A New Quassinoid from *Castela texana*

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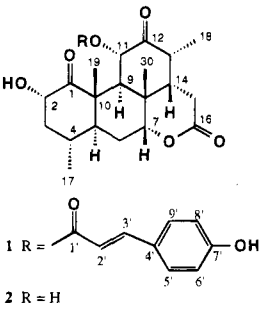
Received April 3, 1995[⊗]

A new quassinoid, 11-*O*-*trans*-*p*-coumaroyl amarolide (**1**) was isolated from *Castela texana*, and the structure was elucidated by spectroscopic analysis. Compound **1** is the first coumaroyl quassinoid derivative to have been isolated from nature. The known compounds amarolide (**2**), chaparrinone, chaparrin, glaucarubolone, holacanthone, and 15-*O*-β-D-glucopyranosyl glaucarubol were also isolated. All isolated compounds were tested for their cytotoxicity and antiprotozoal activities.

Castela texana (T. & G.) Rose (syn. *C. tortuosa* Liebm, *C. Nicholsonii* Hook. β *texana* T. & G.; Simaroubaceae) is a small shrub distributed in southwestern Texas and northeastern Mexico.[1] This plant has been used in folk medicine to treat human dysentery,[2] and the stem bark is intensely bitter. In the course of our continuing search for bioactive quassinoids with potential for use in chemotherapy of protozoal infections, the MeOH extract of the aerial part of this plant was investigated. Herein we report the isolation, structure elucidation, and bioactivity of a new quassinoid, 11-*O*-*trans*-*p*-coumaroyl amarolide (**1**), five known quassinoids—amarolide (**2**), chaparrinone, chaparrin, glaucarubolone, and holacanthone—and the quassinoid glycoside, 15-*O*-β-D-glucopyranosyl glaucarubol.



The $CHCl_3$-soluble portion of the MeOH extract of the coarsely powdered aerial parts of *C. texana* upon repeated CC over Si gel and crystallization, afforded seven pure compounds. By means of spectroscopic analysis and comparison with published data or with authentic specimens, six known compounds were identifed: amarolide (**2**),[3] chaparrinone,[4–6] chaparrin,[7,8] glaucarubolone,[4,9] holacanthone,[5,9,10] and 15-*O*-β-D-glucopyranosyl glaucarubol.[11] Glaucarubolone, chaparrinone, and chaparrin were previously isolated from the stem bark of this plant collected in Mexico.[8] A new quassinoid, 11-*O*-*trans*-*p*-coumaroyl amarolide (**1**), was also fully characterized. Compound **1** is the first coumaroyl quassinoid derivative to have been isolated from nature.

Compound **1** was obtained as colorless needles from Me_2CO. The HREIMS spectrum of **1** showed a molecular ion at *m/z* 510.2259 corresponding to the formula $C_{29}H_{34}O_8$. The IR absorptions at 3414, 3270, 1715, 1693, 1604, and 1515 cm^{-1} suggested the presence of hydroxyl groups, carbonyl groups, and an aromatic ring. The EIMS showed a fragment representing a loss of $C_9H_7O_2$. This behavior, together with the presence of a pair of *trans*-coupled olefinic {δ 6.53 and 8.02 (each 1H, d, J = 16 Hz)}, and A_2B_2-type aromatic {δ 7.01 and 7.57 (each 2H, d, J = 8.6 Hz)} protons indicated the presence of a *trans*-*p*-coumaroyl component in compound **1**. The seven carbon signals from δ 114.2 to 166.5, which represented the nine carbons of coumaroyl moiety, were assigned by using COSY, HETCOR, and LR-HETCOR NMR techniques.

The remainder of the molecule of **1** consisted of $C_{20}H_{27}O_6$, the same formula as amarolide less one proton, suggesting that **1** could be the coumaroyl ester of amarolide. The similarity of the ^{1}H- and ^{13}C-NMR spectra of **1** and amarolide (**2**) also supported this suggestion. The ^{13}C-NMR characteristics of the remaining 20 carbon signals showed four methyls (δ 10.6, 13.0, 18.5, 21.5); three methylenes (δ 26.9, 29.2, 49.6); eight methines, including three oxymethines (δ 29.1, 37.3, 43.1, 47.5, 48.2, 70.9, 75.4, 81.9); and five quaternary carbons (δ 35.7, 50.3, 168.7, 203.7, 214.4). Careful comparison of these 20 ^{13}C-NMR signals of **1** with those of amarolide **2**, revealed that the only substantial difference was that the carbon signals assigned to C-9 and C-12 were shifted upfield about 3 ppm and 6 ppm, respectively; other signals were essentially the same. The observed chemical shift changes can be the result of esterification. The *p*-coumaroyl group in **1** was located at the C-11 hydroxyl group of amarolide, since, in the ^{1}H-NMR spectrum of **1**, the signal ascribed to H-11 {δ 5.80 (d, J = 13.1 Hz)} was shifted downfield compared with that of **2**. The relative stereochemistry at C-2, C-4, C-5, C-7, C-8, C-9, C-10, C-11, C-13, and C-14 in compound **1** was the same as in compound **2** and was confirmed with the help of a NOESY spectrum and the coupling constants observed in the ^{1}H-NMR spectrum. The NOESY spectrum of **1** showed strong interactions between the protons at δ 5.80 (H-11) and 3.18 (H-13) and the methyl protons at δ 1.53 (H-30), as well as strong interactions between the protons at δ 4.39 (H-7) and 2.23 (H-14) and the methyl protons at 1.53 (H-30), indicating that all have β orientation; the methyl protons at δ 1.37 (H-19) interacted with the protons at δ 5.06 (H-2), 5.80 (H-11) and the methyl protons at δ

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⊗ Abstract published in *Advance ACS Abstracts,* December 1, 1995.

0163-3864/96/3259-0073$12.00/0

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds 1 and 2 (C_5D_5N)

	1		2	
position	δ_C	δ_H (m, J in Hz)	δ_C	δ_H (m, J in Hz)
1	214.4 (s)		215.9 (s)	
2	70.9 (d)	5.06 (dd, 11.0, 7.5)	70.8 (d)	4.75 (dd, 11.2, 7.8)
3	49.6 (t)	2.45 (m); 1.08 (m)	50.4 (t)	2.50 (m); 1.08 (m)
4	29.1 (d)	1.83 (m)	29.1 (d)	2.02 (m)
5	48.2 (d)	1.58 (ddd, 11.4, 11.4, 4.9)	47.8 (d)	1.39 (m)
6	26.9 (t)	1.75–1.85	26.7 (t)	1.85 (m); 2.00 (m)
7	81.9 (d)	4.39 (t, 2.6)	82.0 (d)	4.28 (dd, 3.7, 2.1)
8	35.7 (s)		35.4 (s)	
9	37.3 (d)	3.58 (d, 13.1)	40.3 (d)	2.60 (d, 12.5)
10	50.3 (s)		50.4 (s)	
11	75.4 (d)	5.80 (d, 13.1)	74.8 (d)	4.36 (d, 12.5)
12	203.7 (s)		210.6 (s)	
13	43.1 (d)	3.18 (m)	42.7 (d)	3.03 (m)
14	47.5 (d)	2.23 (m)	47.8 (d)	2.20 (m)
15	29.2 (t)	2.71 (dd, 6.5, 18.4)	29.1 (t)	2.58 (dd, 12.2, 5.5)
		2.42 (dd, 12.8, 18.4)		2.18 (dd, 12.2, 17.5)
16	168.7 (s)		169.1 (s)	
17	18.5 (q)	0.65 (d, 6.3)	18.5 (q)	0.91 (d, 6.5)
18	10.6 (q)	0.95 (d, 6.6)	10.6 (q)	1.06 (d, 6.8)
19	13.0 (q)	1.37 (s)	13.1 (q)	1.47 (s)
30	21.5 (q)	1.53 (s)	21.5 (q)	1.50 (s)
1′	166.5 (s)			
2′	114.2 (d)	6.53 (d, 16.0)		
3′	146.6 (d)	8.02 (d, 16.0)		
4′	126.2 (s)			
5′, 9′	130.9 (d)	7.57 (d, 8.6)		
6′, 8′	116.7 (d)	7.01 (d, 8.6)		
7′	161.5 (s)			

Table 2. Cytotoxicity and Antimalarial Activity of Some Quassinoids (IC_{50} μg/mL)

compound	K562	KB	BT-549	SK-OV-3	Vero	*P. falicparum* (D6 clone)	*P. falciparum* (W2 clone)
11-*O*-*trans*-*p*-coumaroylamarolide {**1**}	NA[a]	NA	NA	NA	NA	0.92	0.75
amarolide {**2**}	NA	NA	NA	NA	NA	NA	NA
chaparrinone	1.80	1.20	0.80	0.50	1.50	0.25	0.20
glaucarubolone	0.60	1.00	0.40	0.80	1.50	0.125	0.20
chaparrin	NA	NA	NA	NA	NA	0.25	0.35
holacanthone	1.00	0.50	0.30	0.50	1.00	0.010	0.012
15-*O*-glucopyranosyl glaucaroubol	NA	NA	NA	NA	NA	NA	NA
doxorubicin	0.035	0.12	0.008	0.01	1.5	NT[b]	NT
chloroquine	NT	NT	NT	NT	NT	0.006	0.1

[a] NA = No activity at concentration 10 μg/mL or lower. [b] NT = Not tested.

1.53 (H-30), indicating H-2 and the C-19 methyl group also have β orientation. Interactions between protons at δ 1.58 (H-5) and 3.58 (H-9) and no interaction of H-5 and H-9 with the protons of methyl groups 19 and 30, indicated H-5 and H-9 have α orientation. The multiplicity of the α-oriented H-5 (ddd, J = 4.9, 11.4, 11.4 Hz) suggested that the coupling constant between H-4 (δ 1.83, m) and H-5 is 11.4 Hz, and this indicated that H-4 has a β orientation. On the basis of the preceding spectral evidence, the structure of **1** was established as 11-*O*-*trans*-*p*-coumaroyl amarolide. Complete ^{1}H- and ^{13}C-NMR assignments of **1** and **2** were made by using 1D and 2D NMR techniques and are listed in Table 1.

All seven of the isolated compounds were submitted for antiprotozoal (anti-*Plasmodium*, anti-*Giardia*, and anti-*Crithidia*) and anticancer bioassays. These compounds did not have antigiardial or trypanocidal activity at concentrations of 20 μg/mL or lower. Testing in the anticancer bioassay showed chaparrinone, glaucarubolone, and holacanthone exhibited potent general cytotoxicity without selectivity. The remaining compounds showed no cytotoxicity at the highest concentration tested (10 μg/mL). Testing in the antimalarial bioassay showed that **1** and chaparrin possessed moderate antimalarial activity without potent cytotoxicity. Structure–activity relationship studies of quassinoids have suggested than an α,β-unsaturated ketone in ring-A and an oxymethylene bridge in ring-C are generally considered necessary for anticancer and antimalarial activities[12]; however, exceptions do exist. For example, neoquassin glucoside (picrasinoside B) has antileukemic activity, while neoquassin itself is not active.[13] Compound **1** is another example of a quassinoid that has moderate antimalarial activity without having the structural features generally considered necessary for this bioactivity. With the exception of 15-*O*-β-D-glucopyranosyl glaucaroubol and **2**, the remaining compounds exhibited antimalarial activity. However, these compounds also showed potent cytotoxicity, and only holacanthone had good selectivity against *Plasmodium falciparum*. The IC_{50} of holacanthone in our antimalarial assay was very comparable with that previously reported by O'Neill *et al.*[10] Our results are shown in Table 2.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover Uni-melt capillary apparatus or a Fisher-Johns digital melting point analyzer model 355 and were not corrected. Optical rotations were determined on a Perkin-Elmer 141 automatic polarimeter using MeOH solutions. UV

spectra were taken on a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer in MeOH solutions. IR spectra were taken as KBr pellets on a Perkin-Elmer 1600 Series FT-IR spectrometer. NMR spectra were recorded at 300 MHz (proton) and 75 MHz (carbon) on a Varian VXR-300 instrument, and the solvent signal was used as reference. Low resolution EIMS and the HRMS were obtained in the Department of Chemistry, University of Kansas, Lawrence, KS. Si gel (230–400 mesh, Merck) was used for CC. All solvents used for chromatographic purposes were AR grade.

Plant Material. The *C. texana* plant material used in this study was collected in July 1992, in Terrell County, Texas, and a voucher specimen is deposited at the Institute for Botanical Exploration, Starkville, Mississippi.

Extraction and Purification. The ground twigs and leaves of *C. texana* (6.2 kg) were extracted with MeOH five times. The MeOH extract, after removal of the solvent *in* vacuo at 50 °C, afforded a dark-green residue (438 g). A portion (390 g) of this residue was partitioned between hexane and MeOH-H_2O (9:1), and the aqueous methanolic solution was evaporated *in vacuo* to yield a viscous green-brown residue (335 g). This residue was further partitioned between $CHCl_3$ and MeOH-H_2O (1:9). Evaporation of the $CHCl_3$-soluble fraction *in vacuo*, gave a $CHCl_3$ extract (70 g). A portion (40 g) of the $CHCl_3$ extract was subjected to CC over 400 g Si gel and eluted with a hexane/EtOAc gradient and then MeOH, and was separated into seven crude fractions (fractions 1–7). Fraction 5 was further separated by CC over Si gel with elution by $CHCl_3$-MeOH (100:1–5:1), and the fractions obtained were pooled based on their similarity by TLC. After crystallization, amarolide (**2**) (490 mg), chaparrinone (212 mg), glaucarubolone (330 mg), and chaparrin (60 mg) were obtained. The mother liquid of chaparrinone gave 450 mg residue, and rechromatography on Si gel with elution by EtOAc-Me_2CO gave **1** (25 mg) and holacanthone (30 mg). Fraction 6 was similarly rechromatographed with elution by $CHCl_3$-MeOH (10:1-1:1) to afford 15-*O*-β-D-glucopyranosyl glaucarubol (32 mg).

Known compounds, amarolide (**2**), chaparrinone, chaparrin, glaucarubolone, holacanthone, and 15-O-β-D-glucopyranosyl glaucarubol, were identified by comparison of their corresponding properties (mp, IR, MS, and NMR) with literature values; chaparrinone and holacanthone were compared with authentic samples.

11-*O-TRANS-p*-Coumaroyl Amarolide (1): mp 281–282 °C (dec) (colorless needles from Me_2CO); $[\alpha]^{25}$ D-80° (c 0.1, MeOH); UV λ_{max} (MeOH) (log ϵ) 312 (4.40), 300 (4.33), 227 (4.08) nm; IR (KBr) ν max 3414, 3270, 1715, 1693, 1604, 1515, 1443, 1400, 1378, 1354, 1260, 1240, 1199, 1165, 1115, 1085, 1026, 1009, 968, 826, and 693 cm^{-1}; HREIMS *m/z* $[M]^+$ 510.2259 (calcd for $C_{29}H_{34}O_8$, 510.2254); EIMS *m/z* $[M]^+$ 510 (29), $[M - HOC_6H_4CH{=}CHCO]^+$ 363 (18), 335 (14), 318 (10), 301 (14), 287 (36), 274 (100), 257 (50), 241 (40), 229 (41), 164 (15), $[HOC_6H_4CH{=}CHCO]^+$ 147 (100), $[HOC_6H_4CH{=}CH]^+$ 119 (26), 91 (36); ^{1}H- and ^{13}C-NMR data, see Table 1.

Amarolide (2): mp 260–262 °C (colorless needles from MeOH); IR (KBr) ν max 3508, 3472, 2954, 2858, 1726, 1700, 1452, 1412, 1350, 1265, 1225, 1118, 1077, 1035, 1008, 969, 826, and 700 cm^{-1}; EIMS *m/z* $[M]^+$ ($C_{20}H_{28}O_6$) 364 (5), 318 (52), 274 (25), 259 (8), 214 (25), 199 (15), 187 (75), 171 (20), 159 (45) 147 (60), 133 (30), 119 (35), 49 (100); ^{1}H- and ^{13}C-NMR data see Table 1.

Bioassay Sample Preparation. The compounds were solubilized initially in DMSO. Serial dilutions of these compounds were made using a Matrix Electrapette Liquid Handling System (Matrix Technologies, Lowell, MA) and then added in duplicate to the 96-well microtiter plates containing the appropriate organism. The samples were diluted to contain a concentration of DMSO that had no effect on the growth of the organism being tested.

Cytotoxicity Assays. The human cell lines used in the cytotoxicity assays were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cell lines used were KB (CCL 17), epidermoid carcinoma; BT-549 (HTB 122), breast carcinoma; K-562 (CCL 243), leukemia; SK-OV-3 (HTB 77), ovary carcinoma; Vero cells (CCL 81), fibroblast cell line from African green monkey kidney (non-transformed). All cell lines were adapted for growth in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO) supplemented with glutamine, sodium bicarbonate, and 10% fetal calf serum (Hyclone Laboratories, Logan, UT) at 37 °C in 5% CO_2 humidified atmosphere.

Cytotoxicity assays were performed in 96-well microtiter plates. Approximately 100 μL of the cells were added to each well of a 96-well microtiter plate to achieve a final concentration of 50 000 cells per well. The plate containing the cells was incubated for approximately 24 h. At 24 h, serial dilutions of the compounds to be tested were made and added to duplicate wells. RPMI 1640 medium was added to bring the total volume in each well to 250 μL. The cells were incubated with the test compounds for an additional 2–7 days, depending on the growth characteristics of the particular cell line. At the end of the incubation period, viability of the cells was determined relative to control wells using the neutral red assay.[14] Doxorubicin was used as a positive control. The plate were read on a Bio-Tek Model 312e microplate reader at 490 nm with a reference wavelength of 630 nm.

Antimalarial Assays. Stock cultures of the Sierra Leone D6 clone (chloroquine-sensitive) and Indochina W2 clone (chloroquine-resistant) of *Plasmodium falciparum* were maintained using the method of Trager and Jensen.[15] The cultures were obtained from Dr. Michael Makler (Flow, Inc., Portland, OR).

Each clone was adjusted with fresh A+ uninfected red blood cells (Mississippi Blood Services, Jackson, MS) and complete growth medium to yield a 2% hematocrit and a 2.0% parasitemia. Each compound was tested against the D6 and W2 clones of *P. falciparum*. The suspensions of each clone were added in 200-μL aliquots to all wells of the 96-well plates, except for wells used for medium blanks and uninfected-red-blood-cell controls. Of each drug dilution 10-μL aliquots were added in duplicate to adjacent wells on each microtiter plate using a Matrix Electrapette liquid handling system (Matrix Technologies, Lowell, MA). The plates were then placed in an airtight, humidified Modular incubator (Billups-Rothenberg Del Mar, CA), flushed with a gas mixture of 90% N_2, 5% O_2, 5% CO_2, and incubated at 37 °C for approximately 48 h. Chloroquine and arteether were used as positive controls. After a 48-h incubation, the plates were analyzed for antimalarial

activity using the parasite LDH method of Makler *et al.*[16] The plates were analyzed by reading the endpoint at 630 nm on a Biotek 312e microplate reader (Fisher Scientific, Norcross, GA).

Antigiardial Assay. Stock cultures of *Giardia intestinalis* (ATCC 30888, ATCC, Rockville, MD) were grown as described in the *ATCC Catalogue of Protists*.[17] Approximately 1×10^5 organisms were inoculated per well in a Corning 96-well microtiter plate. The plate was placed into a Billups-Rothenberg modular incubator for 24 h at 37 °C to allow the organisms to enter the logarithmic phase of growth. At 24 h, serial dilutions of the compounds to be tested were added to each well in duplicate. The organisms were incubated for an additional 24 h. The viability of the organisms was determined using a modification of the XTT procedure developed by Wright *et al.*[18] XTT was prepared at 1 mg/mL in warm phosphate-buffered saline (PBS). A 1.53-mg/mL solution of phenazine methosulfate (PMS) was prepared in PBS and 50 μL was added per 5 mL of XTT solution. After removal of the growth medium and rinsing of the *Giardia* plates with warm PBS, 100 μL of warm 1% dextrose in PBS was added to each well of the microtiter plate. To each well, 25 μL of the XTT/PMS solution was added. The remainder of the procedure was the same as previously described.[18] Metronidazole and furazolidone were used as positive controls. The plates were then read on a Bio-Tek Model EL 312e microplate reader at 450 nm with a reference wavelength of 630 nm.

Trypanocidal Assay. Stock cultures of *Crithidia fasciculata* (ATCC 11745, ATCC, Rockville, MD) were grown as described in the *ATCC Catalogue of Protists*.[19] Approximately 1×10^5 cells were inoculated per well into a 96-well microtiter plate. The plate was sealed with parafilm and incubated at 25 °C for 24 h. At 24 h, serial dilutions of the compounds to be tested were added to each well in duplicate. A modification of the sulforhodamine B protein assay was used to determine the viability of organisms remaining at the end of 48 h. The modification involved centrifuging the microplates after addition of 80% trichloroacetic acid to each well of the microplate. The remainder of the procedure was as previously described.[20] Pentamidine and berenil were used as positive controls. The plates were then read on a Bio-Tek Model EL 312e microplate reader at 490 nm with a reference wavelength of 630 nm.

Bioassay Data Analysis. Dose-response curves were generated by analysis with Microsoft Excel version 4.0 (Microsoft Corporation) for all biological assays. The percentage relative to controls was plotted versus the log of the concentration of compound tested to determine the IC_{50} values for each compound.

Acknowledgment. The authors wish to thank Dr. Sidney McDaniel, Institute for Botanical Exploration, Starkville, Mississippi, for identification and collection of the plant material. Dr. A. Douglas Kinghorn kindly provided the authentic samples of chaparrinone and holacanthone. Mr. Frank Wiggers, Department of Pharmacognosy, School of Pharmacy, University of Mississippi, determined NMR spectra. We are especially grateful to Adria Laboratories, Dublin, Ohio, for their generous gift of doxorubicin and for the technical assistance of Mr. John Trott and Mr. Scott Janus in the bioassay portion of this study. Jinhui Dou would like to thank the Department of Pharmacognosy and the Research Institute of Pharmaceutical Sciences, University of Mississippi, for fiancial support.

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NP960013J