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# ISOLATION AND STRUCTURE OF THE CYTOSTATIC LIGNAN GLYCOSIDE PHYLLANTHOSTATIN A[1]

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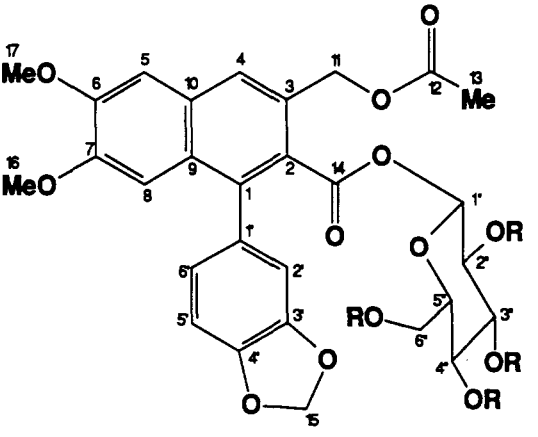
ABSTRACT.—An unusual cytostatic (PS $ED_{50}$ 4 μg/ml) lignan ester has been isolated from the Central American tree *Phyllanthus acuminatus* and designated phyllanthostatin A [**1**]. Separation of an MeOH extract of the root by size exclusion chromatography, high speed countercurrent distribution, and semipreparative hplc afforded glycoside **1** in 0.007% yield. In solution, phyllanthostatin A was slowly transformed into justicidin B [**4**]. The structure of lignan glycoside **1** was determined by hrfabms and 2D-nmr spectroscopy.

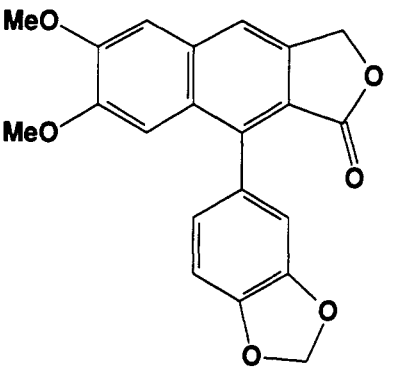
The potentially important phyllanthostatin-type antineoplastic glycosides were discovered during our previous investigations (2) of the Costa Rican tree *Phyllanthus acuminatus* Vahl (Euphorbiaceae). Presently the orthoacid equilibrium product phyllanthostatin 1-phyllanthoside (3) is undergoing preclinical development by the U.S. National Cancer Institute, and total syntheses of phyllanthoside (4) and the aglycone phyllanthocin (5) were completed recently. Because of the easily promoted orthoacid rearrangement (3) uncovered during structural elucidation (2) of the phyllanthostatins, it became necessary to reevaluate their abundance, and possibly detect new members of the series, in relatively fresh *P. acuminatus* roots using hplc analyses at an early stage of separation. Phyllanthoside was revealed as the most prominent phyllanthostatin-type constituent, accompanied by a previously undetected minor amount of a lignan glycoside, herein named phyllanthostatin A [**1**].

Roots of *P. acuminatus* (345 g) were extracted successively with hexane, $CH_2Cl_2$, and MeOH. The MeOH extract was analyzed by hplc with photodiode array detection (hplc-uv/vis) (6,7). The crude extract chromatogram displayed the more abundant phyllanthostatin-type glycosides such as phyllanthoside [λ max = 277 nm (1)] and a previously unknown minor constituent **1** with uv absorption maxima at 248 and 288 nm. Hplc-uv/vis and cell growth inhibition against the P-388 murine lymphocytic




**1** R=H
**2** R=Ac
**3** $R=COCH_2CH_3$

[1]Part 164 of the series Antineoplastic Agents. For Part 163, see deVries *et al.* (1).

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leukemia (in vitro PS system) were used to guide the isolation of lignan **1**. The MeOH extract (22 g) was separated by size exclusion chromatography (Sephadex LH-20, MeOH) and a phyllanthostatin-A-rich fraction was further separated by high-speed countercurrent distribution [HSCCD (8)] with a multilayer coil planet centrifuge (9). Aliquots of 2 g (or less) of the active fraction were separated using the system *n*-hexane-$CH_2Cl_2$-MeOH-$H_2O$ (1:5:4:3) where the lower phase was employed as the mobile solvent. The HSCCD separations were completed in less than 3 h and led to nearly pure phyllanthostatin A. These fractions were combined and further separated by reversed-phase semi-preparative hplc (50% aqueous MeOH to MeOH) yielding 25 mg of phyllanthostatin A [**1**] (0.007% yield). Additional phyllanthostatin A was subsequently isolated from fractions placed aside during an earlier large-scale isolation of the phyllanthostatins (2).

The structure elucidation of phyllanthostatin A [**1**] was not readily accomplished because of its instability in solution and the appearance of split or doubled signals in the nmr spectrum of this glycoside. By hrfabms the molecular formula was determined to be $C_{29}H_{30}O_{13}$. The uv spectrum with absorption maxima at 248 and 288 nm was similar to that recorded for justicidin B [**4**] previously found in *P. acuminatus* (2). However, the ir spectrum contained an ester band at 1738 $cm^{-1}$, and the lactone absorption typical of lignan **4** was absent.

The fabms spectrum of phyllanthostatin A showed a molecular ion at *m/z* 586 $[M]^+$ and a fragmentation pattern with ions at *m/z* 424 and 365. The latter were explained by elimination of glucose $[M-162]^+$ and acetate/$H_2O$ $[(M+H)-162-60]^+$, respectively, from the parent species. The ion at *m/z* 424 also indicated that the acetyl group ($^1H$ nmr $\delta$ 2.09 ppm) was not bonded to the glucose unit. Such an interpretation was further supported by the fabms spectra of the peracetate derivative **2** and the perpropionate derivative **3** of phyllanthostatin A. Both showed the ion at *m/z* 424 and suggested that glycoside **1** was a justicidin B precursor bearing groups derived from HOAc and D-glucose.

Acid hydrolysis of glycoside **1** afforded a $CHCl_3$-soluble degradation product identified as justicidin B by comparison with an authentic sample (co-tlc, mp, uv, ir, eims, $^1H$ nmr). Justicidin B was also detected in MeOH solutions of glycoside **1** stored at room temperature for 2 days. The sugar moiety obtained by acid hydrolysis was identified as D-glucose by peracetylation and comparison (ir, eims, $^1H$ nmr) with authentic D-glucose pentaacetate. The glucose $\beta$ configuration was deduced from the $^1H$-nmr doublet at $\delta$ 5.34 and 5.30 ppm (doubled signal, $2\times J=8.0$ Hz; DMSO-$d_6$) and from the doublet at $\delta$ 5.58 and 5.55 ppm (doubled signal, $2\times J=8.0$ Hz; $CDCl_3$). In addition, the $^{13}C$-nmr chemical shifts corresponded to those reported for $\beta$-D-glucose esters

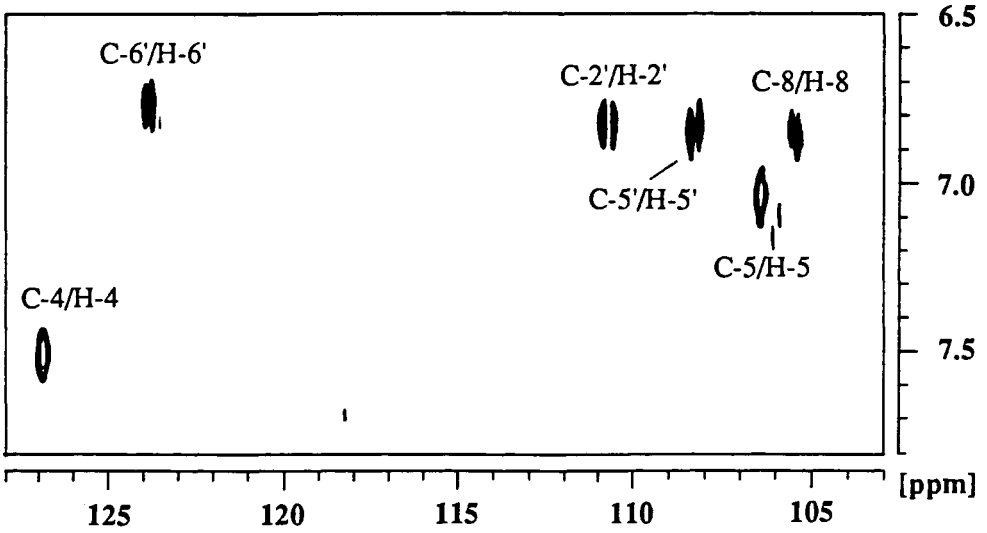


FIGURE 1. A downfield segment of the $^{1}H$-, $^{13}C$-COSY spectrum of phyllanthostatin A [**1**] in $CDCl_3$.

(2,4,5,10–12), and the shift of the anomeric carbon (δ 94.37 ppm) indicated an ester linkage through the C-1″ hydroxyl (2).

Interestingly, the nmr spectra of glycoside **1** recorded in three different solvents ($CDCl_3$, $CD_3OD$, and DMSO-$d_6$) and at different sample concentrations all showed several split or doubled resonances, (cf. Figures 1 and 2 and Tables 1–3) including those

TABLE 1. High-field (400 MHz) $^{1}H$-nmr Chemical Shifts for Phyllanthostatin A [**1**] and Justicidin B [**4**] at 25–145°.[a]

| Proton | Phyllanthostatin A [1] | | | | | Justicidin B [4] | |
|---|---|---|---|---|---|---|---|
| | 25° | 65° | 105° | 135° | 145° | 25° | 145° |
| H-4 | 7.85 s | 7.83 s | 7.81 s | 7.80 s<br>7.90 s[b] | 7.79 s<br>7.88 s[b] | 7.95 s | 7.88 s |
| H-5 | 7.45 s | 7.41 s | 7.40 s | 7.39 s<br>7.44 s[b] | 7.37 s<br>7.43 s[b] | 7.52 s | 7.43 s |
| H-8 | 6.92–6.89 | 6.89 s | 6.89 s | 6.90 s<br>7.06 s[b] | 6.90 s<br>7.06 s[b] | 7.00 s | 7.07 s |
| H-11 | 5.29–5.14 m | 5.29, 5.26<br>5.23, 5.19<br>4 × d (~12) | 5.27, 5.22<br>2 × d (~12) | 5.28, 5.23<br>2 × d (~12)<br>5.39 br s[b] | 5.23, 5.28<br>2 × d (~12)<br>5.37 s[b] | 5.44 s | 5.38 s |
| H-13 | 2.09 s | 2.06 s | 2.06 s | 2.05 s<br>1.88 s[c] | 2.05 s<br>1.88 s[c] | — | — |
| H-15 | 6.18–5.98 | 6.18–5.92 | ~6.06 br<br>6.07 s[b] | 6.03 br m<br>6.06 s[b] | 6.02 br d | 6.14 s | 6.06 s |
| H-16 | 3.66 s | 3.66 s | 3.66 s | 3.67 s<br>3.69 s[b] | 3.67 s<br>3.69 s[b] | 3.67 s | 3.70 s |
| H-17 | 3.92 s | 3.92 s | 3.92 s | 3.92 s<br>3.96 s[b] | 3.92 s<br>3.96 s[b] | 3.95 s | 3.96 s |
| H-2′ | 6.86, 6.84 | ~6.83 br<br>2 × d (1.7) | 6.82 br | 6.81, 6.85<br>6.86 d (1.5)[b] | 6.93 d (1.5)<br>6.85 d (1.5)[b] | 6.85 d (1.5) | |
| H-5′ | 6.92–6.89 | 6.97, 6.91<br>2 × d (7.9) | n.d.[d] | n.d.<br>6.99 d (8.0)[b] | 6.91, 6.98<br>6.98 d (8.0)[b] | 7.05 d (7.9) | 6.98 d (8.0) |
| H-6′ | 6.82, 6.80<br>2 × dd (1.7, 7.9) | 6.81, 6.79<br>2 × dd (1.7, 7.9) | ~6.78 br | 6.78 br<br>6.78[b] | 6.77 dd (1.5,8.0)<br>6.78 dd (1.5,8.0)[b] | 6.80 dd (1.5,7.9) | 6.78 dd (1.5,8.0) |
| H-1″[e] | 5.34, 5.30<br>2 × d (8.0) | 5.35, 5.33<br>2 × d (8.0) | ~5.36 | 5.37 d (8.0) | 5.37 d (8.0) | — | — |

[a]Spectra were recorded using DMSO-$d_6$ solutions: chemical shifts are reported in ppm relative to TMS. Figures in parentheses are coupling constants in Hertz.

[b]Signals attributed to justicidin B.

[c]HOAc obtained by hydrolysis of glycoside **1**.

[d]Not detected.

[e]Glucose protons H-2″-6″ appear as broad or split signals between 4.20 and 3.05 ppm.

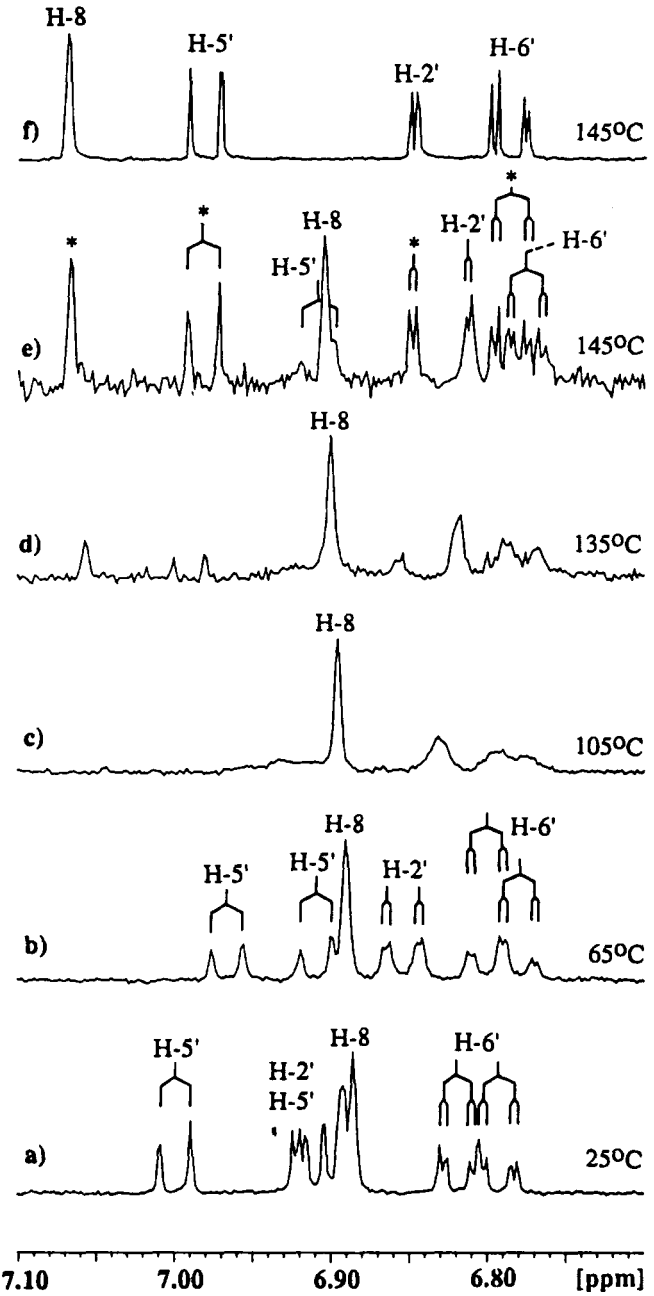


FIGURE 2. Partial $^1$H-nmr spectra of phyllanthostatin A [**1**] (a–e) and justicidin B [**4**] (f) in DMSO-$d_6$ at various temperatures. Signals assigned to justicidin B are marked with an *

assigned to ring-C carbons and protons. These observations are illustrated by the downfield region of the 2D-$^1$H, $^{13}$C-COSY spectrum of phyllanthostatin A [**1**] shown in Figure 1. Connectivities between carbons C-4, C-5 and their attached protons were deduced by the single correlation peaks at 126.92/7.54 and 106.37/7.06 ppm, respectively. But the correlation peaks of carbons C-2', C-5', C-6', C-8, and their corresponding protons were all doubled. Results of 2D homo- and heteronuclear correlation

TABLE 2. High-field (400 MHz) $^1$H-nmr Chemical Shifts for Phyllanthostatin A [**1**], Peracetate **2**, and Perpropionate **3**.[a]

| Proton | Compound | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| H-4 | 7.78 s[b] | 7.72 s[b] | 7.72 s[b] |
| H-5 | 7.17 s | 7.15 s | 7.14 s[b] |
| H-8 | 6.88 s | 6.90–6.73 | 6.92–6.72 |
| H-11 | 5.37, 5.27<br>5.34, 5.29<br>4 × d (12.5) | 5.31–5.04 m | 5.30–5.09 m |
| H-13 | 2.09 s[b] | 2.10–2.00[c] | 2.07, 2.06<br>2 × s |
| H-15 | 6.07–6.05 m | 6.05–6.02 m | 6.05–6.02 m |
| H-16 | 3.79 s | 3.78 s | 3.78 s |
| H-17 | 4.03 s | 4.02 s | 4.01 s |
| H-2′ | 6.85, 6.83<br>2 × d (1.5) | 6.90–6.73 | 6.92–6.72 |
| H-5′ | 6.94–6.92 | 6.90–6.73 | 6.92–6.72 |
| H-6′ | 6.94–6.92 | 6.90–6.73 | 6.92–6.72 |
| H-1″ | 5.58, 5.55<br>2 × d (8.0) | 5.75, 5.74<br>2 × d (8.2) | 5.75, 5.74<br>2 × d (8.0) |
| H-2″–6″ | 3.61–3.33 | 5.31–5.04<br>4.20–4.04 | 5.30–5.09<br>4.20–4.02 |
| -COC*H*$_3$ | | 2.10–2.00[c] | |
| -COC*H*$_2$CH$_3$ | | | 2.40–2.23 |
| -COCH$_2$C*H*$_3$ | | | 1.17–1.04 |

[a]Spectra were recorded using $CDCl_3$ solutions; chemical shifts are reported in ppm relative to TMS; figures in parentheses are coupling constants in Hertz.

[b]Signal split by ~0.005 ppm.

[c]5 acetyl singlets split into 10 signals.

experiments as well as nuclear Overhauser difference spectroscopy (NOEDS) were in agreement with structure **1** and excluded mixtures of possible isomers. The most useful nOe's were observed when protons H-4, H-5, H-8, H-11, H-16, and H-17 were irradiated and led to establishing correlations between H-4 and H-5, H-4 and H-11, H-5 and H-17, H-8 and H-16.

The preceding deductions were further confirmed by the $^{13}$C-nmr spectrum, which showed resonances corresponding to carbons 1, 2, 3, 8, 9, 1′–6′ all split by about 0.1–0.2 ppm. Signals of the remaining carbons appeared as sharp single lines. Perhaps these observations can be explained by hindered rotation around the C-1/C-1′ axis, resulting in conformational isomers. In order to provide evidence for or against this hypothesis, the $^1$H-nmr spectra of glycoside **1** were recorded in DMSO-$d_6$ at different temperatures. Table 1 summarizes the chemical shifts observed at 25, 65, 105, 135, and 145°. Figure 2 shows that part of the spectra representing C-ring protons and the overlapping H-8 proton. At room temperature (Figure 2a) a complex coupling pattern appeared; it became more easily interpretable when the spectrum was recorded at 65° (Figure 2b). Signals due to H-2′, H-5′, and H-6′ broadened and disappeared when the temperature was raised (Figure 2c). At higher temperatures (Figures 2d,e) phyllanthostatin A gradually decomposed, and signals attributable to justicidin B [**4**] appeared. By subtracting these signals (Figure 2f) from the spectrum in Figure 2e, it became possible to assign the doublets δ 6.91 ($J$ = 8.0 Hz, overlapped with the H-8 singlet) and 6.81 ($J$ = 1.5 Hz), as well as the doublet of doublets at 6.77 ($J$ = 1.5 Hz, $J$ = 8.0 Hz), to

TABLE 3. The $^{13}$C-nmr and Heteronuclear Correlation Results Obtained from Phyllanthostatin A [**1**].[a]

| Carbon | Chemical shift[b] | One-bond $^{1}$H, $^{13}$C-correlation[c] | Long-range $^{1}$H, $^{13}$C-correlation[d] |
|---|---|---|---|
| 1 | 137.31/137.16 (2 × s) | | H-2′, H-6′, H-8 |
| 2 | 128.59/128.39 (2 × s) | | H-4, H-11 |
| 3 | 127.70/127.60 (2 × s) | | H-11 |
| 4 | 126.92 (d) | 7.54 | H-5, H-11 |
| 5 | 106.37 (d) | 7.06 | H-4 |
| 6 | 150.63 (s) | | H-8, H-17 |
| 7 | 150.35 (s) | | H-5, H-16 |
| 8 | 105.45/105.35 (2 × d) | 6.88[e] | |
| 9 | 127.70/127.60 (2 × s) | | H-4, H-5 |
| 10 | 129.70 (s) | | H-8 |
| 11 | 65.26 (t) | 5.25, 5.12 | |
| 12 | 172.92 (s) | | H-13 |
| 13 | 21.00 (q) | 1.99 | |
| 14 | 167.42 (s) | | H-1″, H-4[f] |
| 15 | 101.28 (t) | 5.98, 5.91 | |
| 16 | 55.79 (q) | 3.71 | |
| 17 | 55.94 (q) | 4.00 | |
| 1′ | 131.17/131.10 (2 × s) | | H-5′ |
| 2′ | 110.84/110.56 (2 × d) | 6.83[e] | H-6′ |
| 3′ | 147.58–147.31 (2 × s)[g] | | H-5′, H-15 |
| 4′ | 147.58–147.31 (2 × s)[g] | | H-2′, H-15 |
| 5′ | 108.31/108.15 (2 × d) | 6.90[e] | |
| 6′ | 123.86/123.71 (2 × d) | 6.80[e] | H-2′ |
| 1″ | 94.37 (d) | 5.52 | |
| 2″ | 72.43 (d)[h] | 3.44 | |
| 3″ | 76.42 (d)[h,i] | 3.38 | |
| 4″ | 69.20 (d)[h] | 3.54 | |
| 5″ | 76.32 (d)[h,i] | 3.58 | |
| 6″ | 61.34 (d)[h] | 3.73 | |

[a]50 mg (200 mg for COLOC) sample in $CDCl_3$.

[b]In ppm downfield to TMS; multiplicities determined by APT experiments.

[c]One-bond correlations observed in the $^{1}$H, $^{13}$C-COSY spectrum.

[d]Long-range couplings through two, three and four bonds observed in a $^{1}$H, $^{13}$C-COLOC experiment optimized for $J_{CH} = 5$ Hz.

[e]Correlation peak doubled.

[f]Correlation peak of low intensity.

[g]Overlapping signals.

[h]Signals split by ≤0.08 ppm.

[i]Assignment may be reversed.

the C-ring protons H-5′, H-2′, and H-6′, respectively. Other protons (not shown in Figure 2) also appeared as single resonances at higher temperatures. These experiments served to prove that diester **1** was a single compound and the double signals were due to conformational isomers in solution (13).

Assignment of the ring-A, -B, and -C carbons and protons was achieved by 2D heteronuclear correlation methods, and the conclusions are summarized in Table 3. Quaternary carbon resonances were established by means of long-range coupling (mainly over three bonds) observed in a 2D-$^{1}$H, $^{13}$C-COLOC experiment, optimized for $J = 5$ Hz. Based on this experiment, the signal at δ 172.92 was assigned to the C-12 carbonyl showing a two-bond coupling to the H-13 protons. The second carbonyl appeared at δ 167.42 ppm and was characterized by a small W path coupling with H-4

and by a pronounced correlation peak with the anomeric glucose proton (three-bond coupling). In turn this confirmed the glucose ester linkage and allowed assignment of structure **1** to phyllanthostatin A.

Phyllanthostatin A was found to inhibit growth of the PS leukemia cells in vitro at a concentration of $ED_{50} = 4$ μg/ml, and in vivo studies are in progress. Here it is noted that 31 of the 33 lignan biosynthetic products known by 1984 to have cytostatic and/or antineoplastic properties contained a methylenedioxy system, an obvious structural feature of phyllanthostatin A and the related active (and unlactonized) picropodophyllic acid (14). Given the useful human anticancer activity of glycosides derived from the related lignan podophyllotoxin, such as etoposide (VP-16-213), phyllanthostatin A may prove to be a useful model for structural modification in this area. Some recent advances in this field are detailed in Glinski *et al.* (15).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All solvents employed were redistilled. Adsorption cc was performed with Si gel 60 (70–230 mesh, E. Merck, Darmstadt, Germany). Reversed-phase chromatography was accomplished with RP-8 Lobar columns (size B, 40–63 μm, from E. Merck) and size exclusion chromatography with Sephadex LH-20 (particle size 25–100 μm, supplied by Pharmacia Fine Chemicals, Uppsala, Sweden). Tlc was carried out with Si gel GHLF Uniplates (Analtech Inc.) and with RP-8 precoated plates (layer thickness 0.25 mm) from E. Merck. The tlc plates were examined under uv light and developed with anisaldehyde spray reagent.

HSCCD was carried out with an Ito Multilayer Coil Extractor-Separator (P.C. Inc., Potomac, MD, using 2.6-mm i.d. tubing), an FMI Lab Pump, a Linear recorder, and Gilson Model Holochrome uv/vis detector (2.5 mm/3.2 μl cell) and Micro Fractionator. Analytical hplc with photodiode array detection (hplc-uv/vis) was accomplished with an Ultremex RP-8 column (100 × 4.6 mm i.d., 3 μm, Phenomenex, Rancho Palos Verde, CA) at a flow rate of 1 ml/min and a linear gradient of aqueous MeCN (20% to 70% in 15 min). The mobile phase was delivered by two Gilson Model 302 pumps using an Apple IIe programmer. Chromatograms and spectra were recorded with an HP 1040A photodiode array detector and an HP 79994 work station (Hewlett-Packard). Semi-preparative hplc was performed with a Prepex RP-8 column (250 × 10 mm i.d., 5–20 μm, Phenomenex) with aqueous MeOH (50% to 100% in 1 h) at a flow rate of 2 ml/min. Melting points are uncorrected and were determined on a Kofler-type hot-stage apparatus. Optical rotations were measured using a Perkin-Elmer Model 241 Automatic Polarimeter. Uv were recorded by a Hewlett-Packard Model 8450A uv/vis spectrophotometer and ir spectra with a Nicolet Ft-ir Model MX-1 instrument. Nmr spectra were measured using a Bruker AM-400 instrument and are recorded in ppm downfield to TMS. The $^{13}C$-nmr multiplicities were determined with APT experiments based on an average coupling constant of 135 Hz. Both 2D homo- and heteronuclear shift correlated spectra were recorded using standard pulse sequences (16–18). Eims spectra were obtained using a Varian MAT 312 spectrometer. Fabms spectra were recorded with an MS-50 instrument at the NSF Regional Facility, University of Nebraska, Lincoln, Nebraska.

PLANT MATERIAL, EXTRACTION, AND COUNTERCURRENT DISTRIBUTION.—Roots of *P. acuminatus* were collected by Dr. J. Saenz-Renauld in Costa Rica in 1986. A voucher specimen (B680433) is maintained at our Institute. The air-dried roots (345 g) were powdered and extracted (at room temperature) successively with *n*-hexane, $CH_2Cl_2$, and MeOH (3 × 6 liters each), yielding 0.6, 3.6, and 25.0 g of extracts, respectively. An aliquot of the MeOH extract (22 g, $ED_{50} = 15$ μg/ml) was separated by size exclusion chromatography (Sephadex LH-20, 100 × 10 cm i.d., MeOH), and 6 fractions were collected. Fraction 5 (elution vol 6700–8700 ml, 4174 mg, $ED_{50} = 20$ μg/ml) was separated by HSCCD with the solvent system *n*-hexane-$CH_2Cl_2$-MeOH-$H_2O$ (1:4:5:3). Samples (≤2 g) were dissolved in about 10 ml of both phases and introduced into the coil through the head-inlet. Elution was conducted through the same inlet at flow rates between 200 and 240 ml/h with the lower (mobile) phase. Rotation of the coil was at 800 rpm, and retention of the stationary phase was usually about 80%. Uv detection was carried out at 254 nm, and fractions were collected every 2 min.

ISOLATION OF PHYLLANTHOSTATIN A.—Fractions (48 mg) from the HSCCD separation containing phyllanthostatin A were combined and further purified by semi-preparative hplc, affording 25 mg of pure glycoside **1**. In addition, phyllanthostatin A was isolated as follows from a fraction obtained during the large-scale separation of *P. acuminatus* (2). Phyllanthostatin A was detected by hplc-uv/vis, and the corresponding fractions (380 g) were dissolved in 1 liter of MeOH and separated by size exclusion chromatography (Sephadex LH-20, 100 × 10 cm i.d.). The phyllanthostatin-A-rich fractions were combined (8.5 g, elution vol 6–7 liters, $ED_{50} = 3.1$ μg/ml) and further separated (≤2 g samples) by HSCCD using *n*-

hexane-$CH_2Cl_2$-MeOH-$H_2O$ (2:5:4:3) as solvent system. The organic layer was used as the mobile phase. The combined fractions were finally purified by reversed-phase liquid chromatography (Lobar, size B) with aqueous MeOH (60%) as mobile phase, affording 1.06 g ($7 \times 10^{-5}$% yield) of phyllanthostatin A [**1**]: amorphous solid; mp 127–130°; tlc on Si gel $R_f$ 0.38, $CH_2Cl_2$-MeOH (9:1) $R_f$ 0.25, *n*-hexane-$CH_2Cl_2$-MeOH-$H_2O$ (1:4:5:3, lower phase); $[\alpha]^{26}D$ +24.5 ($c$ = 1.5, $CH_2Cl_2$); hrfabms *m/z* $[M]^+$ 586.1703 (calcd for $C_{29}H_{30}O_{13}$, 586.1677; $\Delta$ = 4.4 ppm), $[M-162]^+$ 424, $[(M+H)-162-50]^+$ 365; uv max 248 (log $\epsilon$ 4.72), 288 (4.02) nm; ir $\nu$ max 3525, 2850, 1738, 1507, 1492, 1471, 1433, 1240, 1155, 1060, 1038 $cm^{-1}$; $^1H$ and $^{13}C$ nmr see Tables 1–3. NOED spectra were recorded without degassing, and the following enhancements (in parentheses; negative values) were observed upon irradiation: H-4 (H-5: 6.2%; H-11: 1.2%, 1.5%), H-5 (H-4: 8.1%; H-17: 8.6%), H-8 (H-16: 6.2%), H-11 (H-4: 2.3%), H-16 (H-8: 11.0%), H-17 (H-5: 9.2%); $^1H$,$^{13}C$-COSY and $^1H$,$^{13}C$-COLOC see Table 3.

HYDROLYSIS OF PHYLLANTHOSTATIN A.—A solution of glycoside **1** (50 mg) in MeOH (5 ml) and 2 N HCl (25 ml) was heated at reflux for 30 min, diluted with $H_2O$, and extracted with $CHCl_3$. The chlorocarbon phase was washed with $H_2O$ and solvent evaporated to dryness. The residue was recrystallized from MeOH to afford pure justicidin B [**4**], mp 244–247° [lit. (2) mp 263–265° from $CH_2Cl_2$/MeOH], identical (by ir and $^1H$ nmr) with an authentic specimen. The aqueous phase was neutralized ($NaHCO_3$), solvent evaporated, and the glucose extracted with pyridine. Glucose was detected in the extract by tlc on Si gel using the solvent system EtOAc-MeOH-$H_2O$-HOAc (65:15:15:30) followed by spraying with anisaldehyde reagent and heating to reveal a grayish green spot ($R_f$ 0.61) characteristic of D-glucose. The pyridine solution was treated with $Ac_2O$ (room temperature, 24 h). The product was purified by Si gel cc in $CH_2Cl_2$-MeOH (95:5), and 8 mg of glucose pentaacetate was obtained. Identical $^1H$-nmr chemical shifts and eims spectra were observed with an authentic sample of D-glucose pentaacetate prepared under the same conditions.

PHYLLANTHOSTATIN A PERACETATE [**2**].—Glycoside **1** (5 mg) was acetylated in pyridine/$Ac_2O$ and the product purified by Si gel cc with $CH_2Cl_2$-MeOH (98:2) as eluent (cf. 2) to yield the peracetate as an amorphous solid (4 mg): tlc on Si gel, $R_f$ 0.24, *n*-hexane-EtOAc (1:1); hrfabms *m/z* $[M+Li]^+$ 761.2228 (calcd for $C_{37}H_{38}O_{17}Li$, 761.2270; $\Delta$ = 5.5 ppm), $[(M+Li)-390]^+$ 371, $[(M+H)-390]^+$ 365; ir $\nu$ max 2860, 1758, 1506, 1493, 1434, 1370, 1237 (br), 1156, 1059, 1039; $^1H$ nmr see Table 2.

PHYLLANTHOSTATIN A PERPROPIONATE [**3**].—A solution of glycoside **1** (4 mg) in pyridine (0.2 ml) and propionic anhydride (0.2 ml) was stored at room temperature. After 2 days, solvent was evaporated (under $N_2$) while MeOH was repeatedly added. The residue was chromatographed as noted above for obtaining peracetate **2** to afford 3.5 mg of perpropionate **3** as an amorphous solid: tlc on Si gel, $R_f$ 0.59, *n*-hexane-EtOAc (1:2); hrfabms *m/z* $[M]^+$ 810.2734 (calcd for $C_{41}H_{46}O_{17}$, 810.2721; $\Delta$ = 1.6 ppm), $[(M+H)-59]^+$ 752, $[M-386]^+$ 424.1172 (calcd for $C_{23}H_{20}O_8$, 424.1162; $\Delta$ = 4.7 ppm), [glucose tetrapropionate] 387, $[(M+H)-446]^+$ 365; $^1H$ nmr see Table 2.

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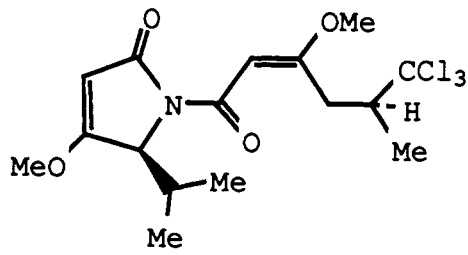
## ERRATUM

The authors have requested the following correction for the paper entitled "Sesterterpenes from a Pacific Sponge, *Carteriospongia flabellifera*," *J. Nat. Prod.*, **51**, 745 (1988).

The corrected structure of compound **3** is as follows:




**3**

## ERRATUM

The authors have requested the following corrections for the paper entitled "Prenylated 4-Hydroxybenzoic Acid Derivatives from *Piper marginatum*," *J. Nat. Prod.*, **51**, 370 (1988).

The corrected uv data for compound **1** should be uv (MeOH) λ max 255, 216 nm (ε 11000, 16200), and those for compound **2** should read uv (MeOH) λ max 254, 215 nm (10200, 17100).