Synthetic Studies on Quassinoids: Transformation of (−)-Glaucarubolone into (−)-Peninsularinone. In Vivo Antitumor Evaluation of (−)-Glaucarubolone, (−)-Chaparrinone, and (−)-Peninsularinone

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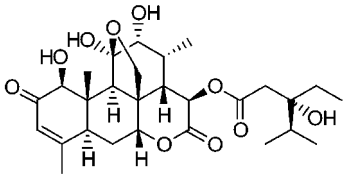
Peninsularinone (**1**), isolated from the roots of *Castela peninsularis*,[1] was identified by our solid tumor selective in vitro assay[2] as a candidate for potential in vivo activity. Subsequent in vivo trials employing a variety of murine solid tumor models[3] demonstrated that peninsularinone was both a very active and a very potent antitumor agent. To optimize therapeutic efficacy and, more importantly, probe the activity of **1** against both human tumor xenografts and murine p-glycoprotein-containing tumors, we required substantial quantities of peninsularinone. We detail below an efficient four-step sequence for the transformation of (−)-glaucarubolone (**2**) into (−)-peninsularinone (**1**) which constitutes a total synthesis of **1**.[4] In addition, the in vivo activity data for **1** against early stage murine pancreatic ductal adenocarcinoma P03, colon adenocarcinoma C38, and adriamycin-resistant mammary adenocarcinoma Mam 17/Adr implanted subcutaneously in mice, along with in vivo data for (−)-glaucarubolone (**2**) and (−)-chaparrinone (**3**), is presented.



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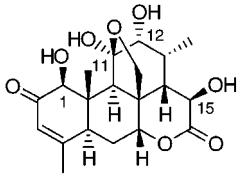

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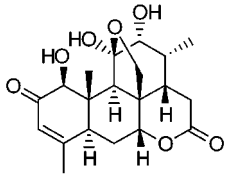

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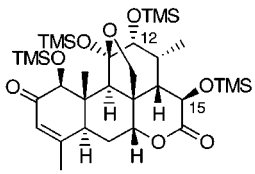
In view of the ready availability of (−)-glaucarubolone (**2**) from the root bark of *Castela polyandra*,[5] we set out to convert **2** into (−)-peninsularinone (**1**) by developing a protocol which would allow for the selective protection of the hydroxyl groups at C(1), C(11), and C(12) in **2** so as to permit exclusive acylation of the C(15) hydroxyl group. Subsequent removal of the protecting groups would provide **1**. Toward this end, a 0.1 M solution of glaucarubolone in pyridine containing 12 equiv of triethylamine at 0 °C was treated with 6.0 equiv of trimethylsilyl trifluoromethanesulfonate. After 2 h at ambient temperature, the fully protected tetra-*O*-trimethylsilyl glaucarubolone **4** was obtained. The tetra-*O*-trimethylsilyl compound **4** was not isolated but directly treated (0 °C) over a 3.0 h period employing a syringe pump with 4.0 equiv of tetra-*n*-butylammonium fluoride (1.0 M solution in tetrahydrofuran) which selectively cleaved the C(12) and C(15) *O*-trimethylsilyl groups. Workup gave rise to an 85% yield of **5** as a highly crystalline compound, mp 228−230 °C. Cleavage of the C(12) *O*-trimethylsilyl group was of no consequence, since subsequent acylation occurred exclusively at the more readily accessible C(15) hydroxyl group.



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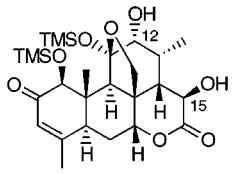

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Acylation of the C(15) hydroxyl in **5** was realized in 82% yield by exposure of **5** to a solution of racemic 3-ethyl-3-hydroxy-4-methylpentanoic acid in methylene chloride containing 4-(dimethylamino)pyridine and dicyclohexylcarbodiimide. The 1:1 mixture of diastereomers was readily separated by thin-layer chromatography, giving rise to **6** and **7**. The structural assignments were made after the more polar diastereomer **6** was transformed into **1**. Thus, exposure of **6** to citric acid in methanol afforded (98%) (−)-peninsularinone (**1**) whose physical and spectral properties were identical in all respects with those of an authentic sample. Similar treatment of **7** with citric acid gave rise to 3′-*epi*-peninsularinone (**8**).

Table 1 summarizes the in vivo data for (−)-peninsularinone against three tumors of murine origin. In addition, Table 1 contains the in vivo data [colon adenocarcinoma C38 and adriamycin-resistant mammary adenocarcinoma Mam 17/Adr] for (−)-glaucarubolone and (−)-chaparrinone, originally obtained by total synthesis.[4] All three quassinoids had therapeutic activity against C38. (−)-Chaparrinone (**3**) was found to be more active than **1** and **2**,[6] exhibiting a *T*/*C* value of 0% and a gross log cell kill of 3.9, indicative of potential clinical activity.[7]

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(1) Grieco, P. A.; Moher, E. D.; Seya, M.; Huffman, J. C.; Grieco, H. J. *Phytochemistry* **1994**, *37*, 1451.

(2) (a) Valeriote, F.; Corbett, T.; Edelstein, M.; Baker, L. *Cancer Invest.* **1996**, *14*, 124. (b) Corbett, T. H.; Valeriote, F. A.; Polin, L.; et al. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*; Valeriote, F. A., Corbett, T. H., Baker, L. H., Eds.; Kluwer Acadademic Publishers: Norwell, MA, 1992; pp 35−87.

(3) (a) Corbett, T.; Valeriote, F.; LoRusso, P.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Knight, J.; Demchik, L.; Jones, J.; Jones, L.; Lisow, L. In *Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval*; Teicher, B., Ed.; Humana Press Inc.: Totowa, NJ, pp 75−99. (b) Corbett, T.; Valeriote, F.; LoRusso, P.; et al. *Int. J. Pharmacognosy* **1995**, *33* (Supplement), 102.

(4) For a total synthesis of (−)-glaucarubolone and (−)-chaparrinone, see: Grieco, P. A.; Collins, J. L.; Moher, E. D.; Fleck, T. J.; Gross, R. S. *J. Am. Chem. Soc.* **1993**, *115*, 6078.

(5) Grieco, P. A.; Vander Roest, J. M.; Piñeiro-Nuñez, M. M. *Phytochemistry* **1995**, *38*, 1463.

(6) In determining which substrate is more active, the highest nontoxic dosages are compared. This is standard NCI practice.

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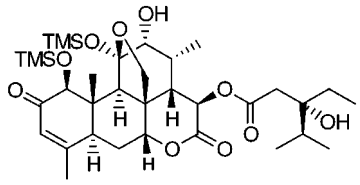
Table 1. In Vivo Activity of Quassinoids Administered Intravenously against Subcutaneous Transplanted Solid Tumors

expt no.	quassinoid	SC tumor[a]	no. of inj IV[b]	total dosage (mg/kg)	mean body wt loss in g/mouse	drug deaths	*T/C* (%)	gross log cell kill
1916	(−)-peninsularinone	C38	12	3.2	−2.4	0/5	23	0.4
1708	(−)-peninsularinone	P03	10	4.3	−1.6	0/5	3	2.0
1787	(−)-peninsularinone	MAM 17/Adr	6	1.2	−2.8	0/5	43	<1
1384	(−)-glaucarubolone	C38	3	151.0	−0.4	0/5	14	1.5[c]
1442	(−)-glaucarubolone	Mam 17/Adr	8	153.0		0/5	100	<1
1530	(−)-chaparrinone	C38	6	232.0	−2.0	0/5	0	3.9[c]
1544A	(−)-chaparrinone	Mam 17/Adr	10	260.0	+3.4	0/5	42	<1

[a] Tumors were implanted as 30−60 mg size fragments bilaterally on day 0. For Colon 38 and Pancreas 03, treatment was started on day 3. For Mam 17/Adr, a more rapidly growing tumor, treatment was started on day 1. [b] IV daily; dose was escalated throughout the treatment. [c] There were mice in two trials [1384 (1 out of 5) and 1530 (3 out of 5)] that remained tumor free for an extended period of time; however, all mice eventually regrew tumors. Cures are defined as long-term tumor free survivors (>100 days post last dose).



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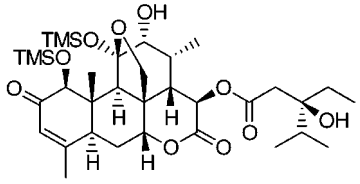

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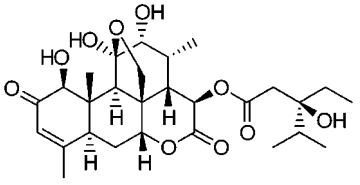

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It was of interest to find that **1** was at least 50-fold more potent than either **2** or **3** in this assay.[8] Both (−)-peninsularinone and (−)-chaparrinone showed marginal activity against Mam 17/Adr. (−)-Peninsularinone exhibited excellent activity against P03 showing a *T/C* value of 3 and gross log cell kill of 2.0, both values indicative of potential clinical activity.[7]

Experimental Section[9]

1,11-Bis(trimethylsilyloxy)glaucarubolone (5). A solution of 500 mg (1.27 mmol) of glaucarubolone (**2**) in 13.0 mL of pyridine containing 2.1 mL (15.0 mmol) of triethylamine at 0 °C was treated with 1.5 mL (7.5 mmol) of trimethylsilyl trifluoromethanesulfonate. After the solution was warmed to ambient temperature and stirred for 2 h, the reaction was recooled to 0 °C and was treated via the aid of a syringe pump over a 3 h period with 5.2 mL (5.2 mmol) of a 1.0 M solution of tetra-*n*-butylammonium fluoride in tetrahydrofuran. After the solution was warmed to ambient temperature over a 1 h period, the reaction mixture was diluted with water and washed with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo giving rise to a brown oil. The oil was chromatographed on 130 g of flash silica gel. Elution with hexanes−ethyl acetate, 2:1, afforded 593 mg (85%) of 1,11-bis(trimethylsilyloxy)glaucarubolone (**5**) as a white solid: R_f 0.72 (ethyl acetate−hexanes, 4:1); IR (KBr) 3595 (m), 3545 (w), 1720 (s), 1690 (s) cm^{-1}; 400 MHz ^1H NMR (C_5D_5N) δ 7.93 (d, 1H, J = 5.2 Hz), 6.04 (br s, 1H), 5.88 (d, 1H, J = 4.8 Hz), 5.32 (dd, 1H, J = 11.2, 5.2 Hz), 4.57 (br s, 1H), 4.23 (s, 1H), 4.00 (d, 1H, J = 8.2 Hz), 3.88 (t, 1H, J = 4.8 Hz), 3.73 (d, 1H, J = 8.2 Hz), 3.11−3.02 (m, 1H), 3.06 (s, 1H), 2.54 (m, 1H), 2.26 (dd, 1H, J = 11.2, 6.4 Hz), 2.06 (dt, 1H, J = 14.2, 2.8 Hz), 1.88 (t, 1H, J = 14.2 Hz), 1.71 (s, 3H), 1.66 (d, 3H, J = 7.2 Hz), 1.32 (s, 3H), 0.36 (s, 9H), 0.32 (s, 9H); 100 MHz ^{13}C NMR (C_5D_5N) δ 198.49, 174.06, 160.92, 127.08, 113.61, 88.04, 81.02, 77.96, 71.71, 68.58, 49.83, 47.26, 46.04, 44.28, 44.09, 33.55, 25.85, 22.36, 16.25, 10.65, 3.01, 1.55; high-resolution MS (EI) calcd for $C_{26}H_{42}O_8Si_2$ (M) *m/e* 538.2419, found 538.2393. An analytical sample was prepared by recrystallization from ethyl acetate: mp 228−230 °C (dec); $[\alpha]^{25}_D$ −12 (*c* 0.55, pyridine). Anal. Calcd for $C_{26}H_{42}O_8Si_2$: C, 57.96; H, 7.86. Found: C, 57.85; H, 8.09.

1,11-Bis(trimethylsilyloxy)peninsularinone (6) and 3′-*epi*-1,11-Bis(trimethylsilyloxy)peninsularinone (7). A solution of 150 mg (0.28 mmol) of 1,11-bis(trimethylsilyloxy)glaucarubolone (**2**), 112 mg (0.70 mmol) of racemic 3-ethyl-3-hydroxy-4-methylpentanoic acid, and 68 mg (0.56 mmol) of 4-(dimethylamino)pyridine in dry methylene chloride was treated with 142 mg (0.69 mmol) of solid 1,3-dicyclohexylcarbodiimide in a

(7) *T/C* values that are less than 42% are considered to be active by NCI standards; *T/C* values that are less than 10% are considered to have excellent activity and potential clinical activity by NCI standards. Gross log cell kill is defined as $T - C/3.2T_d$, where T is the median time in days for the tumors of the treated group of mice to reach 750 or 1000 mg, C is the median time in days for the tumors of the control group to reach 750 or 1000 mg, and T_d is the tumor volume doubling time. Gross log cell kill values of >2.8, 2.0−2.8, 1.3−1.9, 0.7−1.2, and <0.7 with duration of drug treatment of 5−20 days are scored ++++, +++, ++, +, and − (inactive), respectively. An activity rating of +++ to ++++, which is indicative of clinical activity, is needed to effect partial or complete regression of 100−300 mg sized masses of most transplanted solid tumors of mice.

(8) Potency refers to toxicity (usually the dose that produces lethal toxicity) and has nothing to do with antitumor activity. Since compound **1** is 50 times more potent than compound **3**, this means that a dose of say 6 mg/kg of **1** would kill 50% of the mice but it would require a dose of 300 mg/kg of **3** to accomplish the same effect. Antitumor activity is evaluated at the maximum tolerated nonlethal dose and fractions thereof. With the case in point, **3** was more active in terms of antitumor activity (log cell kill) than **1**. This is common in antitumor testing; frequently the most potent analogues are not the most active in terms of antitumor activity.

(9) Proton and carbon nuclear magnetic resonance spectra were recorded on Varian VXR-400 MHz or a Bruker DPX 300 MHz spectrometers. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (δ 0.0). Infrared (IR) spectra were taken on a Perkin-Elmer Model 298 spectrophotometer, a Mattson Galaxy 4020 series FTIR spectrometer, or on a Perkin-Elmer model 1600 FT-IR. Absorption intensities are indicated as strong (s), medium (m), or weak (w). High-resolution mass spectra were obtained on a VG Instruments 70E-HF mass spectrometer. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ. Melting points were obtained on a Fisher-Johns hot-stage or on a MEL-TEMP capillary melting point apparatus. Optical rotations were obtained on a Perkin-Elmer Model 241 polarimeter. Reactions were monitored by thin-layer chromatography (TLC) using E. Merck precoated silica gel 60 F-254 (0.25 mm) plates. E. Merck precoated silica gel 60 F-254 (0.50 mm) plates were used for preparative plate chromatography. Merck silical gel 60 (230−400 mesh) was used for flash chromatography.

single portion. The reaction was allowed to stir at ambient temperature for 12 h. The resulting heterogeneous reaction mixture was filtered through a small plug of Celite with methylene chloride, and the filtrate was concentrated in vacuo. Chromatography of the residue on 100 g of silica gel, eluting with hexanes−ethyl acetate (2:1), provided 158 mg (82%) of the desired diastereomers **6** and **7** as a white foam.

The mixture of **6** and **7** was dissolved in 2.0 mL of methylene chloride and partitioned into six fractions. Each fraction was applied to a silica gel preparative plate (500 μm thickness) and eluted five times with hexanes−ethyl acetate (2:1). The less polar material was isolated with 20 mL of a 10% solution of methanol in ethyl acetate and filtered through a pad of Celite. The filtrate upon concentration in vacuo afforded 72 mg (39%) of **7** as a white foam: R_f 0.18 (hexanes−ethyl acetate, 2:1); $[\alpha]^{25}_D$ +47.1 (*c* 1.0, $CHCl_3$); IR (film) 3588 (w), 1735 (s), 1685 (s) cm^{-1}; 300 MHz ^1H NMR ($CDCl_3$) δ 5.96 (bs, 1H), 5.60 (bd, 1H, J = 11.0 Hz), 4.54 (bs, 1H), 4.00 (s, 1H), 3.94 (d, 1H, J = 8.7 Hz), 3.62 (d, 1H, J = 8.7 Hz), 3.51 (bd, 1H, J = 2.6 Hz), 2.82 (bd, 1H, J = 12.6 Hz), 2.68 (s, 1H), 2.53 (AB quartet, 2H, $\Delta\nu_{AB}$ = 32.8 Hz, J = 15.2 Hz), 2.31 (m, 2H), 2.17 (dt, 1H, J = 14.6, 3.1 Hz), 2.02−1.84 (m, 3H), 1.90 (s, 3H), 1.60 (m, 2H), 1.13 (s, 3H), 1.08 (d, 3H, J = 6.7 Hz), 0.95−0.87 (m, 9H), 0.17 (s, 9H), 0.15 (s, 9H); 75 MHz ^{13}C NMR (C_5D_5N) δ 198.5, 171.8, 168.1, 160.7, 127.1, 113.4, 87.9, 80.5, 78.2, 75.3, 71.5, 70.6, 47.8, 46.2, 46.1, 43.8, 43.8, 40.8, 34.8, 33.2, 29.3, 25.5, 22.2, 17.4, 17.3, 15.4, 10.6, 8.2, 2.9, 1.5; high-resolution MS (EI) calcd for $C_{34}H_{56}O_{10}Si_2$ (M) *m/e* 680.3412, found 680.3415.

The more polar compound was isolated with a 10% solution of methanol in ethyl acetate and was filtered through a pad of Celite. The filtrate upon concentration in vacuo gave 77 mg (40%) of **6** as a white foam: R_f 0.15 (hexanes−ethyl acetate, 2:1); $[\alpha]^{25}_D$ +42.6 (*c* 0.77, $CHCl_3$); IR (film) 3579 (w), 3517 (w), 1734 (s), 1685 (s) cm^{-1}; 300 MHz ^1H NMR ($CDCl_3$) δ 5.97 (bs, 1H), 5.63 (bd, 1H, J = 11.0 Hz), 4.53 (bs, 1H), 4.00 (s, 1H), 3.93 (d, 1H, J = 8.7 Hz), 3.61 (d, 1H, J = 8.7 Hz), 3.51 (bd, 1H, J = 3.2 Hz), 2.82 (bd, 1H, J = 12.1 Hz), 2.69 (s, 1H), 2.53 (AB quartet, 2H, $\Delta\nu_{AB}$ = 37.8 Hz, J = 15.1 Hz), 2.30 (m, 2H), 2.17 (dt, 1H, J = 14.7, 3.0 Hz), 2.03 (bs, 1H), 2.02−1.84 (m, 3H), 1.90 (s, 3H), 1.62 (q, 2H, J = 7.5 Hz), 1.13 (s, 3H), 1.08 (d, 3H, J = 6.6 Hz), 0.95−0.86 (m, 9H), 0.17 (s, 9H), 0.15 (s, 9H); 75 MHz ^{13}C NMR (C_5D_5N) δ 198.5, 171.9, 168.2, 160.7, 127.1, 113.4, 87.9, 80.5, 78.2, 75.3, 71.5, 70.6, 47.8, 46.2, 46.1, 43.8, 43.8, 40.8, 34.6, 33.2, 29.5, 25.5, 22.2, 17.4, 17.1, 15.4, 10.6, 8.2, 2.9, 1.5; high-resolution MS (EI) calcd for $C_{34}H_{56}O_{10}Si_2$ (M) *m/e* 680.3412, found 680.3423.

(−)-Peninsularinone (1). A solution of 75 mg (0.11 mmol) of **6** in 2.2 mL of methanol was treated with 85 mg (0.44 mmol) of citric acid at ambient temperature. After 3 h, the reaction was diluted with ethyl acetate and was filtered through a plug of silica gel. The silica gel was washed with ethyl acetate. The combined filtrates were concentrated in vacuo, and the residue was chromatographed on 40 g of silica gel. Elution with ethyl acetate afforded 57 mg (97%) of (−)-peninsularinone (**1**). Recrystallization from ethyl acetate provided analytically pure **1**, mp 230−232 °C [lit.[1] mp 221−223 °C]; $[\alpha]^{25}_D$ −20.9 (*c* 1.1, pyridine) (lit.[1] $[\alpha]^{25}_D$ −22.6 (*c* 0.19, pyridine)). The ^1H NMR (400 MHz), ^{13}C NMR (75 MHz), and IR spectra of this material were superimposable with the spectra of natural peninsularinone. Anal. Calcd for $C_{28}H_{40}O_{10}$: C, 62.67; H, 7.51. Found: C, 62.39; H, 7.65.

(−)-3′-*epi*-Peninsularinone (8). A solution of 72 mg (0.11 mmol) of **7** in 2.1 mL of methanol was treated with 81 mg (0.42 mmol) of citric acid at ambient temperature. After 3 h, the reaction mixture was diluted with ethyl acetate and filtered through a plug of silica gel. The silica gel was washed with ethyl acetate. The combined filtrates were concentrated *in vacuo* and the residue was chromatographed on 40 g of silica gel. Elution with ethyl acetate afforded 55 mg (96%) of 3′-*epi*-peninsularinone (**8**) as a white solid: R_f 0.26 (chloroform−methanol, 95:5); IR (KBr) 3448 (m), 1735 (s), 1676 (s) cm^{-1}; 300 MHz ^1H NMR (C_5D_5N) δ 7.46 (bs, 1H), 6.44 (bd, 1H, J = 11.1 Hz), 6.08 (bs, 1H), 4.79 (bs, 1H), 4.21 (s, 1H), 4.14 (d, 1H, J = 8.7 Hz), 4.02 (bs, 1H), 3.84 (d, 1H, J = 8.7 Hz), 3.38 (s, 1H), 3.07 (bd, 1H, J = 12.3 Hz), 2.89 (AB quartet, 2H, $\Delta\nu_{AB}$ = 12.8 Hz, J = 15.0 Hz), 2.65−2.55 (m, 2H), 2.25−2.10 (m, 2H), 2.08−1.87 (m, 3H), 1.70 (s, 3H), 1.54 (s, 3H), 1.37 (bd, 3H, J = 6.3 Hz), 1.10−1.02 (m, 9H); 75 MHz ^{13}C NMR (C_5D_5N) δ 197.4, 172.0, 168.3, 162.2, 126.1, 110.7, 84.3, 79.9, 78.5, 75.3, 71.3, 70.7, 48.0, 46.0, 45.5, 45.4, 42.1, 40.9, 34.8, 32.7, 29.3, 25.9, 22.3, 17.4, 17.3, 15.5, 10.7, 8.2; high-resolution MS (EI) calcd for $C_{28}H_{40}O_{10}$ (M) *m/e* 536.2621, found 536.2632. An analytical sample was prepared by recrystallization from ethyl acetate: mp 224.7−226.0 °C; $[\alpha]^{25}_D$ −10.2 (*c* 0.64, pyridine). Anal. Calcd for $C_{28}H_{40}O_{10}$: C, 62.67; H, 7.51. Found: C, 62.48; H, 7.53.

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