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ANTINEOPLASTIC AGENTS, 177.[1] ISOLATION AND STRUCTURE OF PHYLLANTHOSTATIN 6

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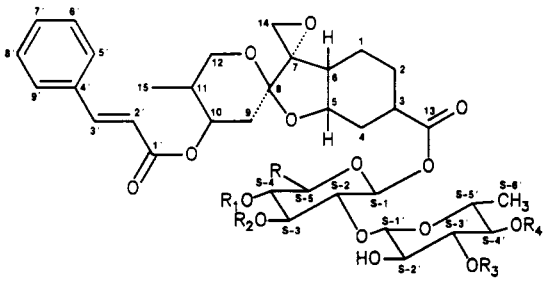
ABSTRACT.—The isolation and structural elucidation of a new *Phyllanthus* glycoside, phyllanthostatin 6 [**7**], was summarized. Phyllanthostatin 6 [**7**] was isolated from the roots of *Phyllanthus acuminatus* (Euphorbiaceae) and was found to inhibit (ED_{50} = 0.35 μg/ml) growth of the murine P-388 lymphocytic leukemia cell line. Two other new constituents were shown to be didesacetylphyllanthostatin 3 [**9**] and descinnamoylphyllanthocindiol [**10**]. Structure determinations were achieved employing hrfabms and 2D-nmr spectroscopy. Application of an hplc separation technique to the *Phyllanthus* glycosides and development of a new isolation procedure for the major antineoplastic constituent, phyllanthoside [**1**], are also described.

The Central American tree *Phyllanthus acuminatus* Vahl (Euphorbiaceae) has been found to produce a new series of potentially useful antineoplastic glycosides. From 1978 to 1986, Costa Rican collections of the roots and stems of this tree were investigated; these investigations led to the isolation and structural elucidation of phyllanthoside [**1**], phyllanthostatin 1 [**2**], the related phyllanthostatins 2 [**6**], 3 [**8**], 4 [**3**], and 5 [**4**] (2,3), and two cytostatic lignans (4). Recently, three new lignans were isolated from the Indian medicinal plant *Phyllanthus niruri* (5). Because of strong activity against human neoplastic cell lines representing breast, CNS (TE671), colon (Colo 205), lung, ovary, and melanoma (Lox) cancers combined with curative levels of activity against the U.S. National Cancer Institute's (NCI) murine B16 melanoma, the phyllanthoside-phyllanthostatin 1 ortho acid equilibrium product has been undergoing preclinical development by the NCI Division of Cancer Treatment and is now in phase 1 clinical trial. Subsequently, Smith and colleagues completed the first total synthesis of phyllanthoside (6), phyllanthostatin 1 (7) and phyllanthostatin 2 (8). A variety of syntheses are now available for the aglycone, phyllanthocin (9).



	R	R_1	R_2	R_3	R_4
1	Me	H	Ac	Ac	H
2	Me	Ac	H	Ac	H
3	Me	H	Ac	H	Ac
4	Me	Ac	H	H	Ac
5	Me	H	H	H	H
6	CH_2OH	H	Ac	Ac	H
7	CH_2OH	H	H	H	H

[1]For Part 176, see Kamano *et al.* (1).

The present report summarizes a procedure for improving the yield of phyllanthoside [**1**]. In addition, a sixth member [**7**] of the cytostatic phyllanthostatin series and two inactive (NCI murine P-388 lymphocytic leukemia cell line, PS system) transformation products have been discovered. Earlier (2) phyllanthoside was isolated in yields ranging from 7.4×10^{-4}% to 1.4×10^{-2}%. In the present study we found that extraction of the dry root with CH_2Cl_2 is an efficient and selective way to obtain a phyllanthoside-rich (corresponding to ~1% of the root) crude extract. The extract was efficiently separated by size exclusion chromatography on Sephadex LH-20 [elution with *n*-hexane–CH_2Cl_2 (1:3) and *n*-hexane–CH_2Cl_2–Me_2CO (1:3:1)] followed by high-speed countercurrent distribution (hsccd) with *n*-hexane–CH_2Cl_2–MeOH–H_2O (2:4:5:2) as solvent system. By this means phyllanthoside [**1**] was quickly isolated (only 4 steps) in yields as high as 0.2%, and rearrangement and degradation of glycoside **1** were minimized.

The hplc technique we previously developed for detection of phyllanthostatin A (4) was very helpful in developing the new isolation procedure for phyllanthoside. Inspection of the hplc analyses corresponding to crude CH_2Cl_2 (Figure 1a) and MeOH (Figure 1b) extracts of *P. acuminatus* illustrates this point. Both extracts displayed a large peak assigned to phyllanthoside [**1**], the most dominant phyllanthostatin constituent in crude extracts of *P. acuminatus*.

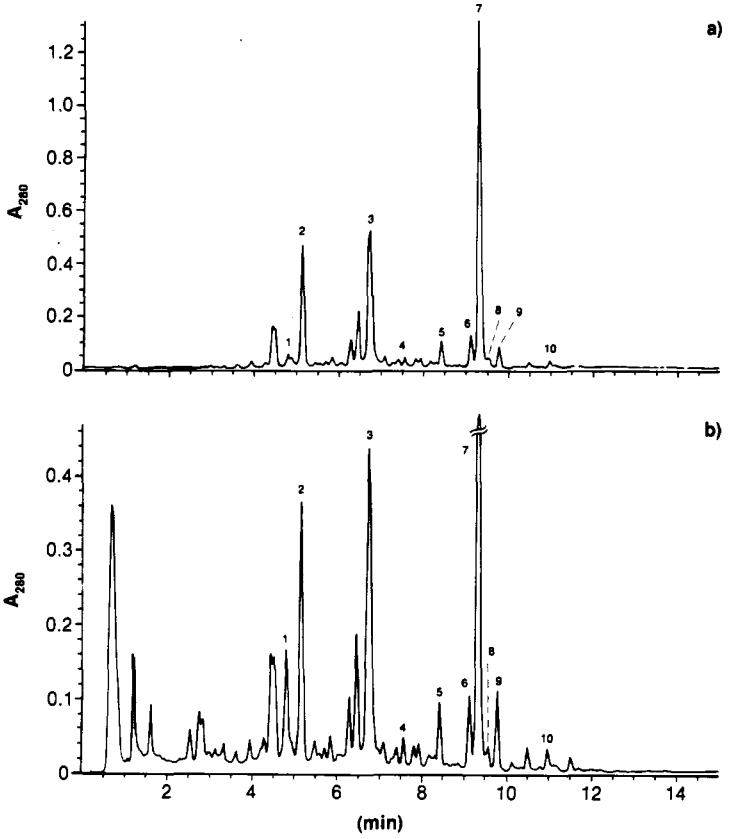


FIGURE 1. Hplc separation of a *Phyllanthus acuminatus* CH_2Cl_2 extract (a) and an MeOH extract (b) using RP-18 Si gel with a linear gradient of MeCN-H_2O (3:7→7:3) (photodiode array detector). Identified peaks are noted with corresponding structure numbers.

Figure 2 illustrates the separation of a mixture of phyllanthoside [**1**] and its isomers **2–4**. *Phyllanthus* glycosides **7/9** and **6/8**, which coeluted (Figure 1) on an RP-18 hplc column, were easily separated on RP-8 (aqueous MeOH). However, the latter system was less powerful for the separation of complex samples such as total extracts. Finally, a freshly prepared CH_2Cl_2 extract of the original *P. acuminatus* roots (collected in 1978) was analyzed as just described. Again, large amounts of phyllanthoside and only traces of phyllanthostatin 1 were detected. Compared to a 1986 sample, the chromatogram (not shown) obtained from the 1978 sample displayed a much larger peak at Rt 6.7 min, assigned to phyllanthostatin 3 [**8**], as well as additional peaks between Rt 4 and 6 min (nonidentified degradation products).

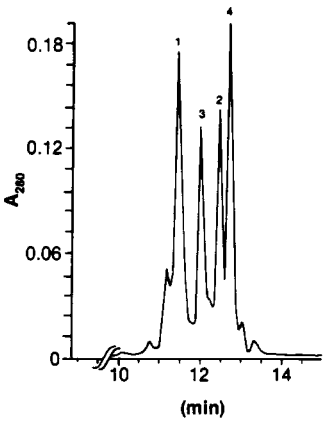
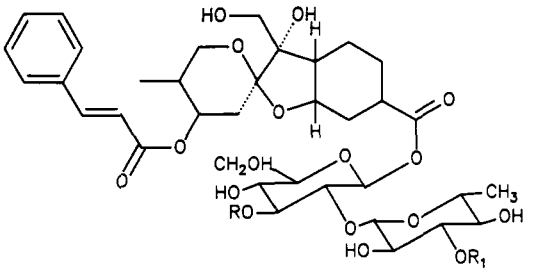


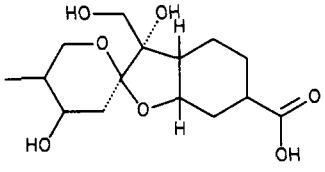
FIGURE 2. Hplc separation of phyllanthoside [**1**] isomers on a column of RP-8 Si gel using a MeOH-H_2O (2:3↦9:1) gradient.

Phyllanthostatin 6 [**7**] was isolated from a fresh 1986 MeOH extract of *P. acuminatus*. The MeOH extract (22 g) was separated by size exclusion chromatography (Sephadex LH-20; MeOH), affording a phyllanthostatin-6-rich fraction (4.95 g), which was further purified by hsccd (4, 10–12) using a CH_2Cl_2-MeOH-H_2O (5:5:3) solvent system. Semi-preparative reversed-phase hplc finally afforded 12 mg of phyllanthostatin 6 [**7**] (3.7×10^{-3}% yield, PS ED_{50} 0.35 μg/ml). Didesacetylphyllanthostatin 3 [**9**] and descinnamoylphyllanthocindiol [**10**] were both isolated from a fraction prepared during an earlier (1983) large-scale isolation of the phyllanthostatins (2). Separation of these relatively polar *Phyllanthus* constituents was accomplished by a size exclusion (Sephadex LH-20), hsccd, and reversed-phase liquid chromatographic sequence. The pure compounds **9** (105 mg) and **10** (0.75 g) were found to be inactive against the PS cell line. Structural determinations were conducted as follows.

Phyllanthostatin 6 [**7**] showed spectroscopic (uv, ir, nmr) properties similar to those of the phyllanthostatins. Acid hydrolysis of the glycoside **7** afforded two hexoses with the same tlc mobility as glucose and 6-deoxyglucose. By hrfabms the molecular formula was established as $C_{36}H_{48}O_{16}$. The ion observed at *m/z* 613 $[(M + Na) - 146]^+$ indicated that deoxyglucose was the terminal hexose of the disaccharide moiety of phyllanthostatin 6. Both the ^1H- and ^{13}C-nmr chemical shifts were assigned based on 2D nmr experiments (^1H,^1H-COSY and ^1H,^{13}C-COSY). Chemical shifts of the cinnamoyl-sesquiterpene moiety were identical to those previously assigned to the phyllanthostatin aglycone (2). For example, the epoxide was confirmed by carbon resonances at δ 49.80 (C-14), 71.02 (C-7), and 102.08 (C-8) and by the C-14 protons at δ 2.93 ppm.



	R	R_1
8	Ac	Ac
9	H	H



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From nmr and ms spectra it became apparent that the phyllanthostatin 6 disaccharide was not acetylated. The disaccharide proton resonances were fully assigned by $^1H,^1H$-COSY and double-quantum filtered phase-sensitive COSY experiments (13,14). Figure 3 shows the sugar resonances between δ 2.9 and 4.2 ppm with the corresponding correlation peaks (double-quantum filtered phase-sensitive COSY spectrum). Interpretation of the latter spectrum compared to a normal $^1H,^1H$-COSY was simplified by less overlapping of the correlation peaks on and close to the diagonal. The C-14 protons, for example, appeared as a very weak signal, whereas the normal $^1H,^1H$-COSY spectrum showed a prominent signal at δ 2.93 ppm. Complete correlation between sugar protons was observed from S-1 through S-6 and from S-1′ through S-6′, respectively. The $^1H,^{13}C$-COSY spectrum showed that the S-2 proton was correlated to the carbon resonance at δ 82.15 ppm typical of glycosylation at this position and confirming glucose as the inner sugar. Chemical shifts assigned to the terminal sugar were typical of 6-deoxy-D-glucose (2). The coupling constants ($J = 8$ Hz) of both anomeric

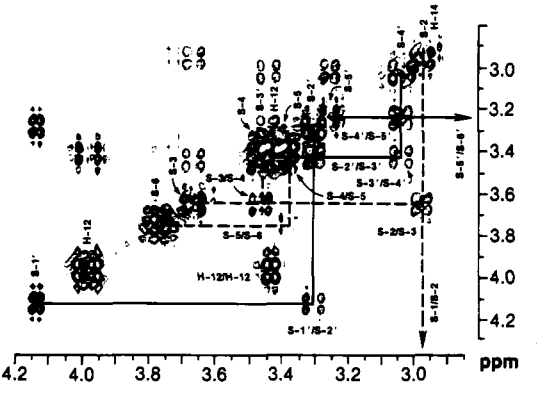

FIGURE 3. Double-quantum-filtered phase-sensitive COSY spectrum of phyllanthostatin 6 [7] carbohydrate moiety (400 MHz, $CDCl_3$).

protons confirmed the β linkage of the 2-*O*-(6-deoxy-D-glucopyranosyl)-D-glucopyranosyl unit. The anomeric proton and carbon of the inner glucose unit displayed chemical shifts (δ 5.48/92.40 ppm) identical to those observed with phyllanthostatin 3 [**8**]. Overall assignment of the phyllanthostatin 6 [**7**] chemical shifts were in agreement with those reported (2) for the other phyllanthostatins. Thus, structure **7** was assigned to this new member of the series.

The molecular formula of didesacetylphyllanthostatin 3 [**9**] was determined by hrfabms to be $C_{36}H_{50}O_{16}$. Except for ^{13}C-nmr chemical shifts recorded for C-7 (85.33 ppm), C-8 (106.31 ppm), and C-14 (66.61 ppm), indicating a diol unit at C-7–C-14 (2), glycoside **9** displayed spectroscopic properties similar to those of phyllanthostatin 6 [**7**]. The ^{1}H- and ^{13}C-nmr resonances assigned to positions 1–15 and 1′–9′ were in accord with those of phyllanthostatin 3, but chemical shifts of the sugar moiety were more typical of a β-linked phyllanthose unit and indeed agreed with the corresponding data for didesacetylphyllanthoside [**5**] (2). Hence, this component was assigned to didesacetylphyllanthostatin 3 [**9**].

Descinnamoylphyllanthocindiol [**10**] gave the same tlc color reaction (brown-gray→pink after 24 h) upon development with anisaldehyde, as observed with the phyllanthostatins. Lack of uv absorption suggested absence of the cinnamoyl ester, and the molecular formula (by hrfabms), $C_{15}H_{24}O_7$, suggested lack of a disaccharide unit (confirmed by nmr analyses). Assignments for the ^{1}H and ^{13}C chemical shifts were achieved by 2D nmr techniques and indicated a phyllanthocindiol (2) analogue with a hydroxyl at C-10 and a carboxyl group at C-3. Compared to glycoside **9**, carbon resonances C-9 to C-11 of glycoside **10** appeared at higher (C-11 at δ 34.22, C-9 at δ 36.76 ppm) and lower (C-10 at δ 68.22 ppm) fields in agreement with a hydroxyl group at C-10. Structure **10** was thereby identified as descinnamoylphyllanthocindiol.

Both the improved procedure herein summarized for isolation of phyllanthoside and its useful total synthesis (6,7) have diminished the problem of future supplies of this substance and the isomeric phyllanthostatin 1. Isolation of phyllanthostatin 6 appears to complete the series of principal antineoplastic and/or cytostatic glycosides produced by *P. acuminatus*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All hplc-grade solvents (Omnisolv) were obtained from EM Science, and all other solvents were redistilled. Adsorption cc was performed with Si gel 60 (70–230 mesh, E. Merck, Darmstadt, Germany). Reversed-phase Si gel chromatography was accomplished with RP-8 Lobar columns (size B, 40–63 μm, 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.). The tlc plates were examined under uv light and developed with an anisaldehyde spray reagent. High-speed countercurrent distribution (hsccd) was performed with an Ito Multilayer Coil Extractor-Separator (P.C. Inc., Potomac, Maryland) using 2.6 mm i.d. tubing, FMI Lab Pump, Linear recorder, and Gilson Model Holochrome uv/vis detector (2.5 mm/3.2 μl cell) with a Micro Fractionator. The hplc-uv/vis separations were accomplished with Ultremex 3 μm RP-8 and RP-18 columns (100 × 4.6 mm i.d.; Phenomenex, Rancho Palos Verde, California). The mobile phase was delivered by two Gilson Model 302 pumps using an Apple II programmer and a Rheodyne 7161 injector with a 0.5 μm in-line precolumn filter (Rainin). Linear gradient elution was carried out with MeCN-H_2O (3:7→7:3) (RP-18) and MeOH-H_2O (2:3→9:1) (RP-8) within 15 min at a flow rate of 1 ml/min. All pure compounds for hplc analyses were dissolved in MeOH (~0.1 mg/ml). Roots of *P. acuminatus* (1 g powder) were extracted at room temperature with MeOH or CH_2Cl_2 (3 × 30 ml solvent). The MeOH extract (78 mg) and the CH_2Cl_2 extracts (16 mg of 1978 samples and 8 mg of 1986 specimens) were dissolved or suspended in 2 and 1 ml of MeOH, respectively. These solutions were each passed through a Sep-Pak C-18 cartridge (Waters). The cartridges were washed with MeOH until 4 ml (MeOH extract) and 2 ml (CH_2Cl_2) eluates were collected. These solutions (10 μl) were injected for hplc analyses. Signals were identified by comparison of retention times with those of authentic samples by co-injection. More than 20 non-identified minor peaks with uv spectra typical of the phyllanthostatins were observed. The same results were obtained when crude extracts were separated on RP-8 Si gel with MeOH-H_2O (2:3→9:1) as eluent.

Melting points are uncorrected and were determined using a Kofler-type hot-stage apparatus. Optical rotations were measured with a Perkin-Elmer Model 241 Automatic Polarimeter. The uv spectra were recorded employing 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. Assignments bearing the same superscript may be reversed. The ^{13}C-nmr multiplicities were determined with APT experiments based on an average coupling constant of 135 Hz. Normal 2D homonuclear and heteronuclear shift correlated spectra were recorded using standard pulse sequences (15–17). The double-quantum filtered phase-sensitive COSY experiment was pursued following the procedure of Wuethrich and co-workers (13,14). Eims spectra were obtained using a Varian MAT 312 spectrometer. Fabms spectra were recorded with a MS-50 instrument at the NSF Regional Facility, University of Nebraska, Lincoln.

PLANT MATERIAL AND EXTRACTION.—The 1986 collection of *P. acuminatus* roots was obtained in Costa Rica, and a voucher specimen is preserved in our Institute. The dry powdered roots (345 g) were extracted at room temperature successively with *n*-hexane, CH_2Cl_2, and MeOH (3 × 6 liters each solvent), yielding 0.6, 3.6, and 25.0 g extracts, respectively. Extraction, solvent partitioning, and chromatographic separation of *P. acuminatus* (1978 collection) were performed as described by Pettit *et al.* (2).

ISOLATION OF PHYLLANTHOSIDE [1].—The 1986 CH_2Cl_2 extract (3.6 g) was separated on a column of Sephadex LH-20 (60 × 4 cm i.d.) with *n*-hexane–CH_2Cl_2 (1:3) as initial solvent system. After eluting with 2.5 liters, the solvent was changed to *n*-hexane–CH_2Cl_2–Me_2CO (1:3:1), and a phyllanthoside-rich (0.833 g) fraction was eluted. An aliquot of this fraction (106 mg) was purified by hsccd with the solvent system *n*-hexane–CH_2Cl_2–MeOH–H_2O (2:4:5:2). The sample was dissolved in a mixture (4 ml) of stationary and mobile phase (6:1) and introduced in the coil through the head inlet. The coil was rotated at 800 rpm, and the mobile phase (lower layer) was pumped at a flow rate of 200 ml/h. Detection (uv) was set at 280 nm and fractions collected every 1.5 min. Retention of the stationary phase was 45%. Pure (by hplc) phyllanthoside (93 mg) was obtained (elution volume 265–335 ml) and its identity confirmed by comparison with an authentic sample (ir, ^{1}H nmr, and ^{13}C nmr).

ISOLATION OF PHYLLANTHOSTATIN 6 [7].—The MeOH extract (22 g) of *P. acuminatus* (1986 collection) was separated by size exclusion chromatography on Sephadex LH-20 (100 × 10 cm i.d., MeOH), and 7 fractions were collected. Fraction 5 (4.95 g, elution volume 6150–6700 ml) was further separated by hsccd with the solvent system CH_2Cl_2-MeOH-H_2O (5:5:3). Samples (2 g) were dissolved in a mixture of upper (13 ml) and lower (2 ml) phase. The hsccd was conducted with the lower, organic phase and a flow rate of 200 ml/h. A uv detector was set at 280 nm and fractions collected every 1.5 min. Fractions eluted between 120 and 150 min after sample introduction were combined and afforded 38 mg of almost pure phyllanthostatin 6 [7]. Combined fractions were purified by semi-preparative hplc (RP-8, Prepex 5-20 μm, 250 × 10 mm, Phenomenex) with aqueous MeOH-H_2O (3:7) at a flow rate of 2 ml/min, yielding 12 mg (3.7×10^{-3}% yield) of phyllanthostatin 6 [7]: amorphous solid; mp 136–139°; tlc on Si gel R_f 0.12 [CH_2Cl_2-MeOH (9:1)], R_f 0.25 [CH_2Cl_2-MeOH-H_2O (5:5:3) (lower phase)]; $[\alpha]^{25}D$ +12.0° (c = 0.25, CH_2Cl_2); hrfabms m/z $[M+Na]^+$ 759.2839 (calcd for $C_{36}H_{48}O_{16}Na$, 759.2840) with Δ = 0.1 ppm, $[(M+Na)-146]^+$ 613; uv λ max (MeOH) 277 nm; ir (KBr) ν max 3422, 2940, 1745, 1707, 1635, 1450, 1315, 1281, 1169, 1123, 1075, 1021 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.83 (3H, d, J = 6 Hz, H-15), 1.20 (3H, d, J = 6 Hz, S-6′), 1.27 (2H, m, H-2), 1.57 (H-1), 1.63 (H-9), 1.76 (H-4), 1.91 (H-9), 1.94 (H-11), 1.98 (H-1, H-6), 2.32 (H-4), 2.50 (H-3), 2.93 (2H, br s, H-14), 2.97 (S-2), 3.04 (S-4′), 3.25 (S-5′), 3.30 (S-2′), 3.37 (S-5), 3.43 (H-12), 3.44 (S-3′), 3.46 (S-4), 3.66 (S-3), 3.75 (S-6), 3.98 (1H, dd, J = 11.5 Hz, H-12), 4.13 (1H, d, J = 8 Hz, S-1′), 4.42 (H-5), 5.14 (H-10), 5.48 (1H, d, J = 7.7 Hz, S-1), 6.56 (1H, d, J = 16.1 Hz, H-2′), 7.39 (3H, br s, H-5′, H-7′, H-9′), 7.56 (2H, br s, H-6′, H-8′), 7.78 (1H, d, J = 16.1 Hz, H-3′); ^{13}C nmr ($CDCl_3$) δ 12.73 (q, C-15), 17.76 (q, S-6′), 21.84 (t, C-1), 25.62 (t, C-2), 29.47 (t, C-4), 33.14 (d, C-11), 34.31 (t, C-9), 37.04 (d, C-3), 38.21 (d, C-6), 49.80 (t, C-14), 61.64 (t, S-6), 62.79 (t, C-12), 69.15 (d, S-4), 69.70 (d, C-10), 71.02 (s, C-7), 72.16 (d, S-5′)[a], 72.61 (d, C-5), 74.87 (d, S-2′)[a], 75.15 (d, S-4′), 76.03 (d, S-3′)[b], 76.10 (d, S-3), 76.30 (d, S-5)[b], 82.15 (d, S-2), 92.40 (d, S-1), 102.08 (s, C-8), 104.50 (d, S-1′), 118.57 (d, C-2′), 128.29 (2 × d, C-6′, C-8′), 129.24 (2 × d, C-5′, C-9′), 130.61 (d, C-7′), 134.34 (s, C-4′), 145.01 (d, C-3′), 166.99 (s, C-1′), 174.38 (s, C-13).

HYDROLYSIS.—A solution of phyllanthostatin 6 [7] (2 mg) in MeOH (2 ml) and 2 N HCl (10 ml) was heated at reflux for 30 min, diluted with H_2O, and extracted with $CHCl_3$. The aqueous phase was neutralized ($NaHCO_3$), the solvent was evaporated, and the sugars were extracted with pyridine. Glucose and 6-deoxyglucose were 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 spots at R_f 0.58 and R_f 0.70 characteristic of D-glucose and 6-deoxy-D-glucose, respectively.

ISOLATION OF DIDESACETYLPHYLLANTHOSTATIN 3 [**9**] AND DESCINNAMOYLPHYLLANTHOCINDIOL [**10**].—A fraction obtained from an earlier large-scale isolation of phyllanthoside (2) was separated by size exclusion chromatography on Sephadex LH-20 in MeOH (100 × 10 cm i.d.; 100 g and 92 g samples) yielding 11 fractions. Part (6 g) of the major fraction (111 g; elution volume 4550–5925 ml) was further separated by hsccd with the solvent system CH_2Cl_2-MeOH-H_2O (5:5:3). The organic layer was used as mobile phase and was passed at a flow rate of 400 ml/h. Retention of the stationary phase was about 50%. Samples (3 × 2 g) were dissolved in a 20 ml mixture of both phases, and fractions were collected every minute. Fractions eluted between volumes 150 and 250 ml were combined (0.36 g) and further purified by reversed-phase liquid chromatography with MeOH-H_2O (3:2→7:3) (Lobar RP-8, size B) to afford 105 mg of didesacetylphyllanthostatin 3 (7×10^{-6}% yield). Another aliquot (6.3 g) of the main fraction was separated by reversed-phase liquid chromatography with MeOH-H_2O (1:3) (Lobar RP-8, size B, 3 × 2.1 g samples). Fractions containing diol **10** were combined in MeOH solution and further purified on a column of Sephadex LH-20, yielding 0.75 g of descinnamoylphyllanthocindiol [**10**] (5×10^{-5}% yield).

Didesacetylphyllanthostatin 3 [**9**] was isolated as an amorphous solid: mp 135–139°; tlc on Si gel R_f 0.08 [CH_2Cl_2-MeOH (9:1)]; $[\alpha]^{25}D$ +9.1° ($c = 0.11$, CH_2Cl_2); hrfabms *m/z* $[M+Li]^+$ 745.3236 (calcd for $C_{36}H_{50}O_{16}Li$, 745.3260), $\Delta = 3.3$ ppm; uv λ max (MeOH) 277 nm; ir (KBr) ν max 3433, 2940, 1745, 1707, 1635, 1445, 1309, 1281, 1233, 1169, 1117, 1074 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.85 (3H, d, $J = 5.6$ Hz), 1.21 (3H, d, $J = 5.5$ Hz, S-6')[a] 1.25 (3H, d, $J = 5.5$ Hz, S-6)[a], 1.32 (H-2), 1.38 (H-1), 1.59 (H-1), 1.73 (H-4), 1.82 (H-6), 1.94 (H-9, H-11), 2.02 (H-2), 2.14 (H-9), 2.17 (H-4), 2.51 (H-3), 3.00 (S-2), 3.04 (S-4), 3.05 (S-4'), 3.23 (S-5')[b], 3.25 (S-2'), 3.38 (S-5)[b], 3.42 (S-3'), 3.49 (H-12, H-14), 3.57 (S-3), 3.93 (H-14), 4.01 (H-12), 4.16 (1H, d, $J = 7.7$ Hz in C_5D_5N, S-1'), 4.18 (H-5), 5.13 (H-10), 5.45 (1H, d, $J = 8.1$ Hz in C_5D_5N, S-1), 6.50 (1H, d, $J = 15.8$ Hz, H-2'), 7.46 (H-5', H-7'), 7.55 (H-6', H-8'), 7.75 (1H, d, $J = 15.8$ Hz, H-3'); ^{13}C nmr ($CDCl_3$) δ 12.67 (q, C-15), 17.61 (q, S-6')[a], 17.82 (q, S-6)[a], 20.47 (t, C-1), 26.11 (t, C-2), 29.47 (t, C-4), 33.21 (d, C-11), 35.32 (t, C-9), 36.87 (d, C-3), 43.22 (d, C-6), 62.77 (t, C-12), 66.21 (t, C-14), 70.06 (d, C-10), 72.10 (d, S-2'), 72.70 (d, S-5)[b], 72.83 (d, C-5), 74.58 (d, S-5')[b], 75.07 (2d, S-4, S-4'), 75.88 (d, S-3'), 76.23 (d, S-3), 81.70 (d, S-2), 85.33 (s, C-7), 92.28 (d, S-1), 104.15 (d, S-1'), 106.31 (s, C-8), 118.56 (d, C-2'), 128.32 (2 × d, C-6', C-8'), 129.22 (2 × d, C-5', C-9'), 130.52 (d, C-7'), 134.35 (d, C-4'), 145.15 (d, C-3'), 167.22 (s, C-1'), 174.72 (s, C-13).

Descinnamoylphyllanthocindiol [**10**] was obtained as an amorphous solid: mp 60–65°; tlc on Si gel R_f 0.18 [CH_2Cl_2-Me_2CO-H_2O (20:80:5)]; $[\alpha]^{25}D$ +92° ($c = 0.25$, MeOH); hrfabms *m/z* 323.1688 $[M+Li]^+$ (calcd for $C_{15}H_{24}O_7Li$, 323.1683), $\Delta = 1.5$ ppm; ir (KBr) ν max 3456, 2954, 1707, 1455, 1417, 1390, 1121, 1082, 1040, 1022, 985 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.90 (3H, d, $J = 6.8$ Hz, H-15), 1.30–1.39 (H-2), 1.51–1.60 (H-1), 1.72–1.82 (H-1, H-4, H-6, H-9, H-11), 2.05–2.13 (H-2, H-9), 2.18–2.22 (H-4), 2.59–2.65 (H-3), 3.44 (1H, d, $J = 11.5$ Hz, H-14), 3.51 (H-12), 3.79 (H-12), 3.86 (H-10), 4.00 (1H, d, $J = 11.5$ Hz, H-14), 4.20 (H-5); ^{13}C nmr [$CDCl_3$-MeOD (9:1)] δ 12.56 (q, C-15), 20.21 (t, C-1), 25.74 (t, C-2), 29.33 (t, C-4), 34.22 (d, C-11), 36.76 (t, C-9), 36.84 (d, C-3), 42.71 (d, C-6), 62.03 (t, C-12), 65.19 (t, C-14), 68.22 (d, C-10), 74.01 (d, C-5), 84.11 (s, C-7), 107.52 (s, C-8), 180.00 (s, C-13, in MeOD).

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