As a check on the rescaling, the two partial data sets from which the rescaling factor was calculated were averaged. The 160 averaged reflections have $R = 5.5\%$ based on F_o. The merged data sets provided 2363 unique data. No absorption correction was applied. The 1302 reflections having $F_o^2 > 3.0\sigma(F_o^2)$ were used in the final refinement.

Structure Solution and Refinement. Both structures were solved by direct methods. Hydrogen atoms were not included in either structure. For **1**, all atoms were treated anisotropically. For **2**, only one of the two molecules present in the asymmetric unit was treated anisotropically. Atomic scattering[6] and anomolous depression[7] factors were from usual sources. The final full-matrix least-squares refinements converged to $R = \sum||F_o| - |F_c||/\sum|F_o| = 0.102$ for **1** and 0.100 for **2** and $R_w = [\sum w(|F_o| - |F_c|)^2|\sum w/F_o|^2]^{1/2} = 0.135$ for **1** and 0.138 for **2**. For **1** there were 61 parameters and 502 observations; for **2** there were 131 parameters and 1302 observations. The weighting scheme used in the minimization of $\sum w(|F_o| - |F_c|)^2$ is defined as $w = 1/\sigma^2(F_o)$ where $\sigma^2(F_o)$ includes a factor ($p = 0.06$) introduced to downweight intense reflections, in addition to the contribution from counting statistics. In both structures, the residuals showed no anomalies. The largest peak in the final difference Fourier map was 0.30 e/Å^3 for **1** and 0.47 e/Å^3 for **2**.

Measurement of the Photoelectron Spectra. The He I (21.2 eV) photoelectron spectra were recorded using a spectrometer whose configuration has been described previously.[8,9] The instrument was initially calibrated using a 50/50 mixture of Xe/Ar gas and in all cases a resolution of 35 meV or better was achieved relative to Xe (fwhm). Individual band positions were determined using Xe (12.13 and 13.44 eV) and Ar (15.76 eV) as *A* references. All three samples were air-stable at room temperature and it was necessary to apply heat in order to produce sufficient vapor pressure to obtain a spectrum. Minimal temperatures necessary to achieve sublimation were used. These temperatures were 75 °C for **1**, 50 °C for **2**, and 30 °C for **3**. In all cases, samples sublimed cleanly with no evidence of decomposition being seen, i.e., production of HCN.

Supplementary Material Available: A figure showing the UV absorption spectra of **1–3**, ORTEP drawings of **1** and **2**, expansions of the first bands of the PE spectra of **1–3**, tables of the MO energies of **1–11**, table of crystal data for **1** and **2**, and tables of positional parameters, general temperature factor expressions (*B*'s and *U*'s), refined temperture factor expressions (*β*'s), and root-mean-square applitudes of thermal vibrations (12 pages). Ordering information is given on any current masthead page.

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Phyllanthostatin 1-Phyllanthoside Orthoacid Rearrangement[1]

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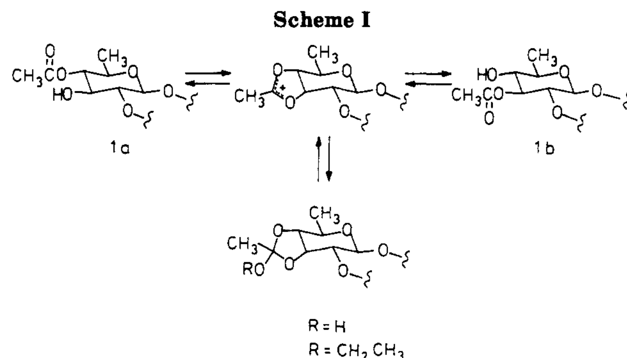
Received February 21, 1985

Solvolysis of phyllanthoside (**1b**) in 1:9 ethanol–water (14 days at room temperature) consumed about 54% of the starting glycoside and afforded the S3′-monoacetyl derivative **1f** and phyllanthostatins 1 (**1a**) and 4 (**1e**) in 8–10% yields accompanied by five related products of orthoacid rearrangement and/or deacetylation. Several additional minor transformation products were detected by HPLC analyses. When phyllathostatin 1 (**1a**) was subjected to the same solvolysis reaction for 21.5 h, approximately half rearranged to phyllanthoside (**1b**). After 4 days the product composition resembled that from phyllanthoside. Discovery of the phyllanthostatin 1 ⇄ phyllanthoside orthoacid rearrangement revealed a very important aspect of *Phyllanthus* glycoside chemistry and provided the first examples of O-3 ⇄ O-4 acetyl migrations in a glucopyranoside.

The central American tree *Phyllanthus acuminatus* Vahl (Euphorbiaceae) has been found[2] to contain a series of potentially useful antineoplastic (murine P388 lymphocytic leukemia and B16 melanoma) glycosides. Presently, we have completed the isolation and structural elucidation of four principal members, namely, phyllanthostatins 1–3 (**1a**,**c**, **2**) and phyllanthoside (**1b**).[2] Glycosides **1a** and **1b** have been undergoing detailed evaluation by the U.S. National Cancer Institute (NCI). The major glycoside **1b** has recently been selected for further development toward an eventual clinical trial. During our structural studies of glycosides **1a** and **1b** it became clear that both substances were interconvertible by virtue of intramolecular acetyl group migrations[3] of the orthoacid[4] type (Scheme I). Such neighboring group participation reactions involving acetoxonium ion[5] intermediates (Scheme I) are well known,[6] and recent examples include a very pertinent and interesting study by Weidmann[7] and colleagues of O-4 → O-6 and O-3 ⇄ O-4 acetyl migrations

Scheme I



* Author to whom correspondence should be addressed.

(1) Contribution 112 of Antineoplastic Agents. The previous report appears in: Pettit, G. R.; Holzepfel, C. W. *J. Org. Chem.*, in press.

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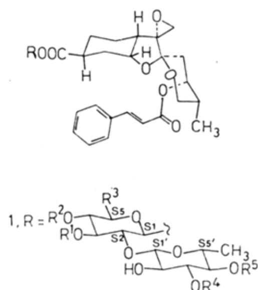
in glucosides such as methyl 3-*O*-acetyl-2-benzamido-6-bromo-2,6-dideoxy-α-D-glycopyranoside in aqueous pyridine (or other examples in 0.1 N sodium hydroxide–ethanol).

Because the closely related phyllanthostatins presented challenging separation problems we employed silica gel chromatography extensively, and it was observed that the relative amounts of phyllanthoside and phyllanthostatin 1 isolated varied markedly depending on time used for the



a, $R^1 = R^5 = H; R^2 = R^4 = COCH_3; R^3 = CH_3$: phyllanthostatin 1
b, $R^1 = R^4 = COCH_3; R^2 = R^5 = H; R^3 = CH_3$: phyllanthoside
c, $R^1 = R^4 = COCH_3; R^2 = R^5 = H; R^3 = CH_2OH$: phyllanthostatin 2
d, $R^1 = R^4 = H; R^2 = R^5 = COCH_3; R^3 = CH_3$
e, $R^1 = R^5 = COCH_3; R^2 = R^4 = H; R^3 = CH_3$
f, $R^1 = R^2 = R^5 = H; R^4 = COCH_3; R^3 = CH_3$
g, $R^1 = R^4 = R^5 = H; R^2 = COCH_3; R^3 = CH_3$
h, $R^1 = COCH_3; R^2 = R^4 = R^5 = H; R^3 = CH_3$
i, $R^1 = R^2 = R^3 = R^4 = R^5 = H; R^3 = CH_3$

chromatographic procedures. Indeed, when a solution of phyllanthoside (**1b**) in methylene chloride–methanol–water (97:3:0.2; the solvent used as eluent) was stirred with silica gel 60 at room temperature, HPLC analysis indicated that significant amounts of phyllanthostatin 1, together with other high retention volume products, were formed with increasing time. Interestingly, similar results were observed in the absence of silica gel and this led to an ex-

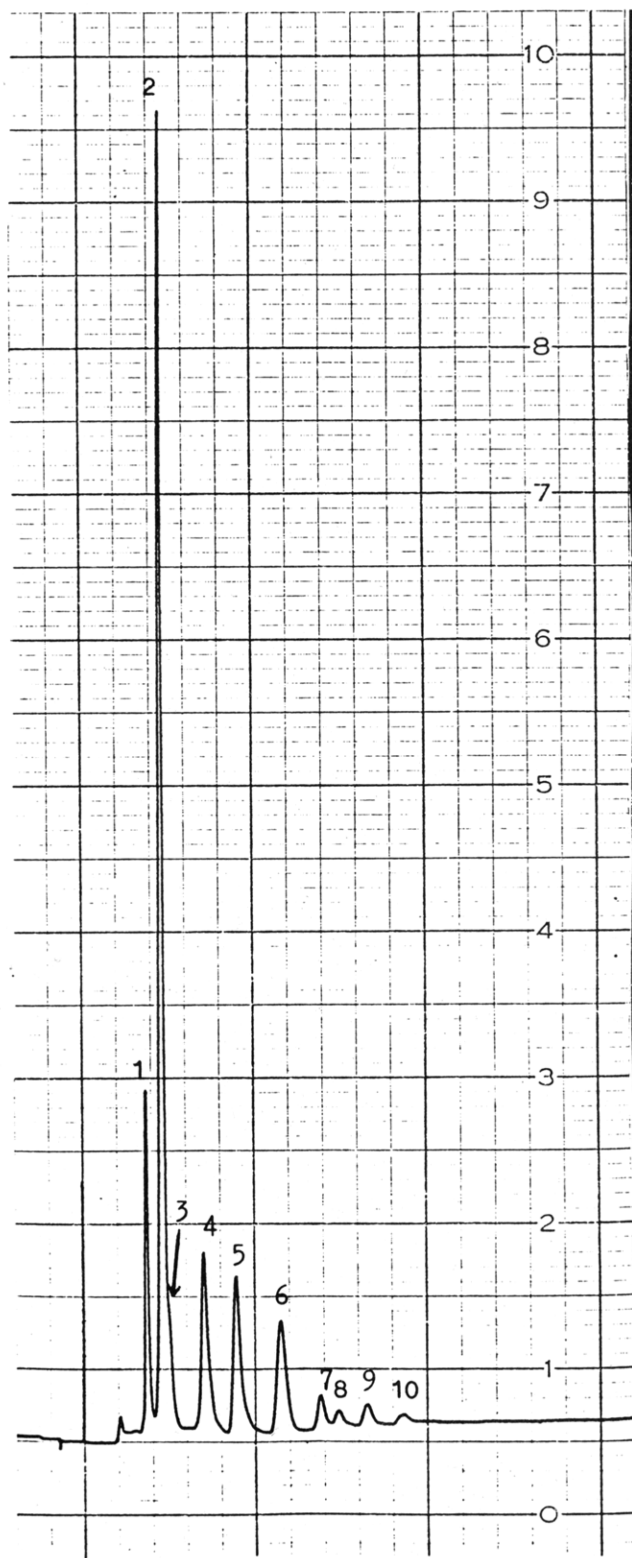


Figure 1. HPLC analysis of the solvolysis products from phyllanthoside in 1:9 ethanol–water employing 97:5.0:0.2 methylene chloride–methanol–water with μ-Porasil and 10 μL of a solution containing 1 mg/L mL.

amination of phyllanthoside stability in solvents of the type used for anticancer substance administration in the NCI P388 in vivo test system.

Solutions of phyllanthoside (**1b**) in 0.9% saline solution containing 10% ethyl alcohol (used for biological evaluation) were prepared at concentrations corresponding to the optimum dose levels[2a] used for P388 leukemia in vivo evaluation. The composition of these solutions was mon-

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A combination of these total syntheses based on (*S*)-(−)-perilla aldehyde and natural tartaric acid, combined with our recent x-ray crystal structure determination (and conformational analysis) of (+)-phyllanthocindiol methyl ester (**2**; R = CH_3) confirm structures 1 and 2 as the correct absolute configurational and conformational assignments: Nassimbeni, L. R.; Niven, M. L.; Cragg, G. M.; Pettit, G. R. *Acta Crystallogr., Sect C.; Cryst. Struct. Commun.* **1984**, *C40*, 146.

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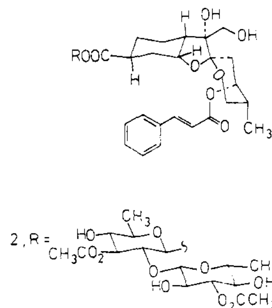
Table I. Summary Results from Solvolysis of Phyllanthoside (1b) in Ethanol–Water (1:9)

HPLC analysis,[a] Figure 1 peak no. (retention vol, mL)	isolated mg[b]	solvolysis product composition	acetate location(s)
1 (5.8)	99	phyllanthostatin 1 (**1a**)	S4, S3′
2 (7.2)	390	phyllanthoside (**1b**)	S3, S3′
3 (7.7)	18	phyllanthostatin 5 (**1d**)	S4, S4′
4 (10.8)	84	phyllanthostatin 4 (**1e**)	S3, S4′
5 (13.7)	71	S3-monodeacetylphyllanthoside (**1f**)	S3′
6 (17.6)	46	phyllanthostatin 3 (**2**)	S3, S3′
7 (21.9)[c]	39	S3′-monodeacetylphyllanthostatin 1 (**1g**)	S4
8 (24.7)			
9 (28.9)	106[d]	S3′-monodeacetylphyllanthoside (**1h**)	S3
10 (34.7)		S3,S3′-dideacetylphyllanthoside (**1i**)	S3, S3′

[a] Figure 1 peak numbers and HPLC conditions. The identity of components 1, 2, 6, 7, 9, and 10 was established by coinjection of the mixture with authentic samples. [b] From a 0.90-g solvolysis product by column chromatography on silica gel 60. [c] Flow rate increased from 1.5 to 2.5 mL/min at 13.3 min. [d] Fractions 8–10 were eluted as a mixture which also contained a number of other minor components.

itored by HPLC. After 16 h at ~25 °C considerable decomposition had occurred, but at −11 °C (over 37 h) phyllanthoside remained essentially unchanged. In the solid state phyllanthoside was found to be stable for more than a year.

When a solution of phyllanthoside (**1b**) in aqueous ethanol[8] (9:1) was allowed to stand at room temperature for 14 days, an HPLC analysis of the mixture showed the presence of 10 products (Figure 1). Identity of the components corresponding to peaks 1, 2, 6, 7, 9 and 10 was established by coinjection of the mixture with authentic samples (Table I). In addition, the products corresponding to peaks 1, 2, 3, 4, 5, 6, and 7 (Figure 1) were isolated by chromatographic separation employing silica gel 60. Structures of the compounds corresponding to peaks 4 and 5 were determined by detailed analysis of their 400-MHz ^{1}H NMR spectra. By this means compound **4** was shown to be a diacetate at S3 and S4′ (**1e**, named phyllanthostatin 4) and **5** as the S3′-monoacetate. Glycoside 3 was clearly separated from phyllanthoside (peak 2; Figure 1) by HPLC using a less polar eluent. On the basis of ^{1}H NMR decoupling studies product 3 was tentatively assigned S4,S4′-diacetate structure **1d** and designated phyllanthostatin 5. Thus, treatment of phyllanthoside with aqueous ethanol (9:1) causes migration of the S3- and S3′-acetyl groups, accompanied by some hydrolysis to form mono- and dideacetyl derivatives as well as opening of the 7,14-epoxide to give the corresponding 7,14-diol, phyllanthostatin 3 (**2**). Relative yields (Table



I) of the solvolysis products appear to differ in some instances from those expected from an estimation of peak areas in the HPLC chromatogram (Figure 1), and this was attributed to further transformations operative during column chromatographic separations.

Acyl group migrations via orthoacid intermediates (Scheme I) of the type experienced with phyllanthoside are frequently observed in carbohydrate chemistry, particularly in the presence of trace amounts of acid or base.[6] Neighboring group rearrangements of this class generally occur quite readily between vicinal gauche hydroxyl groups bearing a relative cis relationship. The acetyl migrations observed in the case of phyllanthoside, however, might be influenced by other factors such as the relative ability of the hydroxyl groups to form intramolecular hydrogen bonds.[6b] As expected, rearrangement of 1b → 1a in 1:9 ethanol–water (4 days) was reversible and phyllanthostatin 1 (**1a**) gave mixtures similar to those derived from phyllanthoside. But following only 21.5 h about half of the phyllanthostatin 1 was converted to phyllanthoside, suggesting that at equilibrium the phyllanthostatin 1 ⇄ phyllanthoside rearrangement predominately favors production of glycoside **1b**. To our knowledge, these glucoside O-3 ⇄ O-4 acetyl migrations communicated (presented in detail here) by us in 1982,[2] and those described in 1983 by the Weidmann group[7] represent the first such examples.

All of the solvolysis products are currently being studied in respect to antineoplastic activity. When completed the resulting structure–activity relationships will be summarized in a future report. Preliminary results indicate that all of the phyllanthostatin glycosides exhibit varying degrees of in vitro and in vivo activity.

Experimental Section

Solvents were redistilled and column chromatography was conducted with prepacked silica gel 60 columns (E. Merck, Darmstadt, Germany). Column chromatography was initiated following equilibration of the silica with the first eluting solvent, and chromatographic separations were monitored (and partially automated) by using Model HM UV–vis Holochrome and Gilson Model FC-220K and Micro fractionators (Model FC-80H). Thin-layer chromatography (TLC) was performed with silica gel GHLF Uniplates (layer thickness 0.25 mm) obtained from Analtech, Inc. and precoated RP-2 (silanized) silica gel 60 F254 plates (layer thickness 0.25 mm) from E. Merck. Visualization of the TLC chromatograms was conducted with anisaldehyde or ceric sulfate spray reagents or by exposure to ultraviolet light. The purity of all products (colorless in each case) was determined by high-performance liquid chromatography on a μ-Porasil column (30 cm × 4 mm) with methylene chloride–methanol–water (97:3.0:0.2 unless otherwise noted) as eluent using a Waters Liquid Chromatograph ALC 2000 series with a Model 440 Absorbance detector and Omniscribe (Houston Instruments) recorder.

Melting points were determined on a Koffler-type hot-stage apparatus and are uncorrected. The ^{1}H NMR spectra (deuteriochloroform solution and tetramethylsilane internal standard) were obtained at 400 MHz. Elemental analyses were determined by Dr. A. W. Spang at the Spang Microanalytical Laboratory,

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Eagle Harbor, Michigan. General experimental procedures used in the present study have been outlined in a preceding contribution.[2d]

Preliminary Solvolysis Experiments with Phyllanthoside (1b). The experimental conditions used for HPLC analyses of the following solvolysis products were similar to those entered in Figure 1 except for composition of the eluent. A solution of phyllanthoside (**1b**, 5 mg) in methylene chloride–methanol–water (97:3:0.2, 10 mL) was stirred with silica gel 60 (1 g). After 96 h an HPLC analysis of the product using the same solvent mixture (97:3:0.2, 3 mL/min) indicated the presence of approximately equal amounts of phyllanthostatin 1 (**1a**) and phyllanthoside (**1b**) together with minor amounts of compounds **1d**, **1e**, and **1f**. Similar results were obtained when a solution of phyllanthoside was stirred in the absence of silica gel 60. Next a solution of phyllanthoside (0.64 mg) in 0.9% saline containing 10% alcohol (1 mL) was allowed to remain at room temperature for 16.5 h. An HPLC analysis using the same conditions as just noted indicated the presence of phyllanthostatin 1 and phyllanthoside in an approximate ratio of 1:3, a significant amount of S3,S4′-diacetate **1e**, minor amounts of S4,S4′-diacetate **1d** and higher retention volume products probably arising from hydrolysis of the acetate groups.

Solvolysis of Phyllanthoside in Ethanol–Water (1:9). One-hundred 10-mg samples of phyllanthoside (**1b**) in 7 mL each of ethanol–water (1:9) were allowed to stand at room temperature (~25 °C) for 14 days. The 100 solution samples were combined, concentrated, and freeze-dried to give the product (1 g). An HPLC analysis using the conditions entered on Figure 1 indicated the presence of ten components. A 0.90-g portion of the mixture was chromatographed on silica gel 60 (two Lobar B column in series). Development with 99:1.0:0.2 methylene chloride–methanol–water (500 mL), followed by 98:2.0:0.2 (1200 mL), and elution with the same solvent gave the following products (elution volume, weight; structure): phyllanthostatin 1 (1200–1320 mL, 99 mg, **1a**); phyllanthoside (1350–1680 mL; 455 mg, **1b**; this fraction contained a small amount of compound **1d**); S3,S4′-diacetate **1e** (2220–2650 mL, 84 mg); S3′-acetate **1f** (3000–3780 mL, 71 mg); phyllanthostatin 3 (4860–5370 mL, 46 mg, **2**) S3′-monodeacetylphyllanthostatin 1 (5380–6330 mL, 39 mg, **1g**). Elution with the same solvents in a ratio of 90:10:0.2 (1000 mL) gave material (106 mg) shown to contain S3′-monodeacetylphyllanthoside (**1h**) and S3,S3′-deacetylphyllanthoside (**1i**).

The new phyllanthostatin glycosides were characterized as follows:

Phyllanthostatin 4 (S3,S4′-diacetate 1e) was obtained as an amorphous solid: mp 122–126 °C; $[\alpha]^{40}_D$ +14.7° (*c* 0.95, $CHCl_3$); IR (KBr) ν_{max} 3450, 1750, 1710, 1695(sh) 1640, 1452, 1380, 1310, 1255, 1240, 1210, 1175, 1125, 1080, 1055, 1025, 950, 905, 767, and 730 cm^{-1}; ^{1}H NMR (400 MHz $CDCl_3$) δ 0.82 (d, J = 6.8 Hz, 15-CH_3), 1.08 (d, J = 6.0 Hz, S6′-CH_3), 1.23–1.27 (m, 2-H's), 1.26 (d, J = 6.0 Hz, S6-CH_3), 1.54–1.64 (m, 1- and 9-H's), 1.78 (dd, J = 14.4 and 10.7 Hz, 4-H), 1.84–2.05 (m, 1-,6-,9- and 11-H's), 2.10 (s, $OCOCH_3$), 2.11 (s, $OCOCH_3$), 2.34 (m, 4-H), 2.50 (m, 3-H), 2.91 (s, 14-H's), 3.08–3.22 (m, S2-,S4-, and *S2′*-H's), 3.26 (m, S5′-H), 3.37–3.46 (m, 12-,S5- and S3′-H's), 3.92 (m, 12-H), 3.93 (d, J = 7.4 Hz, S1′-H), 4.39 (m, 5-H), 4.49 (dd, J = 9.5 and 9.5 Hz, S4′-H), 4.94 (dd, J = 9.4 and 9.4 Hz, S3-H), 5.12 (br s, 10-H), 5.53 (d, J = 8.0 Hz, S1-H), 6.50 (d, J = 16.0 Hz, 2′-H), 7.33–7.57 (m, aromatic H's) and 7.74 (d, J = 16.0 Hz, 3′-H).

Anal. Calcd for $C_{40}H_{52}O_{17}$·H_2O: C, 58.39; H, 6.57. Found: C, 58.69; H, 6.68.

S3′-Monodesacetylphyllanthoside (1f) was isolated as another amorphous solid: mp 121–124 °C; $[\alpha]^{40}_D$ +4.5° (*c* 0.91, $CHCl_3$); IR (KBr) ν_{max} 3450, 1750, 1712, 1690 (sh), 1453, 1380, 1258, 1208, 1170, 1126, 1078, 1022, 948, 905, 768, and 730 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.83 (d, J = 6.8 Hz, 15-CH_3), 1.22 (d, J = 5.8 Hz, S6′-CH_3), 1.25 (d, J = 6.1 Hz, S6-CH_3), 1.28–1.37 (m, 2-H's), 1.58–1.66 (m, 1- and 9-H's), 1.76 (m, 4-H), 1.88–2.02 (m, 1-,6-,9-, and 11-H's), 2.15 (s, $OCOCH_3$), 2.38 (m, 4-H), 2.47 (m, 3-H), 2.78 (dd, J = 8.5 and 8.5 Hz, S2-H), 2.93 (br s, 14-H's), 3.02 (dd, J = 9.0 and 9.0 Hz, S4-H), 3.13 (dd, J = 9.0 and 9.0 Hz, S4′-H), 3.26 (m, S5′-H), 3.36 (m, S5- and S2′-H's), 3.42 (m, 12-H), 3.49 (dd, J = 9.0 and 8.7 Hz, S3-H), 3.97 (dd, J = 11.4 and 11.1 Hz, 12-H), 4.04 (d, J = 7.7 Hz, S1′-H), 4.42 (br s, 5-H), 4.82 (dd, J = 9.0 and 9.0 Hz, S3′-H), 5.09 (br s, 10-H), 5.40 (d, J = 8.1 Hz, S1-H), 6.59 (d, J = 15.9 Hz, 2′-H), 7.39–7.62 (m, aromatic H's) and 7.78 (d, J = 15.9 Hz, 3′-H).

Anal. Calcd for $C_{38}H_{50}O_{16}$·H_2O: C, 58.46; H, 6.67. Found: C, 58.82; H, 6.92.

Phyllanthostatin 5 (S4,S4′-Diacetate 1d). The phyllanthoside (**1b**) fraction isolated by the initial silica gel chromatogram was shown to contain a small amount of diacetate **1d** by HPLC using methylene chloride–methanol–water (97:3.0:0.2; flow rate 3 mL/min, retention volumes; phyllanthoside 22.44 mL; S4,S4′-diacetate **1d**, 38.64 mL). Rechromatrography of the mixture (385 mg) on silica gel 60 (Lobar B column) and elution with methylene chloride–methanol–water (98:2.0:0.2, 1200 mL) gave phyllanthoside (340 mg) followed (between volumes 1440 to 1920 mL) by S4,S4′-diacetate **1d** (19 mg) as an amorphous solid: mp 100–102 °C; $[\alpha]^{24}_D$ +31.48° (*c* 0.54, $CHCl_3$); ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.86 (3 H, d, J = 7 Hz, 15-CH_3), 1.14 (3 H, d, J = 5 Hz, S6′-CH_3), 1.25 (3 H, d, J = 7 Hz, S6-CH_3), 2.5–3.10 (m), 2.15 (3 H, s, $OCOCH_3$), 2.18 (3 H, s, $OCOCH_3$), 2.91 (1 H, m, S2-H), 2.98 (2 H, br s, 14-H's), 3.30–3.84 (m, S3-, S5-, S2′-, S3′-, and S5′-H's), 4.04 (1 H, m, 12-H), 4.07 (1 H, d, J = 7 Hz, S1′-H), 4.42–4.59 (2 H, m, 5-H and S4′-H), 4.63 (1 H, dd, J = 9 and 9 Hz, S4-H), 5.20 (1 H, m, 10-H), 5.51 (1 H, d, J = 8, S-1 H), 6.59 (1 H, d, J = 16 Hz, 2′-H), 7.44–7.88 (aromatic H's) and 7.88 (1 H, d, J = 16 Hz, 3′-H).

Anal. Calcd for $C_{40}H_{52}O_{17}$: C, 59.70; H, 6.47. Found: C, 60.10; H, 7.04.

Solvolysis of Phyllanthostatin 1 (1a) in Ethanol–Water (1:9). Phyllanthostatin 1 in aqueous ethanol (9:1, 1 mg/1mL) was allowed to stand at room temperature. After 21.5 h HPLC (conditions as above) analysis indicated the presence of phyllanthostatin 1 and phyllanthoside in approximately equal amounts. Over a longer period (102 h) significant amounts of S3,S4′-diacetate **1e**, minor amounts of S4,S4′-diacetate **1d** and other (see above) higher retention-volume products were formed.

Acknowledgment. The very necessary financial assistance was provided by Eleanor W. Libby, the Donald Ware Waddell Foundation, Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.), the Fannie E. Rippel Foundation, the Flinn Foundation, the Robert B. Dalton Endowment Fund, the Upjohn Co., Contract NO1-CM-97297 with the Division of Cancer Treatment, NCI, DHHS, and Grants CA16049-06-08 awarded by the National Cancer Institute, DHHS. For other helpful assistance we also wish to thank Drs. P. D. Ellis, D. J. Gust, and the National Science Foundation Regional Facility at the University of South Carolina (CH78-18723).