L-5
Cytotoxic Quassinoids from *Simaba cedron*

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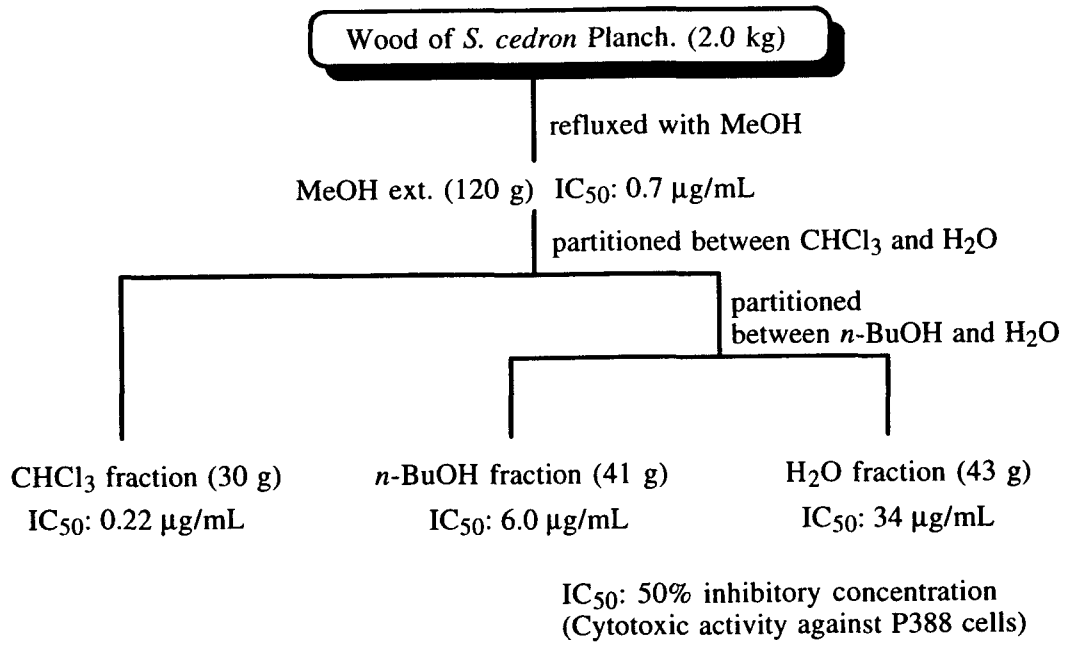
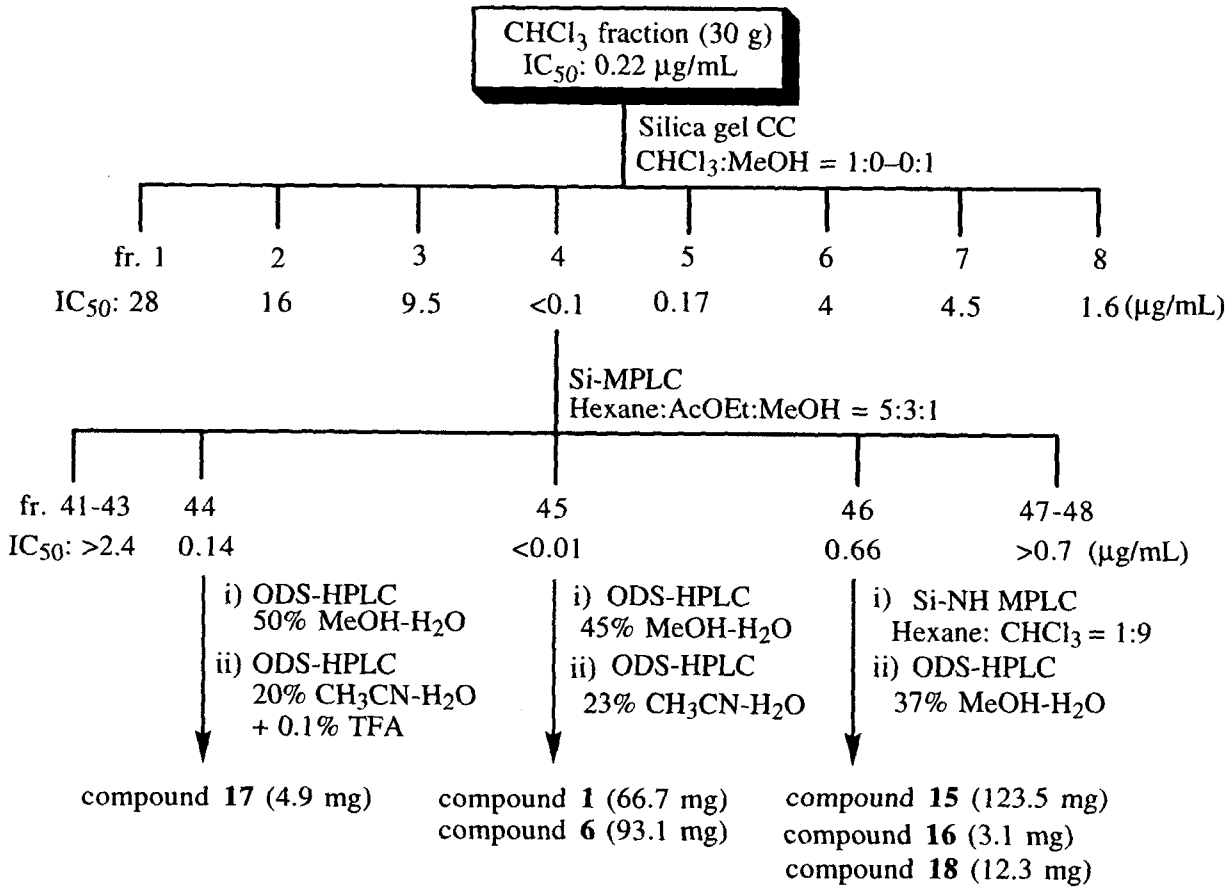
During a survey of new antitumor substances from higher plants, we have found that the crude extract of *Simaba cedron* Planchon (Simaroubaceae) showed cytotoxic activity (IC_{50} 0.7 μg/mL) against P388 leukemia cells. Activity-guided chromatographic purification using P388 cells led to the isolation of five novel quassinoids, cedronolactones A—E (**1—5**) and nine known quassinoids, simalikalactone D (**6**), chaparrinon (**7**), chaparrin (**8**), glaucarubolone (**9**), glaucarubol (**10**), samaderine Z (**11**), guanepolide (**12**), ailanquassin A (**13**), and polyandrol (**14**). In this seminar, the structural elucidation of **1—5** and the cytotoxic activity of the isolated compounds are discussed.

The methanolic extract (120 g, IC_{50} 0.7 μg/mL) prepared from the wood of *S. cedron* (2.0 kg) was partitioned between $CHCl_3$ and H_2O, and then *n*-BuOH and H_2O. The $CHCl_3$-soluble material (30 g, IC_{50} 0.22 μg/mL) was subjected to silica gel column chromatography ($CHCl_3$–MeOH) to give eight fractions. Further purification of the fourth fraction, the most active fraction (IC_{50} <0.1 μg/mL), using medium-pressure liquid chromatography (MPLC) (silica gel) and HPLC (ODS silica gel) furnished a new quassinoid, cedronolactone A (**1**) and a known one, simalikalactone D (**6**). A new quassinoid, cedronolactone B (**2**) and four known ones, chaparrinone (**7**), glaucarubolone (**9**), guanepolide (**12**), and ailanquassin A (**13**) were



Procedures for Extraction and Partition of *S. cedron* Planch.

obtained from this and/or other fractions of the silica gel column chromatography. The *n*-BuOH-soluble material (41 g, IC_{50} 6.0 μg/mL) 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**)—E (**5**), and known compounds, chaparrin (**8**), glaucarubolone (**9**), glaucarubol (**10**), samaderine Z (**11**), and polyandrol (**14**).



Procedures for Separation of Compounds from $CHCl_3$ Fraction

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 (**6**) 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 (**13**). 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 **13**. Furthermore, NOESY correlations were observed between H-5 and H-6α, H-5 and H-9, and H-6α and Me-18. These observations indicated that cedronolactone B (**2**) is the 5*S* epimer of **13**. 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 (Δδ 2.12) and C-15 (Δδ 38.0) NMR resonances compared to those of **2**. Consequently, cedronolactone C (**3**) was deduced to be the 5*S* epimer of polyandrol (**14**). The structure of **3** was confirmed by direct comparison with the authentic compound obtained by selective epimerization of **14** 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 (**11**), the C-7 and C-12 resonances of **11** were observed at δ 83.5 and δ 75.9, respectively, while those of **4** were observed at δ 72.8 and δ 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 compound **4**. Furthermore, the NOESY correlation between H-9 and H-15α, 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.

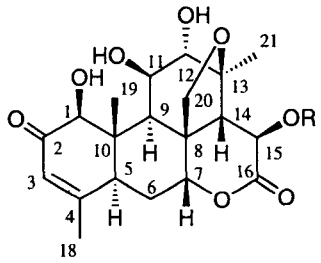
Cedronolactone E (**5**) was characterized as crystalline powder, and its molecular formula was determined by HREIMS as $C_{19}H_{24}O_8$. The IR spectral data suggested the presence of a γ-lactone (1776 cm^{-1}) and a δ-lactone (1747 cm^{-1}). Detailed analysis of 2D NMR spectra (H-H COSY, HMQC, HMBC and NOESY) revealed the unique pentacyclic structure having an ether linkage between C-4 and C-11. The relative stereochemistries of the two methyl groups at positions C-4 and C-10, and H-5 were determined to be all in the β-configurations by observation of NOESY correlations between H-5 and Me-18, H-5 and Me-19, and Me-18 and Me-19.

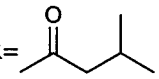
Compounds **6**—**14** were identified as simalikalactone D (**6**), chaparrinone (**7**), chaparrin (**8**), glaucarubolone (**9**), glaucarubol (**10**), samaderine Z (**11**), guanepolide (**12**), ailanquassin A (**13**), and polyandrol (**14**) respectively, by comparing their physical and spectral data with those reported in literature.

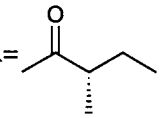
In addition to these quassinoids, three known canthin derivatives, 3-methoxycanthin-2,6-dione (**15**), canthin-6-one-3-oxide (**16**) and infractin (**17**), and a known coumarin, cleomiscosin A (**18**) have been isolated.

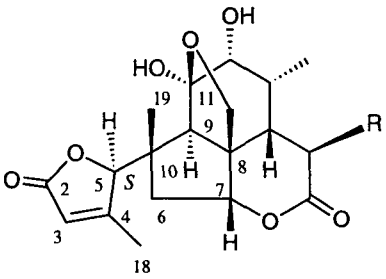
The IC_{50} values (μg/mL) of compounds **1—4**, **6—18** against P388 lymphocytic leukemia cells were 0.0074, 6.5, 49, 38, 0.0055, 0.92, >100, 1.4, >100, 2.4, 70, 39, 17, 5.2, 6.7, 2.0 and 9.0, respectively.

Antimaralial activity was evaluated using *Plasmodium falciparum*. Although compounds **7** and **9** showed fairly potent antimaralial activity, they showed weak cytotoxicity against FM3A cells.

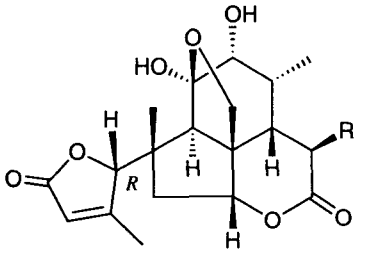


R=  cedronolactone A (**1**)

R=  simalikalactone D (**6**)

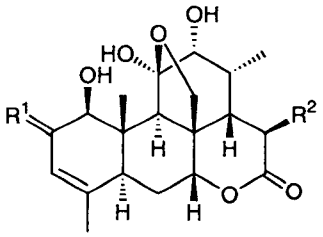


R=H cedronolactone B (**2**)
R=OH cedronolactone C (**3**)

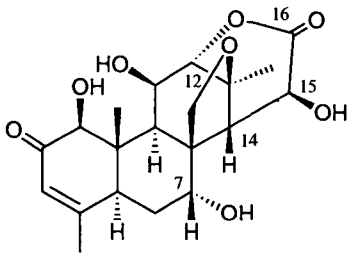


R=H ailanquassin A (**13**)
R=OH polyandrol (**14**)

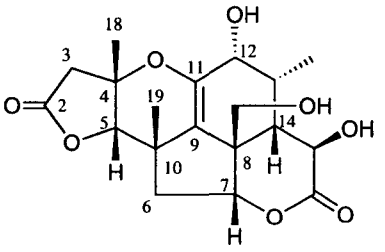
R=H samaderine Z (**11**)



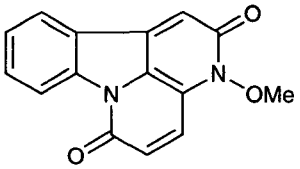
R^1=O; R^2=H chaparrinone (**7**)
R^1=α-OH, β-H; R^2=H chaparrin (**8**)
R^1=O; R^2=OH glaucarubolone (**9**)
R^1=α-OH, β-H; R^2=OH glaucarubol (**10**)



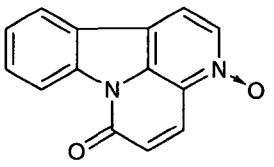
cedronolactone D (**4**)



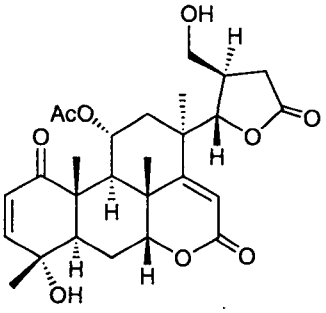
cedronolactone E (**5**)



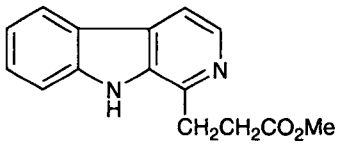
3-methoxycanthin-2,6-dione (**15**)



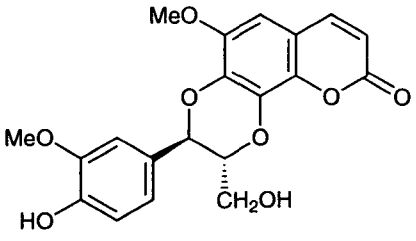
canthin-6-one-3-oxide (**16**)



guanepolide (**12**)



infractin (**17**)



cleomiscosin A (**18**)