ISOLATION AND STRUCTURE OF 14,15β-EPOXYPRIEURIANIN FROM THE SOUTH AMERICAN TREE *GUAREA GUIDONA*[1]

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ABSTRACT.—Cell growth inhibitory constituents of the French Guiana medicinal tree *Guarea guidona* L. Sleumer (also known as *G. trichilioides*, Meliaceae) were investigated by bioassay (PS *in vitro*) directed isolation procedures. A chloroform extract of the root bark was found to contain a new PS *in vitro* (ED_{50} 0.47–0.74 μg/ml) active limonoid characterized (principally by 250 MHz nmr measurements) as 14,15β-epoxyprieurianin (**10a**). The root bark was also found to contain prieurianin (**1**, PS ED_{50} 4.4–7.8 μg/ml) and 7-oxo-gedunin (**9**, PS inactive).

The relatively large Meliaceae family contains some fourteen hundred species distributed among fifty genera. Bark and heartwood triterpenes from a small number of the trees have been very nicely investigated by the Connolly and Taylor groups. These studies have led to the discovery of, e.g., prieurianin (**1**, from *Trichilia prieuriana*, 3), rohitukin (**2**, *Aphanamixis polystacha*, 4), dregeanin (**3**, *e.g.*, *Trichilia dreageana* and *Guarea thompsonii*, 4), glabretal (**4**, *Guarea glabra*, 5) and 3,4-seco-tirucalla-4(28),7,24-triene-3,21-dioic acid (**5**, *Guarea cedrata*, 6). Other recent endeavors in this field include structural determination of the KB (cell line from a human nasopharynx carcinoma) inhibitory hispidins A–C (e.g., A, **6**, *Trichilia hispida*, 7) and discovery of the potent cell growth inhibitors (murine P388 lymphocytic leukemia, *in vitro* PS system) aphanastatin **7a**, amoorastatin **7b** (*Aphanamixis grandifolia*, 8) and related substances (9).[3]

The next part of our program concerned with uncovering Meliaceae antineoplastic constituents was directed at the *Guarea* genus, specifically *Guarea guidona* L. Sleumer, commonly known as *G. trichilioides* L.[4] A number of South American trees are found in the genus *Guarea* and some African species are commercial timber (10). In Brazil, *G. trichilioides* L. is known as *Guaré* and is the principal source of sandalwood oil (11).[5] A preparation of this oil has been used in South America to treat gonorrhea (11). And other extracts of the tree have been used to treat worm infections (12). Most interestingly, *G. trichilioides* extracts, prepared in coconut oil, have been used in Venezuela as a native cancer treatment (13). The only prior chemical studies of *G. guidona* involved toxic components of the fruit (14), isolation of fissinolide (**8**) from the seeds, and β-sitosterol from the wood (and bark, 15, 16).

When the root bark of *Guarea guidona*, collected (1978) in French Guiana, was extracted with hexane (by percolation) followed by chloroform (Soxhlet) removal of solvent from the latter fraction yielded a bright yellow foam that significantly inhibited (ED_{50} 0.29 μg/ml) growth of the National Cancer Institute's (NCI) murine P388 lymphocytic leukemia (PS system) cell line (17). Careful separation

[1]For previous parts in this series refer to (1) and Antineoplastic Agents 79 (2).

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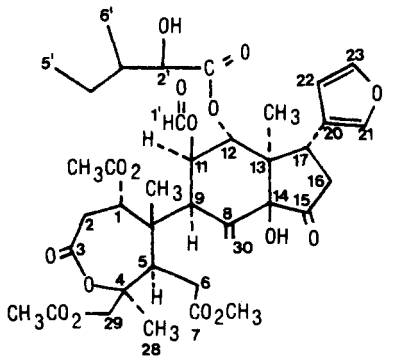
[3]Subsequently, a specimen of our aphanastatin (**7a**) was found by Prof. K. Nakanishi and colleagues to be a potent antifeedant against the Southern army worm (*Spodoptera eridonia*) and Mexican bean beetle (*Epilachna varivestis*).

[4]We wish to thank Dr. T. D. Pennington for determining that *G. guidona* L. is preferable to the frequently used *G. trichilioides* L.

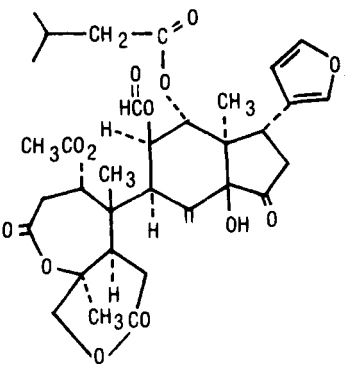
[5]When wounded with an auger or axe the young tree will yield a white to yellow viscous latex with a cinnamon-like odor and irritating taste. Steam distillation of the latex affords the pale yellow essential oil "Oleo de Sandalo" with a rose odor (11).

(guided by PS *in vitro* bioassay) of the chloroform fraction by use of silica gel and silicic acid-Celite chromatography led to 7-oxo-gedunin (**9**, PS *in vitro* inactive, ref., 18–20) and two cytotoxic (PS) constituents: prieurianin (**1**, 3, PS ED_{50} 4.4–7.8 μg/ml) and a new limonoid found, as summarized in the sequel, to be 14,15β-epoxyprieurianin (**10a**, PS ED_{50} 0.47–0.74 μg/ml).

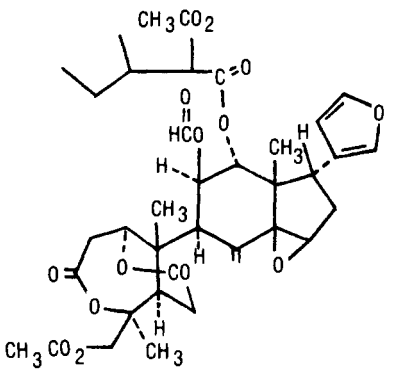
The high resolution mass spectrum of 7-oxo-gedunin (**9**) allowed assignment of the molecular formula $C_{26}H_{30}O_6$ ($M^{+\cdot}$ at *m/e* 438.1994) and displayed the diagnostic fragment ion $C_{20}H_{27}O_3$ (base beak at *m/e* 315.1917). An ^1H nmr (60 MHz) spectrum revealed between 1.1 and 3.3 δ the presence of four methyl groups, singlets at 3.8 and 5.4 δ (H-15 and H-17, respectively), two doublets centered at



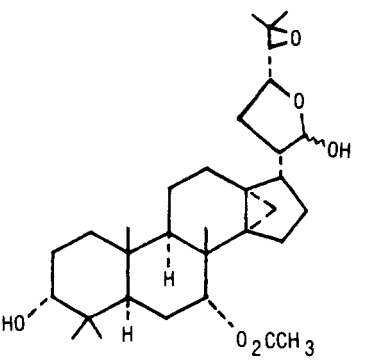
1. Prieurianin



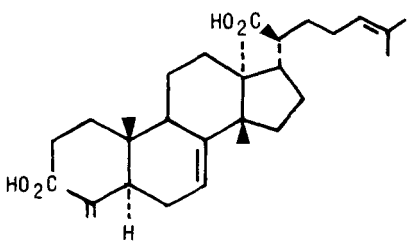
2. Rohitukin



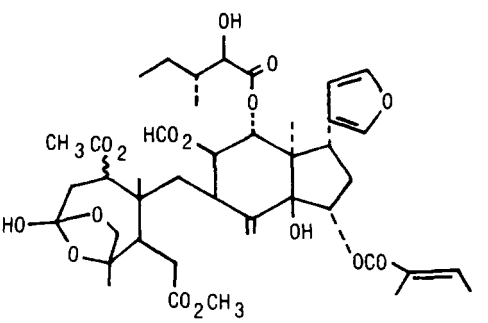
3. Dregeanin



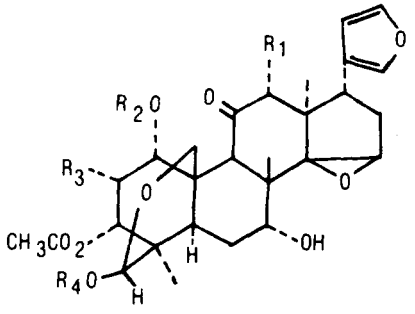
4. Glabretal



5.



6. Hispidin A

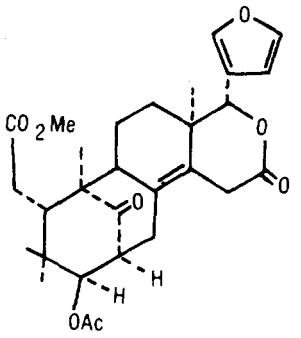


7a. Aphanastatin;

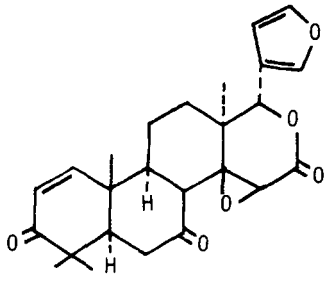
$R_1 = R_3 = OH$; $R_2 = COCH_3$

$R_4 = CO\text{-}CH(CH_3)\text{-}CH_2CH_3$

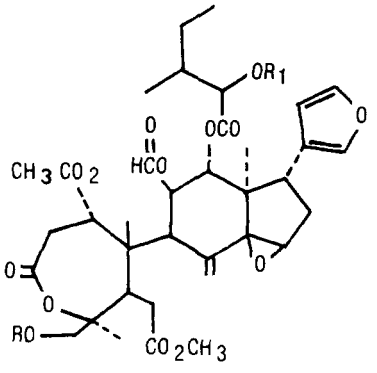
7b. Amoorastatin; $R_1 = R_2 = R_3 = R_4 = H$



8. Fissinolide



9. 7-oxo-Gedunin



10a. R = $COCH_3$, R_1 = H

14, 15 β - epoxyprieurianin

b. $R = R_1 = COCH_3$

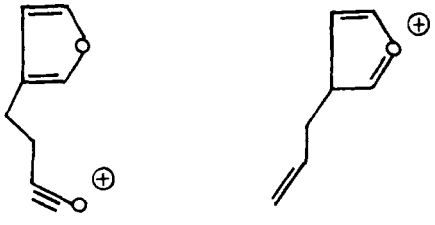
c. $R = R_1$ = H

5.73 and 7.09 (J = 10 Hz, H-2 and H-3, respectively) δ and the furan protons at 6.3 and 7.29 δ. Direct comparison[6] with an authentic specimen completed the characterization of this diketone as 7-oxo-gedunin (**9**).

Interpretation of a prieurianin (**1**) mass spectrum led to assignment of the molecular formula $C_{38}H_{50}O_{16}$ ($M^{+\cdot}$ at *m/e* 762). A high resolution mass spectrum showed fragmentation ions at *m/e* 702.2891 ($C_{36}H_{46}O_{14}$) and 656.2778 ($C_{35}H_{44}O_{12}$) due to the successive loss of one mole of acetic and formic acids from the molecular ion. The base peak at *m/e* 121.0289 ($C_7H_5O_2$) was attributed to ion A[7]. The ultraviolet spectrum did not show any significant absorption above 210 nm. But an infrared spectrum displayed a strong carbonyl band at 1715–1765 cm^{-1}.

[6]The mutual identity was confirmed by mixture melting point determination, thin layer chromatographic behavior, and mass spectral comparison.

[7]The following fragment ions appear to be reasonable assignments:



A B

Presence of a ketone was shown by a circular dichroism curve ($\Delta\epsilon$ −1.45 at 306 nm). The ^{1}H nmr spectrum was badly resolved at room temperature but sharpened on warming. These physical and ^{1}H nmr spectral properties were comparable with those reported for prieurianin (3), and identification was completed by direct comparison[6] with an authentic sample (**1**).

The substance (**10a**) found to most strongly inhibit growth of the PS cell line resisted all attempts at crystallization. Spectral data suggested a relationship to the complex tetranortriterpenoids of the prieurianin-type that give broad (unresolved) ^{1}H nmr spectra at room temperature due to the slow interconversion of rotational and multiple conformational isomers on the nmr time scale (3, 4, 6, 21). The high resolution mass spectrum indicated the molecular formula $C_{38}H_{50}O_{15}$ ($M^{+\cdot}$ at *m/e* 746.3078), bearing one oxygen less than prieurianin (**1**). The mass spectral fragmentation pattern of **10a** was very similar to that of prieurianin and showed ions at *m/e* 686.3010 ($C_{36}H_{46}O_{13}$) and *m/e* 640.2906 ($C_{35}H_{44}O_{11}$) corresponding to the successive loss of 1 mole each of acetic and formic acids from the molecular ion. In contrast to prieurianin, no base peak was found at *m/e* 121 (ion A)[7] but, instead, at 107.0494 (C_7H_7O) and attributed to ion B.[7] The latter fact suggested that the structural difference between prieurianin and new limonoid **10a** resided in the five-membered ring D and was consistent with a 14,15-epoxide replacing the α-ketol group. Also, the circular dichroism curve and ^{13}C nmr spectrum (in pyridine at 60°) that showed no resonance above 180 ppm pointed to the absence of a ketone carbonyl. The structure of limonoid **10a** was completely elucidated by analysis of a 250 MHz ^{1}H nmr spectrum (table 1 and figure 1)

TABLE 1. A summary of the 250 MHz ^{1}H nmr spectra exhibited by prieurianins **10a-b** at 59° in deuteriochloroform. Chemical shifts have been presented in ppm and coupling constants as (Hz).

	(10a)	(10b)
H-1	5.56[a]	5.49[a]
H-9	3.62[b]	3.62[b]
H-11	5.56[a]	5.49[a]
H-12	5.81 d (10.6)	5.79 d (10.2)
H-15	3.77 s	3.76 s
2-H-29	4.54 AB q (12) 4.25	4.51 AB q (12) 4.24
2H-30	5.56[a] 5.32	5.57 5.31
H-23	7.31	7.31
H-21	7.22	7.24
H-22	6.14	6.22
C-CH_3	1.52 1.52 0.98	1.55 1.51 0.97
H-1'	7.92	7.92
CO_2CH_3	3.65[b]	3.65[b]
O_2CCH_3	2.02 2.06	2.06 2.06 2.04
H-2'	3.42 d (3)	4.80 d (4.2)
CH_3-5'	0.84 t (6)	0.80 t (7.2)
CH_3-6'	0.87 d (6)	0.84 d (6.4)

[a]These signals form an unresolved multiplet.
[b]The signal for H-9 is partly obscured by that for the CO_2CH_3.

measured at 59°. A triplet (δ 0.84) and a doublet (δ 0.87) due, respectively, to the 5' and 6'-methyl group were revealed along with signals due to three tertiary methyl, two acetoxy and one methoxy group. The signals assigned the two H-29 protons (AB quartet), the three furan protons and H-1' of the formate (^{13}C,

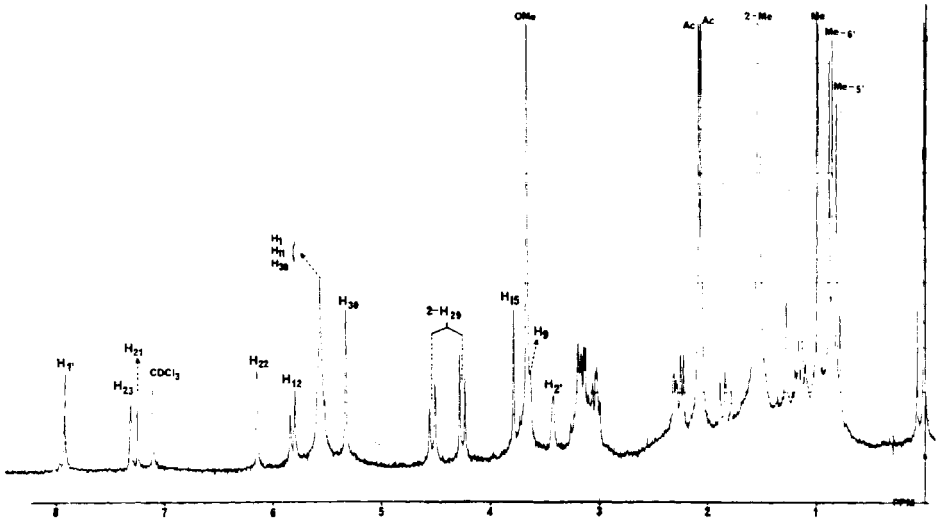


FIG. 1. The 250-MHz ^{1}H nmr spectrum of 14,15β-epoxyprieurianin (**10a**) measured in deuteriochloroform at 59°.

161.5 δ) correspond in chemical shift to those of prieurianin (3, 21). Double resonance experiments revealed the protons H–1, H–11, H–12, H–9 and H–2'. For example, irradiation at the frequency 5.56 δ (multiplet corresponding to H–1, H–11 and H–30) collapsed the doublet at 5.81 ppm (H–12) to a singlet, eliminated the shoulder at 3.62 ppm (H–9) and changed the multiplet at 3.15 ppm (H–2). Conversely, irradiation of H–2, H–12 or H–9 simplified the three proton multiplet at 5.56 δ. The one proton singlet at 3.77 δ was attributed to H–15 (19) and the doublet at 3.42 ppm to H–2'.

Further evidence for the preceding assignments was obtained by interpretation of the 250 MHz ^{1}H nmr spectrum of the acetyl derivative (**10b**), $C_{40}H_{52}O_{16}$, prepared by acetylating alcohol 2' (**10a**) with acetic anhyride-pyridine. The ^{1}H nmr spectrum of acetate **10b** (table 1) was very similar to that of alcohol **10a**. The only significant difference was due to an additional acetoxy group and a downfield shift for the H–2' resonance (d, δ 4.80). Furthermore, the ^{1}H nmr spectral data exhibited by acetate **10b** were almost identical with those reported (21) for the peracetate (**10b**) obtained by acetylation of the limonoid "D–5" (**10c**) recently isolated by Connolly and colleagues from *Trichilia prieuriana*. Based on the above spectral data and correlations, the new cytotoxic (PS) limonoid was assigned structure **10a**, 14,15β-epoxyprieurianin.

Evaluation of 14,15β-epoxyprieurianin (**10a**) against the PS *in vivo* neoplastic disease is in progress. Unfortunately, ketone **1** has been found to be PS inactive up to a toxic dose (8 mg/kg).

EXPERIMENTAL[8]

ISOLATION OF 7-OXO-GEDUNIN (**9**), PRIEURIANIN (**1**) and 14,15β-EPOXYPRIEURIANIN (**10a**).—

[8]A voucher specimen of *Guarea guidona* L. Sleumer has been retained by one of us (CM).[2,3] Chromatographic solvents were redistilled. Silica gel F–254 was employed for thin layer chromatography (tlc). Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Optical rotation measurements were made (at room temperature) on a Roussel-Jouan Quick polarimeter. Infrared spectra (chloroform solutions) were recorded with a Perkin-Elmer model 257 spectrometer. The ultraviolet spectra were obtained with a a Spectronic model 505 spectrometer (Bausch and Lomb). Electron-impact mass spectral studies were conducted on an MS 50–AEI spectrometer. The 60 MHz ^{1}H nmr spectra were obtained with a Varian T60 instrument, the 250 MHz ^{1}H nmr spectra were recorded with a Cameca spectrometer, and both were measured in deuteriochloroform solution with respect to tetramethylsilane as internal standard. The ^{13}C nmr spectrum of limonoid **10a** was measured with a Bruker HXE 90 (22.63 MHz) spectrometer.

Dried and finely ground (milled) root bark of *Guarea guidona* L.[9] (300 g, collected in French Guiana in 1978) was treated by hexane percolation at room temperature. The residual mass was extracted (Soxhlet) with chloroform over 2 days. Evaporation (*in vacuo*) of solvent yielded a bright yellow foam (8.1 g). A portion (5.1 g) of the foam was chromatographed on a column of silica gel 60 (400 g, E. Merck) with chloroform to chloroform-methanol (99:1) as eluant. Fractions displaying similar tlc [chloroform-methanol (98:2)] patterns that contained limonoids **9**, **1** and **10a** were combined to afford a crude mixture weighing 1.45 g. The mixture was subjected to column chromatography on silicic acid-celite (120 g, 2:1) with chloroform as eluant. Fractions (50 ml each) collected and combined on the basis of tlc behavior yielded three main components. The first (0.17 g), when crystallized from ethyl acetate, afforded pure 7-oxo-gedunin (**9**, 83 mg);[6] mp 263–265°(ref. 20, mp 262°), $[\alpha]_D -50°$ (c, 0.9, chloroform); mass spectrum *m/e*, 438.1994 (M^+, required for $C_{26}H_{30}O_6$, 438.1999), base peak at *m/e* 315.1917 (required for $C_{20}H_{27}O_3$, 315.1921).

The second nearly pure fraction (0.14 g), when crystallized (twice) from ethyl acetate, gave pure prieurianin (**10a**, 38 mg);[6] mp 212–214° (Ref. 3, mp 213–214°); mass spectrum, *m/e* 762.3101 (M^+, required for $C_{38}H_{50}O_{16}$, 762.3102), other fragments at *m/e* 702.2891 (required for $C_{36}H_{46}O_{14}$, 702.2890), 656.2778 (required for $C_{35}H_{44}O_{12}$, 656.2783) and base peak at *m/e* 121.0289 (required for $C_7H_5O_2$, 121.0289).

Fraction three (0.45 g) contained epoxide **10a**, the less polar prieurianin, and a more polar, ultraviolet detectable component. Careful rechromatography (with a fraction collector) on silicic acid-celite gave a fraction (0.22 g) of epoxide **10a** which still contained the ultraviolet detectable compound. Finally, preparative tlc (on 4 silica gel plates, 1510 LS 254, Schleicher and Schüll), with chloroform-methanol (97:3) as mobile phase followed by two elutions led to chromatographically homogenous 14,15β-epoxyprieurianin (0.13 g) as a colorless foam; $[\alpha]_D$ +24.5° (c, 1.7 in chloroform) mass spectrum, *m/e*, 746.3078 (M^+, required for $C_{38}H_{50}O_{15}$, 746.3085), 686.3010 (required for $C_{36}H_{46}O_{13}$, 686.3017), 640.2906 (required for $C_{35}H_{44}O_{11}$, 640.2904), and base peak at *m/e* 107.0494 (required for C_7H_7O, 107.0497). The 250 MHz ^1H nmr data has been summarized in table 1 and figure 1.

14,15β-EPOXYPRIEURIANIN ACETATE (**10b**).—Alcohol **10a** (58 mg) in acetic anhydride (1 ml)-pyridine (1 ml) was set aside at room temperature for 24 h. After isolation and purification by preparative tlc (see above) a non-crystalline diacetate (**10b**, 38 mg) was obtained that showed no evidence of hydroxyl absorption in an infrared spectrum. The mass spectrum was consistent with empirical formula $C_{40}H_{52}O_{16}$ and displayed significant ions at *m/e* 788 ($M^{+\cdot}$), 742 ($M^{+\cdot}-42$) and 728 ($M^{+\cdot}-60$). The 250 MHz ^1H nmr data have been recorded in table 1.

THIN LAYER CHROMATOGRAPHIC ANALYSES.—The chloroform-methanol (97:3) solvent system proved to be very effective for tlc (silica gel F-254) analysis of 7-oxo-gedunin (**9**, Rf 0.703), prieurianin (**10a**, Rf 0.55) and 14,15β-epoxyprieurianin (**10a**, Rf 0.505). A spray reagent composed of sulfuric acid containing 1% vanillin (followed by heating) developed epoxide **10a** as a pink spot which changed in several hours to blue.

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[9]A sample (No. CM 878) of the plant studied has been deposited in the "Herbier du Museum d'Histoire Naturelle", Paris.

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