Cytotoxic Quassinoids from *Simaba cedron*

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Four new quassinoids, cedronolactones A−D (**1**−**4**), together with nine known compounds, simalikalactone D (**5**), chaparrinone (**6**), chaparrin (**7**), glaucarubolone (**8**), glaucarubol (**9**), samaderine Z (**10**), guanepolide (**11**), ailanquassin A (**12**), and polyandrol (**13**), were isolated from the wood of *Simaba cedron*. The chemical structures of **1**−**4** were elucidated on the basis of their chemical and spectral properties. Cedronolactone A (**1**) was shown to exhibit a significant in vitro cytotoxicity (IC_{50} 0.0074 μg/mL) against P-388 cells.

During a survey of new antitumor substances from higher plants,[1] especially those belonging to the Simaroubaceae,[2−8] we have found that the crude extract of *Simaba cedron* Planchon (Simaroubaceae) showed cytotoxic activity against P-388 leukemia cells. Activity-guided chromatographic purification using P388 cells led to the isolation of four novel quassinoids, cedronolactones A−D (**1**−**4**) and nine known quassinoids, simalikalactone D (**5**),[9] chaparrinone (**6**),[10,11] chaparrin (**7**),[12] glaucarubolone (**8**),[10,13] glaucarubol (**9**),[14,15] samaderine Z (**10**),[16] guanepolide (**11**),[17] ailanquassin A (**12**),[18] and polyandrol (**13**) (Chart 1).[19] In this paper, the structural elucidation of **1**−**4** and the cytotoxic activity of **1**−**13** are reported.

Results and Discussion

The methanolic extract prepared from the wood of *S. cedron* was partitioned between $CHCl_3$ and H_2O, and then *n*-BuOH and H_2O. The $CHCl_3$-soluble material was subjected to Si gel column chromatography ($CHCl_3$−MeOH) to give eight fractions. Further purification of the fourth fraction using MPLC (Si gel) and HPLC (ODS Si gel) furnished two new quassinoids, cedronolactones A (**1**) and B (**2**), and five known ones, simalikalactone D (**5**), chaparrinone (**6**), glaucarubolone (**8**), guanepolide (**11**), and ailanquassin (**12**). The *n*-BuOH-soluble material was applied to Diaion HP-20 column chromatography (H_2O−MeOH). The fraction eluted with 20−60% MeOH was further chromatographed using MPLC and then HPLC to give the new quassinoids, cedronolactones C (**3**) and D (**4**), and known compounds, chaparrin (**7**), glaucarubolone (**8**), glaucarubol (**9**), samaderine Z (**10**), and polyandrol (**13**).

Cedronolactone A (**1**) was obtained as colorless needles, and its molecular formula was determined to be $C_{25}H_{34}O_9$ by HREIMS. Its IR, UV, and ^{13}C NMR spectra showed the presence of an α,β-unsaturated ketone, a δ-lactone, and an ester carbonyl group. The ^1H and ^{13}C NMR spectra of **1** were very similar to those of simalikalactone D (**5**),[9] except for the ester side-chain moiety at the C-15. Analysis of the H−H COSY, HMBC, and HMQC spectra revealed that compound **1** possesses a 3-methylbutanoyloxy group at C-15 position. From these data and NOESY spectra, the structure of cedronolactone A (**1**) was established as shown.

Cedronolactone B (**2**) was characterized as colorless needles, whose molecular formula of $C_{19}H_{24}O_7$ was determined by HREIMS. The IR, UV, and NMR spectral data showed the presence of an α,β-unsaturated-γ-lactone and a δ-lactone and were very similar to those of ailanquassin A (**12**).[18] However, the proton resonances of Me-18, H-6α, and H-5 were observed at 0.44, 0.39, and 0.12 ppm more upfield, respectively, than analogous data for compound **12**. Furthermore, NOESY correlations were observed between H-5 and H-6α, H-5 and H-9, and H-6α and Me-18 as shown in Figure 1. These observations indicated that cedronolactone B (**2**) is the 5*S* epimer of **12**. This structure was confirmed by direct comparison with the authentic compound obtained by selective epimerization of **12** at the C-5 stereocenter.

Cedronolactone C (**3**) was characterized as colorless needles, and its molecular formula was determined by HREIMS as $C_{19}H_{24}O_8$. Although the IR, UV, MS, and NMR spectral data of **3** were similar to those of **2**, the presence of an additional hydroxyl group was suggested by its molecular formula and NMR spectra. The position of the hydroxyl group was determined by the shifts of H-15 ($\Delta\delta$ 2.12) and C-15 ($\Delta\delta$ 38.0) NMR resonances compared to those of **2**. Consequently, cedronolactone C (**3**) was deduced to be the 5*S* epimer of polyandrol (**13**).[19] The structure of **3** was confirmed by direct comparison with the authentic compound obtained by selective epimerization of **13** at C-5.

Cedronolactone D (**4**) was characterized as an amorphous solid, with its molecular formula determined as $C_{20}H_{26}O_8$ by HREIMS. Although its spectral data were similar to those of samaderine Z (**10**),[16] the C-7 and C-12 resonances of **4** were observed at δ 83.5 and 75.9, respectively, while those of **10** were observed at δ 72.8 and 87.0, respectively, in the ^{13}C NMR spectrum. A long-range coupling was observed between H-12 and C-16 in the HMBC spectrum, which indicated that a lactone linkage exists between C-12 and C-16 in com-

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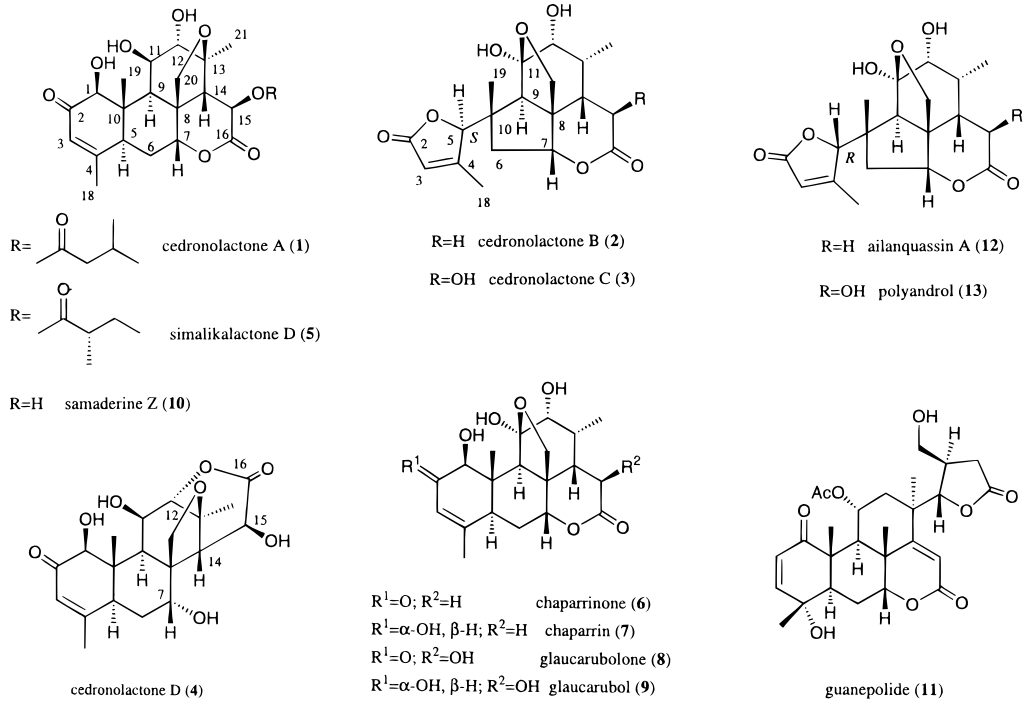
Chart 1



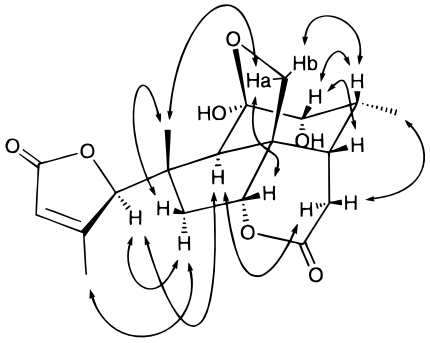


Figure 1. NOESY correlations of **2**.

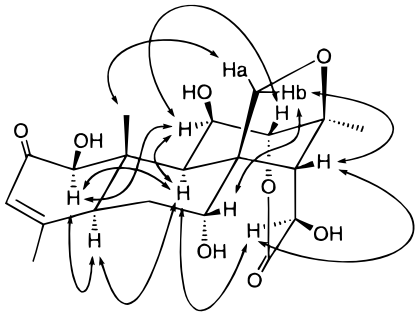


Figure 2. NOESY correlations of **4**.

pound **4**. Furthermore, the NOESY correlation between H-9 and H-15α, as shown in Figure 2, suggested that the configuration of the hydroxyl group at the C-15 was in the β configuration. From the above findings, structure **4** was deduced for cedronolactone D.

Compounds **5**−**13** were identified as simalikalactone D (**5**), chaparrinone (**6**), chaparrin (**7**), glaucarubolone (**8**), glaucarubol (**9**), samaderine Z (**10**), guanepolide (**11**), ailanquassin A (**12**), and polyandrol (**13**) respectively, by comparing their physical and spectral data with those reported in the literature.[9−19] The IC_{50} values (μg/mL) of compounds **1**−**13** against P-388 lymphocytic leukemia cells were 0.0074, 6.5, 49, 38, 0.0055, 0.92, >100, 1.4, >100, 2.4, 70, 39 and 17, respectively.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. UV spectra were taken on a Hitachi 557 spectrophotometer. IR spectra were run on a Perkin−Elmer 1710 or a JASCO A-302 spectrophotometer. ^{1}H, ^{13}C, and 2D (COSY, NOESY, HMBC, and HMQC) NMR spectra were measured by a Bruker AM 400 or a AM 500 spectrometer. ^{1}H NMR chemical shifts are referenced in pyridine-d_5 to residual C_5D_4HN (7.21 ppm); ^{13}C NMR chemical shifts are referenced to the solvent (135.5 ppm). Mass spectra were obtained with a VG AutoSpec E or a Finnigan MAT TSQ-700 spectrometer. Preparative HPLC was carried out on a Shimadzu HPLC system using a Wakosil-II 5C_{18} HG Prep (20 × 250 mm) column with UV detector. MPLC was carried out using a Kusano C. I. G. system (Kusano, Tokyo, Japan).

Plant Material. The wood of *Simaba cedron* Planchon (Simaroubaceae) was purchased at São Paulo, Brazil, in 1991. The botanical identification was made by Dr. S. de M. Alves. A voucher specimen has been deposited in the herbarium of Tokyo University of Pharmacy Life Science.

Extraction and Isolation. The wood of *S. cedron* (2.0 kg) was extracted with MeOH (3 × 4 L). The MeOH extract [120 g, IC_{50} value (μg/mL) against P-388 cells: 0.7] was partitioned between $CHCl_3$ and H_2O, and then between *n*-BuOH and H_2O. The $CHCl_3$-soluble fraction (30 g, IC_{50} 0.22 μg/mL) was subjected to column chromatography over Si gel using a $CHCl_3$−MeOH (1:0−0:1) gradient system to give eight fractions. The fourth fraction (IC_{50} <0.1 μg/mL) was further applied to MPLC (Si gel) using *n*-hexane−EtOAc−MeOH (5:3:1) as solvent system and then to HPLC (ODS Si gel, with mixture of MeOH−H_2O and MeCN−H_2O as solvent

Table 1. ^{13}C and ^{1}H NMR Chemical Shifts (δ) for Cedronolactones A−D (**1**−**4**), Simalikalactone D (**5**), and Ailanquassin A (**12**)[a,b]

	cedronolactone A (**1**)		cedronolactone B (**2**)		cedronolactone C (**3**)		cedronolactone D (**4**)		simalikalactone D (**5**)		ailanquassin A (**12**)	
position	δ_C	δ_H mult. (*J*/Hz)	δ_C	δ_H mult. (*J*/Hz)	δ_C	δ_H mult. (*J*/Hz)	δ_C	δ_H mult. (*J*/Hz)	δ_C	δ_H mult. (*J*/Hz)	δ_C	δ_H mult. (*J*/Hz)
1	82.9 d	4.15 s					84.3 d	4.22 d (2.0)	81.1 d	4.12 s		
2	198.4 s		172.5 s		172.5 s		198.7 s		196.6 s		172.6 s	
3	125.1 d	6.13 s	120.5 d	5.88 s	120.6 d	5.89 t (1.4)	124.7 d	6.13 q (1.3)	123.2 d	6.12 s	119.0 d	5.93 s
4	162.9 s		168.2 s		168.0 s		164.8 s		161.1 s		169.9 s	
5	43.7 d	2.91 br d (12)	91.8 d	4.85 s	91.7 d	4.87 s	43.6 d	3.47 br d (12)	42.0 d	2.90 br d (12)	92.2 d	4.97 br s
6	28.3 t	2.21 dt (2.4, 14.7)	47.1 t	2.54 d (15.7)	46.1 t	2.51 d (15.7)	31.3 t	2.13 dt (2.2, 13.4)	26.5 t	2.20 dt (2.7, 14.6)	46.5 t	2.9 3d (16.1)
		1.72 dt (14.7, 2.4)		2.31 dd (15.7, 5.7)		2.27 dd (15.7, 5.2)		1.67 dt (13.4, 2.2)		1.71 dt (14.6, 2.7)		2.32 dd (16.1, 4.7)
7	84.3 d	4.87 t (2.4)	83.7 d	4.72 d (5.7)	83.4 d	4.76 d (5.2)	72.8 d	4.27 br s	82.4 d	4.87 t (2.7)	80.5 d	4.66 d (4.7)
8	46.6 s		55.6 s		56.7 s		50.3 s		46.5 s		57.0 s	
9	43.0 d	2.72 d (4.4)	45.0 d	3.38 s	46.3 d	3.49 s	44.6 d	2.54 d (4.1)	41.3 d	2.71 d (4.5)	44.1 d	3.34 s
10	48.3 s		46.1 s		45.8 s		48.7 s		44.8 s		46.5 s	
11	75.5 d	5.41 t (4.4)	111.7 s		112.0 s		72.9 d	5.53 dd (2.2, 4.1)	78.3 d	5.40 t (4.5)	111.3 s	
12	80.1 d	4.31 d (4.4)	80.2 d	3.97 t (3.6)	81.0 d	4.12 d (3.8)	87.0 d	4.67 t (2.2)	73.7 d	4.31 d (4.5)	83.8 d	3.95 t (4.3)
13	81.2 s		33.3 d	2.35 m	34.6 d	2.64 m	76.9 s		79.5 s		33.4 d	2.38 m
14	53.1 d	2.83 br d (13)	38.9 d	2.11 m	47.1 d	2.52 dd (10.4, 6.1)	58.1 d	2.84 d (2.5)	51.4 d	2.81 br d (13)	38.6 d	2.19 dd (12.5, 6.3)
15	68.9 d	4.95 d (6.2)	30.5 t	3.26 dd (18.2, 13.1)	68.5 d	5.38 d (10.4)	66.5 d	5.95 s	67.1 d	4.93 d (12.7)	30.5 t	3.28 dd (18.4, 12.5)
				2.76 dd (18.2, 5.8)								2.82 dd (18.4, 6.3)
16	168.7 s		169.6 s		173.9 s		173.7 s		166.8 s		170.0 s	
18	22.1 q	1.72 s	16.5 q	2.05 s	16.5 q	2.01 s	22.5 q	1.76 s	22.2 q	1.71 s	16.1 q	2.49 s
19	11.4 q	1.41 s	20.8 q	1.59 s	20.6 q	1.58 s	11.9 q	1.50 s	10.0 q	1.41 s	18.4 q	1.51 s
20	72.3 t	5.01 d (7.4)	72.1 t	3.94 d (8.7)	72.3 t	3.92 s	74.7 t	4.97 d (7.8)	70.5 t	5.00 d (7.4)	72.0 t	3.94 d (8.7)
		3.72 d (7.4)		3.83 d (8.7)		3.92 s		3.74 d (7.8)		3.72 d (7.4)		3.82 d (8.7)
21	23.9 q	1.79 s	12.9 q	1.08 d (7.2)	15.9 q	1.69 d (7.3)	22.8 q	1.78 s	15.0 q	1.78 s	12.7 q	1.11 d (7.2)
1′	171.6 s								173.3 s			
2′	43.4 t	2.41 dd (7.5, 4.8)							39.7 d	2.60 m		
3′	25.9 d	2.27 m							26.5 t	1.88 m		
4′	22.4 q	1.00 d (6.3)							20.3 q	1.04 t (7.4)		
5′	22.4 q	1.01 d (6.3)							9.6 q	1.26 d (7.0)		

[a] Measurements were performed in pyridine-d_5 at 400 MHz for ^{1}H and 100 MHz for ^{13}C. [b] ^{13}C Multiplicities were established by DEPT pulse sequences.

systems) to give cedronolactone A (**1**, 79 mg) and simalikalactone D (**5**, 93 mg). The fifth fraction (IC_{50} 0.17 μg/mL) was subjected to MPLC (Si gel) using *n*-hexane−EtOAc−MeOH (5:4:1) and then to HPLC (ODS Si gel) using either a MeOH−H_2O or a MeCN−H_2O (20:1−1:1) gradient system to give cedronolactone B (**2**, 25 mg), chaparrinone (**6**, 134 mg), glaucarubolone (**8**, 186 mg), and ailanquassin A (**12**, 40 mg). Repeated MPLC (ODS Si gel) of the sixth fraction using a MeOH−H_2O gradient system (IC_{50} 4.0 μg/mL) furnished guanepolide (**11**, 7.5 mg).

The *n*-BuOH-soluble fraction (41 g, IC_{50} 6 μg/mL) was applied to HP-20 column chromatography using a H_2O−MeOH (1:0−0:1) gradient system to give seven fractions (A−G). Fraction C (IC_{50} 21 μg/mL) was purified by MPLC (Si gel) using $CHCl_3$−MeOH (9:1) and then HPLC (ODS Si gel), using H_2O−MeOH (17:3), to give cedronolactone C (**3**, 257 mg), polyandrol (**13**, 261 mg), and samaderine Z (**10**, 375 mg). Fraction D (IC_{50} 16 μg/mL) was crystallized from MeOH to give a crude crystal, which was then subjected to HPLC (ODS Si gel) to afford chaparrin (**7**, 78 mg), glaucarubolone (**8**, 1.043 g), and glaucarubol (**9**, 1.139 g). Cedronolactone D (**4**, 10 mg) was obtained from the mother liquid by using HPLC (ODS Si gel).

Cedronolactone A (1): colorless needles, mp 185−188 °C; $[\alpha]^{25}_D$ −40° (*c* 0.11, pyridine); UV (EtOH) λ_{max} (log ϵ) 240 (4.02) nm; IR (KBr) ν_{max} 3436, 1752, 1666, 1377, 1346, 1262, 1158, 1118 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 478 $[M]^+$ (13), 3460 (7), 376 (6), 358 (19), 340 (17), 301 (15), 255 (18), 236 (22), 195 (36), 152 (22), 135 (24), 111 (29), 84 (51), 55 (100); HREIMS *m/z* 4782219 (calcd for $C_{25}H_{34}O_9$, 478.2203).

Cedronolactone B (2): colorless needles, mp 194−196 °C; $[\alpha]^{25}_D$ −38° (*c* 0.19, pyridine); UV (MeOH) λ_{max} (log ϵ) 213 (3.98) nm; IR (KBr) ν_{max} 3392, 1742, 1709, 1637, 1322, 1256, 1194, 1119 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 364 $[M]^+$ (49), 346 (11), 333 (8), 318 (20), 305 (65), 292 (13), 267 (29), 231 (17), 207 (23), 191 (25), 173 (27), 145 (33), 125 (41), 97 (82), 68 (100), 53 (91); HREIMS *m/z* 364.1513 (calcd for $C_{19}H_{24}O_7$, 364.1522).

Cedronolactone C (3): colorless needles, mp 99−105 °C; $[\alpha]^{25}_D$ +75° (*c* 0.44, pyridine); UV (MeOH) λ_{max} (log ϵ) 213 (4.04) nm; IR (KBr) ν_{max} 3510, 1736, 1631, 1316, 1231, 1191, 1104 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 380 $[M]^+$ (6), 362 (22), 321 (37), 305 (12), 265 (18), 217 (41), 189 (33), 145 (41), 137 (100), 98 (46), 97 (83), 77 (44); HREIMS *m/z* 380.1468 (calcd for $C_{19}H_{24}O_8$, 380.1471).

Cedronolactone D (4): amorphous solid; $[\alpha]^{25}_D$ −55° (*c* 0.10, pyridine); UV (MeOH) λ_{max} (log ϵ) 241 (3.86) nm; IR (KBr) ν_{max} 3539, 3400, 1724, 1697, 1677, 1262, 1113 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 394 $[M]^+$ (53), 376 (57), 343 (15), 279 (16), 271 (24), 253 (43), 225 (57), 207 (100), 169 (69), 149 (63), 105 (52), 91 (83), 69 (61); HREIMS *m/z* 394.1621 (calcd for $C_{20}H_{26}O_8$, 394.1628).

Selective Epimerization of 12. A solution of **12** (22.2 mg) in pyridine (0.5 mL) was stirred at 150 °C for 24 h under an Ar atmosphere. The solution was evaporated in vacuo. The residue was separated by HPLC (ODS Si gel) using H_2O−MeOH (25:3) to give **2** (6.7 mg) and recovered **12** (11.1 mg).

Selective Epimerization of 13. A solution of **13** (20.5 mg) in pyridine (0.5 mL) was stirred at 150 °C for 24 h under an Ar atmosphere. The solution was evaporated in vacuo. The residue was separated by HPLC (ODS Si gel) using H_2O−MeOH (25:2) to give **3** (4.5 mg) and recovered **13** (12.0 mg).

Cytotoxic Activity Against P388 Cells.[20,21] An MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed in 96-well plates. The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product that can be measured spectrophotometrically. Murine P-388 leukemia cells (3×10^4 cell/mL) were inoculated in each well with 100 μL/mL of RPMI-1640 medium (Nissui Pharmaceutical Company, Ltd., Tokyo, Japan) supplemented with 5% fetal calf serum (Mitsubishi Chemical Industry Co., Ltd., Tokyo, Japan) and kanamycin (100 μg/mL) at 37 °C in a humidified atmosphere of 5% CO_2. Various drug concentrations (10 mL) were added to the cultures at Day 1 after transplantation. At Day 3, 20 μL of MTT solution (5 mg/mL) per well was added to each cultured medium. After a further 4 h of incubation, 100 μL of 10% sodium dodecyl sulfate−0.01 N HCl solution was added to each well, and the formazan crystals in each well were dissolved by stirring with a pipet. The optical density measurements were made using a microplate reader (Tosoh MPR-A4i) at two wavelengths (550 and 700 nm). In all these experiments, three replicate wells were used to determine each data point.

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