s	2.75, s	3.11, s	3.16, s
12	3.58, br d (3.7)	3.60, br s	4.03, br s	3.58, br d (3.3)	4.07, m	4.06, br d (3.1)
13	~2.4 ^c , m	2.41, m	~2.6 ^c , m	~2.4 ^c , m	2.67, m	2.63 ^c , m
14	~2.4 ^c , m	2.48, dd (11.0, 6.4)	~2.6 ^c , m	~2.35 ^c , m	2.78, dd (11.6, 6.2)	2.51, dd (12.0, 6.0)
15	5.48, br d (10.8)	5.17, d (11.0)	6.47, d (11.8)	5.59, d (11.3)	6.08, d (11.6)	6.44, d (12.0)
18	2.01 ^c , s	2.02, br s	1.70, s	2.03, br s	1.71, s	1.54, s
19	1.21 ^c , s	1.21, s	1.56, s	1.21, s	1.55, s	1.69, s
20	3.97, d (9.0)	3.96, d (8.9)	4.16, d (8.7)	3.97, d (9.0)	4.18, d (8.6)	4.20, d (8.7)
	3.71, d (9.0)	3.71, d (8.9)	3.84, d (8.7)	3.69, d	3.75, d (8.6)	3.88, d (8.7)
21	1.21 ^c , br d (5.0)	1.28, d (7.0)	1.4, d (6.6)	1.20, d	1.6, d (7.3)	1.33, d (7.0)
2'				2.44, m		2.55, sext (~7)
3'	1.89, dq (14.6, 7.6)	1.94, dq (14.3, 7.3)	~2.15 ^c , m	1.79, ddq	2.23, dq (14.2, 7.5)	1.88, ddq (~14, 14, 7)
	2.10 ^c , dq (14.6, 7.6)	1.87, dq (14.3, 7.3)	~2.05 ^c , m	1.51, ddq	2.03, dq (14.2, 7.5)	1.57, ddq (~14, 14, 7)
4'	0.98, t (7.6)	0.98, t (7.3)	1.23, t (7.4)	0.97, t	1.05, t (7.5)	1.01, t (7.4)
5'	1.57, s	1.62, s	1.71, s	1.12, d	1.75, s	1.22, d (7.1)
2''	2.09 ^c , s	2.08, s			2.07, s	

^a Measured in CDCl₃. ^b Measured in pyridine-*d*₅. ^c Overlapping signals.

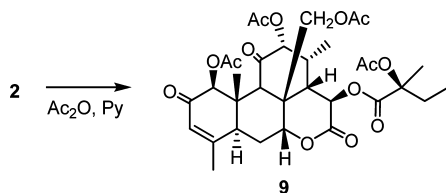
Table 2. ^{13}C NMR Spectroscopic Data of **1–6**

position	δ_{C} , mult.											
	1^a		2^a		3^b		4^a		5^b		6^b	
1	83.0	CH	83.1	CH	84.7	CH	83.0	CH	83.6	CH	83.9	CH
2	196.0	qC	196.0	qC	197.6	qC	196.0	qC	72.9	CH	73.0	CH
3	124.2	CH	124.1	CH	126.5	CH	124.3	CH	126.8	CH	127.3	CH
4	164.7	qC	165.1	qC	162.7	qC	164.7	qC	134.9	qC	135.2	qC
5	41.7	CH	41.7	CH	42.5	CH	41.7	CH	41.4	CH	42.3	CH
6	25.4	CH ₂	25.3	CH ₂	26.2	CH ₂	25.5	CH ₂	25.7	CH ₂	26.2	CH ₂
7	77.3	CH	77.2	CH	78.7	CH	77.3	CH	78.8	CH	79.3	CH
8	47.5	qC	47.4	qC	46.5	qC	47.3	qC	48.1	qC	46.4	qC
9	44.4	CH	44.2	CH	45.9	CH	44.5	CH	45.1	CH	45.7	CH
10	45.3	qC	45.3	qC	45.7	qC	45.2	qC	42.1	qC	41.7	qC
11	108.8	qC	108.7	qC	111.0	qC	108.9	qC	110.7	qC	111.3	qC
12	79.3	CH	79.5	CH	80.3	CH	79.2	CH	80.2	CH	80.4	CH
13	31.4	CH	31.2	CH	33.0	CH	31.4	CH	32.7	CH	33.3	CH
14	45.5	CH	44.8	CH	48.4	CH	45.6	CH	45.7	CH	48.4	CH
15	71.0	CH	71.0	CH	71.8	CH	69.4	CH	73.0	CH	70.6	CH
16	167.0	qC	167.0	qC	168.4	qC	167.3	qC	167.7	qC	168.7	qC
18	23.0	CH ₃	23.0	CH ₃	22.6	CH ₃	23.0	CH ₃	21.0	CH ₃	21.5	CH ₃
19	10.1	CH ₃	10.1	CH ₃	11.2	CH ₃	10.0	CH ₃	11.0	CH ₃	11.3	CH ₃
20	71.0	CH ₂	71.9	CH ₂	71.6	CH ₂	71.0	CH ₂	71.4	CH ₂	71.9	CH ₂
21	14.1	CH ₃	14.1	CH ₃	16.1	CH ₃	14.5	CH ₃	15.6	CH ₃	15.9	CH ₃
1'	171.4	qC	171.4	qC	176.7	qC	175.7	qC	171.4	qC	175.8	qC
2'	80.7	qC	80.6	qC	75.6	qC	41.0	CH	81.1	qC	41.9	CH
3'	30.1	CH ₂	31.7	CH ₂	34.2	CH ₂	26.5	CH ₂	31.4	CH ₂	27.4	CH ₂
4'	7.2	CH ₃	7.2	CH ₃	8.8	CH ₃	11.5	CH ₃	7.5	CH ₃	12.2	CH ₃
5'	21.1	CH ₃	21.1	CH ₃	26.1	CH ₃	16.0	CH ₃	21.0	CH ₃	16.5	CH ₃
1''	170.1	qC	170.3	qC					169.9	qC		
2''	20.9	CH ₃	20.3	CH ₃					21.0	CH ₃		

^a Measured in CDCl_3 . ^b Measured in pyridine- d_5 .

Scheme 1^a

^a Reagents and conditions: (a) TMSOTf, Et_3N , Py, rt, 2.5 h then TBAF, rt, 1 h; (b) AcCl, rt, 2.5 h; (c) **13**, EDCl, DMAP, CH_2Cl_2 , rt, 3 d; (d) citric acid, MeOH, rt, 2.5 h.

Scheme 2. Acetylation of **2**

using 1D and 2D NMR techniques revealed that the previously reported α -orientations at C-2 and C-11 were incorrect. In the ^1H NMR spectrum of **8**, the coupling patterns of H-2 (δ_{H} 4.44, dd, $J_1 = 11.3$, $J_2 = 6.0$) and H-11 (δ_{H} 3.62, td, $J_1 = 9.8$, $J_2 = 3.9$) clearly indicated β -axial orientations. NOE effects observed between H-2 and β -methyl groups at C-19 and C-27, as well as between H-11 and β -methyl groups at C-19 and C-30, indicated α -configurations for the methoxy groups at C-2 and C-11. Accordingly, we consider the structures of **7** and **8** in the literature to constitute a prima facie case of inadvertently misdrawn configurations at C-2 and C-11. The structures of **7** and **8** are presented herein with the correct configurations.

Biological Activity

Quassinoids **2–4** were previously reported to be moderately cytotoxic against the KB cancer cell line.^{17–19} Therefore, quassi-

Table 3. Cytotoxic Activity of Quassinoids **1–6**

compound	ED_{50} (μM) ^a			
	DU145	A549	KB	KB-VIN
1	0.04	0.06	0.05	0.42
2	0.07	0.07	0.07	0.44
3	0.47	0.88	0.44	1.24
4	0.09	0.13	0.18	0.37
5	0.83	3.6	0.67	NA ^b
6	4.7	16	4.3	NA
paclitaxel	0.003	0.008	0.007	>1

^a Values are the mean ED_{50} (concentrations that gave 50% effect under the defined assay conditions) in μM . ^b Tested compounds did not reach 50% inhibition at 10 $\mu\text{g}/\text{mL}$.

noids **1–6** were tested for in vitro cytotoxic activity against KB and three additional human cancer cell lines. The results are summarized in Table 3. Quassinoids **1**, **2**, and **4** showed the highest potency, with EC_{50} values ranging from 0.04 to 0.18 μM against DU145, A549, and KB cell lines and 0.37 to 0.44 μM against KB-VIN. Compounds **3** and **5** showed significant, but lower, cytotoxicity.

The synthesized **1** was further tested against multiple breast cancer cell lines, including MDA-MB-231 [estrogen receptor negative (ER⁻) basal-like breast cancer], MDA-MB-468, MCF-7, MCF/HER2 [MCF-7 overexpressing HER2], SKBR3 (ER⁻, HER2 overexpressing luminal-like breast cancer), and BT474 [ER⁻ and

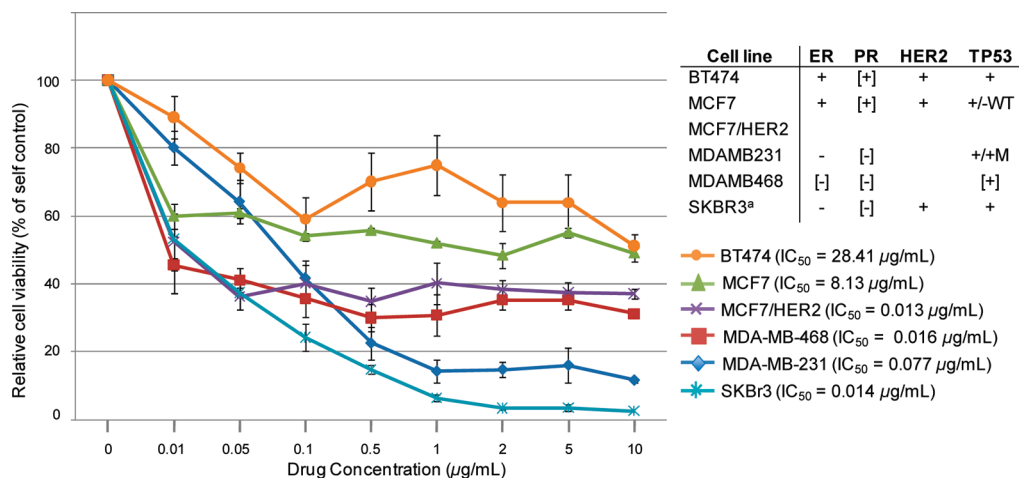


Figure 1. Cytotoxicity of **1** against multiple breast cancer cell lines. The table lists the protein expression status of ER/PR, HER2 overexpression, and wild-type (WR) or mutant (M) TP53 of each cell line.²⁴

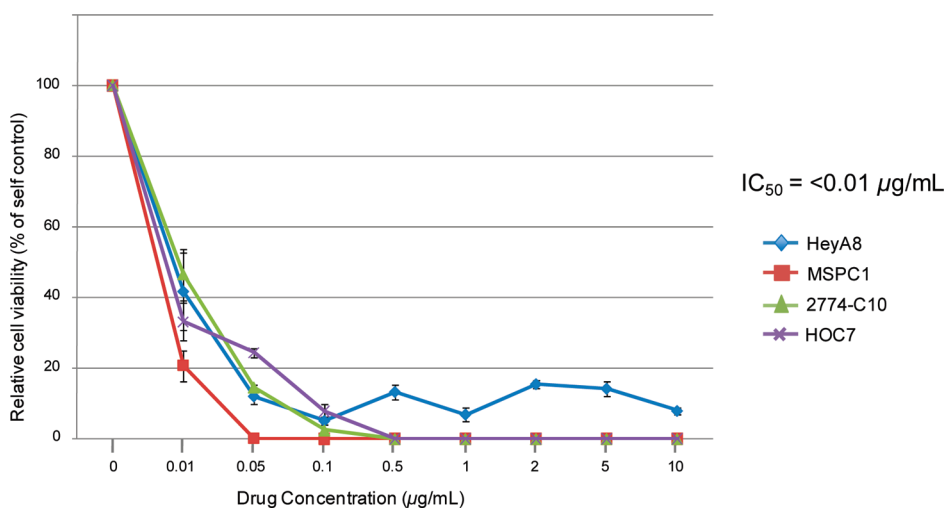


Figure 2. Cytotoxicity of **1** against multiple ovarian cancer cell lines.

progesterone receptor (PR)-positive], as well as ovarian cancer cell lines, including MSPC1, 2774-C10 (ovarian cancer expressing normal level of HER2), HeyA8 (highly metastatic epithelial ovarian), and Hoc7 (human ovarian adenocarcinoma). As shown in Figure 1, compound **1** greatly suppressed tumor cell growth of MCF7/HER2 and SKBR3, as well as triple negative MDA-MB468 and MDA-MB-231 breast cancer cell lines. The efficient suppression of growth of ER-negative MDA-MB468 and MDA-MB-231 cell lines implies that ER and PR may not be major targets of **1**. This is also supported by the observation of low sensitivity of BT474 and MCF7 to **1**. On the other hand, HER2-overexpressing ER/PR-negative SKBR3 cells showed hypersensitivity to compound **1**, suggesting that **1** might interfere with the HER2 signaling pathways. This view was also supported by the higher sensitivity of MCF7/HER2 compared to MCF-7. However, targets and mechanisms of action of **1** required further studies. Compound **1** also demonstrated significant inhibition of ovarian cancer cell lines, MSPC1, 2774-C10, HeyA8, and Hoc7 (Figure 2). Taken together, these results led us to assess the effect of **1** on mammary epithelial proliferation in vivo using a *Brcal/p53*-deficient mice model.

The human tumor suppressor gene *BRCA1* interacts directly with ER and PR and modulates the transcription activities of ER α and PR, as well as the nongenomic function of ER α . Mutations in the *BRCA1* gene are associated with an increased risk of breast and ovarian cancers. Previous studies revealed that mammary epithelial cells from mutant mice express higher levels of PR and show extensive mammary epithelial proliferation.^{20,21}

We have used these mutant and wild-type mice to test antiproliferation effects of neotanshinlactones²² in vivo and found that *Brcal*^{f11/f11}*p53*^{f5&6/f5&6}*Cre*^c mice provide a sensitive readout. The effect of **1** on mammary epithelial proliferation was compared with those of paclitaxel and another quassinoid, bruceantin. Paclitaxel is used in clinical treatment of breast cancer, and bruceantin was evaluated in phase II clinical trials as an anticancer drug candidate. In accordance with prior observations, there was extensive side branching in the mammary glands of vehicle-treated *Brcal*^{f11/f11}*p53*^{f5&6/f5&6}*Cre*^c mice (Figure 3A (d) and B). However, daily peritoneal injection of 0.1 mg of **1** for 7 days reduced branching points to 32% (Figure 3A (a) and B) compared with that of vehicle-treated mice. Importantly, the reduction was more pronounced than results with paclitaxel (Figure 3A (b) and B) or bruceantin (Figure 3A (c) and 1B). Using a two-tail *t* test, **1** ($P = 0.0014$), paclitaxel ($P = 0.0016$), and bruceantin ($P = 0.023$) treatment all led to significant reduction in mammary duct branching. While signaling pathways driving the elevated proliferation in *Brcal/p53*-deficient mammary gland are not well characterized, it is interesting to note that the EGFR pathway was up-regulated in mammary epithelial cells from *BRCA1* carriers.²³ It is known that EGFR family members can form heterodimers. Further studies would be needed to determine whether compound **1** indeed targets the EGFR pathways in the mouse model and the HER2 pathways in the breast cancer cell lines.

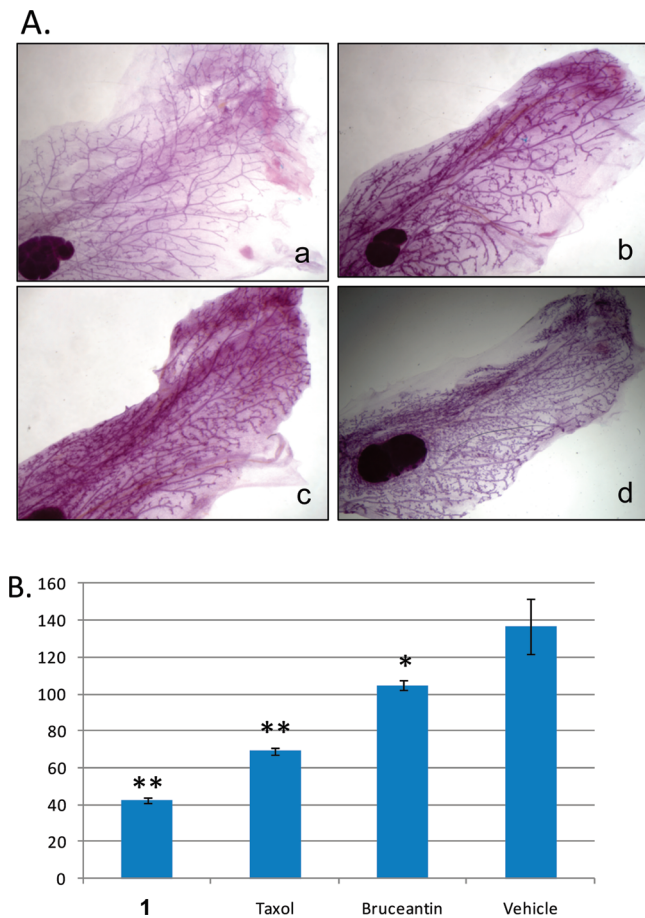


Figure 3. Treatment with **1** leads to decreased mammary ductal branching. Mammary gland whole mounts were prepared from *Brcal^{fl/fl}p53^{fl5&6/fl5&6}Cre^e* mice following treatment with 0.1 mg of **1** and paclitaxel daily for 7 days and bruceantin daily for 5 days. (A) Mammary gland whole mounts of **1**- (a), paclitaxel- (b), bruceantin- (c), and vehicle (d)-treated 3-month-old mice. (B) Number of branching points in the mammary glands of treated mice. The data represent average of branch points in three randomly selected areas \pm SD (* $P \leq 0.02$; ** $P \leq 0.001$).

Conclusions

A new quassinoid, 2'-(*R*)-*O*-acetylglaucaurubinone (**1**), and seven known quassinoids (**2**–**8**) were isolated from the bark of *Odyendyea gabonensis* (Pierre) Engler [syn. *Quassia gabonensis* Pierre] (Simaroubaceae) using bioactivity-guided separation. The structure of **1** was determined by spectroscopic analysis and confirmed by semisynthesis from **10**. We also established the complete assignments of ¹H and ¹³C NMR signals of **4**–**6**, as well as corrected configurations in **7** and **8**. The synthesized **1** showed potent cytotoxic activity against multiple cancer cell lines. The pattern of cytotoxicity against breast and ovarian cancer cell lines suggested that **1** does not target ER or PR. *Brcal/p53*-deficient mice treated with **1** showed significant reduction in mammary ductal branching, indicating that **1** could be a promising antitumor lead compound.

Experimental Section

General Experimental Procedures. Proton nuclear magnetic resonance (¹H NMR) spectra were measured on a Varian Gemini 2000 (300 MHz) or Unity Inova-500 (500 MHz) NMR spectrometer. All chemical shifts are reported in δ (ppm). Mass spectra were obtained on a JEOL JMS-700 (2) mass spectrometer. IR spectra were measured on a JEOL FT/IR-680 Plus spectrophotometer. Analytical thin-layer chromatography (TLC) was performed on Merck precoated aluminum silica gel sheets (Kieselgel 60 F 254). HPLC experiments were performed by using a Shimadzu LC-10 or LC-6 with UV detection at

254, 240, or 210 nm. An Alltech C18 column (22 mm diameter \times 250 mm) or YMC C18 column (46 mm diameter \times 250 mm) was used in RP-HPLC.

Plant Collection and Extract Preparation. Bark of *Odyendyea gabonensis* was collected in Gabon on February 6, 1991. The bark was extracted with MeOH/CH₂Cl₂ (1:1).

Isolation of Quassinoids. The raw extract (QG; 9.9 g) (ED₅₀ against MCF-7 <2.5 μ g/mL) was separated into a hexane-soluble fraction (QGP; 475 mg), an EtOAc-soluble fraction (QGE; 4.28 g), and residue (QGI_n). Then, the EtOAc fraction (ED₅₀ <0.37 mg/mL) was separated by silica gel CC (eluent, hexane/EtOAc = 4:1–2:1–1:1 to MeOH) to afford fractions QGE-S8 (110 mg) and QGE-S9 (1.4 g). Fraction QGE-S9 was separated by silica gel CC (eluent, MeOH/CHCl₃ gradient) to afford fractions QGE-S9-4 (12 mg), QGE-S9-5 (414 mg), QGE-S9-6 (32 mg), QGE-S9-7 (259 mg), and QGE-S9-11 (41 mg). QGE-S9-6 was applied to RP-HPLC (column, YMC C18; eluent, MeOH/H₂O, 6:4; flow rate, 3 mL/min) to afford **2** (7.5 mg). QGE-S8, QGE-S9-5, and QGE-S9-7 were combined (784 mg) and applied to silica gel CC (eluent, 8% MeOH/CHCl₃) to give fractions QGE-S9-5-3 (284 mg) and QGE-S9-5-4 (163.5 mg). A portion of QGE-S9-5-3 (144.6 mg) was applied to RP-HPLC (column, YMC C18; eluent, MeOH/H₂O, 6:4; flow rate, 3 mL/min) to afford **2** (36.6 mg) and **4** (15.1 mg). The remainder (110 mg) was subjected to RP-HPLC (column, Alltima C18; eluent, CH₃CN/H₂O gradient; flow rate, 5 mL/min) to give **1** (5.0 mg). QGE-S9-5-4 was separated by HPLC (column, Alltima C18; eluent, CH₃CN/H₂O gradient; flow rate, 5 mL/min) to give **3** (11.5 mg). QGE-S9-11 was applied to RP-HPLC (column, YMC C18; eluent, MeOH/H₂O, 6:4; flow rate, 3 mL/min) to give **5** (16.8 mg) and **6** (3.1 mg). QGE-S9-4 was separated by preparative TLC (eluent, 5% MeOH/CH₂Cl₂) to afford **7** (9.5 mg) and **8** (2.5 mg).

2'-(*R*)-Acetylglaucaurubinone (1**):** IR (liquid film) ν_{\max} 3447 (OH), 1741 (C=O), 1636 (C=C) cm⁻¹; ¹H NMR, see Table 1; ¹³C NMR, see Table 2; HREIMS *m/z* 536.2258 [M]⁺ (calcd for C₂₇H₃₆O₁₁ 536.2260).

Acetylation of 2'-(*S*)-Acetylglaucaurubinone (2**).** Acetic anhydride (0.1 mL) was added to a pyridine solution (0.5 mL) of **2** (9.3 mg), and the reaction mixture was stirred at rt overnight, then extracted with EtOAc/water. The organic layer was dried over anhydrous Na₂SO₄, filtered, and evaporated to give a residue, which was then purified by silica gel CC (eluent, EtOAc/hexane, 1:10) to afford pure **9** (4.1 mg).

Semisynthesis of 2'-(*R*)-Acetylglaucaurubinone (1**) from Glaucaurubinone (**10**).** TMSOTf (0.15 mL, 0.75 mmol) was added to a solution of **10** (50 mg, 0.13 mmol) in pyridine (1.3 mL) and Et₃N (0.21 mL, 1.5 mmol) at 0 °C. After stirring for 2.5 h at rt, the mixture was cooled to 0 °C, and a 1.0 M TBAF in THF solution (0.52 mL, 0.52 mmol) was added dropwise. The mixture was warmed to rt over 1 h, diluted with water, and extracted with EtOAc. The organic phase was washed with brine, dried over Na₂SO₄, and concentrated. The residue was chromatographed on silica gel eluting with EtOAc/hexane (1:4) to obtain di-TMS ether **11** (60 mg, 0.11 mmol, 86%), which was dissolved in CH₂Cl₂ (2.5 mL). EDCI (150 mg, 0.78 mmol), DMAP (27 mg, 0.22 mmol), and **13** (110 mg, 0.68 mmol) were added to the solution, which was stirred at rt overnight. The mixture was partitioned between water and CH₂Cl₂. The organic phase was washed with brine, dried over Na₂SO₄, and concentrated. The residue was chromatographed on silica gel eluting with EtOAc/hexane (1:4) to give ester **14** (26 mg, 0.04 mmol, 35%) along with **11** (32 mg, 53%). A solution of **14** (24 mg, 0.035 mmol) in MeOH (1.0 mL) was treated with citric acid (25 mg, 0.13 mmol) at rt for 2.5 h. After addition of EtOAc, the mixture was filtered through SiO₂. The SiO₂ was washed with EtOAc, and the combined filtrates were concentrated. The residue was chromatographed on silica gel eluting with EtOAc/hexane (1:4) to give **1** (14 mg, 0.026 mmol, 75%).

***Brcal^{fl/fl}p53^{fl5&6/fl5&6}Cre* Mutant Mice.** Generation of *Brcal^{fl/fl}p53^{fl5&6/fl5&6}Cre* and *p53^{fl5&6/fl5&6}Cre* mice has been described previously.^{17,18} The mice were in a C57BL/6 and 129/Sv mixed background. All animal experiments were in accordance with guidelines of the Federal and Institutional Animal Care and Use Committee at the University of California, Irvine.

Treatment with 2'-(*R*)-Acetylglaucaurubinone (1**).** Three-month-old mice were treated with 0.1 mg of **1** or vehicle daily for 7 days. Stock solution was 1 mg/mL in dimethylsulfoxide (DMSO). A mixture of 10 μ L of stock solution, 30 μ L of 40% polyethylene glycol (PEG), and 60 μ L of 0.9% NaCl solution was prepared at the time of treatment.

Vehicles include DMSO, PEG, and NaCl solution. Vehicle or compound was administered ip every day as described.

Histology and Immunohistochemistry. The fourth pair glands were dissected and spread on a glass slide. After fixation with Carnoy's fixative for 3 h, the tissues were hydrated and stained in Carmine alum overnight as described at <http://mammary.nih.gov/tools/histological/Histology/index.html#a1>. Branching points in three random areas totaling approximately 2 mm² were counted.

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Supporting Information Available: HPLC analysis of **1** (synthetic and natural), **2**, and **4** and NMR spectra of compound **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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