mass spectrum, exact mass calcd for $C_{15}H_{22}O_3$ m/e 250.1569, found m/e 250.1560.

(1S*,2R*,5S*,7S*,8R*,11R*)-1-Hydroxy-2-methyl-8-acetoxytricyclo[5.3.1.0^{5,11}]undecan-6-one (24). To a stirred solution of the acetate **23** (175 mg, 0.70 mmol) and trimethylamine *N*-oxide dihydrate (100 mg, 0.90 mmol) in 3 mL of THF/acetone/water (3:2:1, v/v/v) was added a small crystal of osmium tetraoxide under nitrogen. The solution was stirred for 12 h, ethyl acetate and solid sodium dithionite were added, and a black particulate suspension formed. The aqueous layer was separated, and the organic phase was filtered through anhydrous magnesium sulfate and concentrated to a clear viscous oil. This oil was dissolved in 3.5 mL of dry benzene, lead tetraacetate (350 mg, 0.79 mmol) was added, under nitrogen, and the mixture was stirred for 1 h. Ethyl acetate and saturated sodium bicarbonate solution were added. The organic layer was separated, filtered through anhydrous magnesium sulfate, and concentrated to afford 110 mg of crude product as colorless plates. The solid was recrystallized from ether–hexane to give 40 mg of pure product. The mother liquors were concentrated and the residue purified by flash chromatography to give an additional 47 mg of pure product as colorless plates (49% yield overall): mp 155–156 °C; IR ($CHCl_3$) 3590, 3500, 1770, 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.05 (d, t, 1, J = 2.9, 8.3 Hz), 3.91 (d, t, 1, J = 2.6, 8.3), 3.43 (t, 1, J = 10.0 Hz), 2.36 (t, 1, J = 10.0 Hz), 2.09 (s, 3), 1.95–2.21 (m, 2), 1.24–1.80 (m, 8), 0.96 (d, 3, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$) δ 207.2, 171.3, 71.0, 67.9, 56.4 (overlapping resonances), 40.4, 35.7, 27.8, 23.1, 22.8, 21.0, 19.6, 14.4; mass spectrum (CI), M + 1, 253.

(1R*,2R*,5S*,7S*,11R*)-2-Methyl-8-acetoxytricyclo[5.3.1.0^{5,11}]undecan-6-one (27). To a stirred solution of cyclobutanone **24** (40 mg, 0.16 mmol), 4-(dimethylamino)pyridine (70 mg, 0.60 mmol), and triethylamine (100 mg, 1.0 mmol) in 2 mL of dry distilled dichloromethane was added distilled phosphorus oxychloride (80 mg, 0.52 mmol) at 0 °C under nitrogen. The solution became faintly purple colored and was allowed to warm to room temperature. Ethyl acetate and saturated sodium bicarbonate solution were added and the organic layer was separated. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated to afford a crystalline material. This material was dissolved in 3.5 mL of ethyl acetate with 5 mg of 10% palladium-on-alumina under an atmosphere of hydrogen. The solution was stirred for one day, purged with nitrogen, and filtered through Celite. The filtrate was concentrated to give a white crystalline material which was purified by flash chromatography to yield 36 mg (95% overall) of product as white needles: mp 95–97 °C; IR ($CHCl_3$) 1780, 1735 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.97 (t, 1, J = 8.0 Hz), 3.86 (dt, 1, J = 2.3, 9.4 Hz), 3.13 (t, 1, J = 9.4 Hz), 2.57 (q, 1, J = 9.4 Hz), 2.09 (s, 3), 1.15–2.05 (m, 10), 0.92 (d, 3, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 208.4, 171.3, 69.0, 55.8, 53.0, 37.8, 32.3, 29.2, 26.5, 24.1, 20.9, 20.4, 19.8, 16.5; mass spectrum, exact mass calcd for $C_{14}H_{20}O_3$ m/e 236.1413, found m/e 236.1412.

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Registry No. (±)-**1** (Y′ = OH; R = CH_2OH), 91949-72-3; (±)-**11** (isomer 1), 91949-73-4; (±)-**11** (isomer 2), 91949-74-5; **14**, 30332-99-1; **15**, 5557-87-9; **16**, 32442-48-1; **17**, 69225-59-8; **18**, 69519-94-4; (±)-**19**, 91949-75-6; (±)-**20**, 91993-70-3; (±)-**21**, 91949-76-7; (±)-**22**, 91949-77-8; (±)-**23**, 91949-78-9; (±)-**24**, 91949-79-0; (±)-**25**, 91949-80-3; (±)-**26**, 91949-81-4; (±)-**27**, 91949-82-5; $(EtO)_2POCH_2CO_2Et$, 867-13-0; propargyl alcohol, 107-19-7; triethyl orthoacetate, 78-39-7.

Supplementary Material Available: Tables of atomic positional and thermal parameters (18 pages). Ordering information is given on any current masthead page.

Antineoplastic Agents. 104. Isolation and Structure of the *Phyllanthus acuminatus* Vahl (Euphorbiaceae) Glycosides[1a]

George R. Pettit,* Gordon M. Cragg, Matthew I. Suffness,[1b] Devens Gust, Fred E. Boettner,[1c] M. Williams,[1c] J. A. Saenz-Renauld,[1d] Peter Brown,[1e] Jean M. Schmidt, and Paul D. Ellis[1f]

Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287

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Three new antineoplastic glycosides, phyllanthostatins 1 (**2a**), 2 (**2c**), and 3 (**3a**), as well as phyllanthoside (**2b**), have been isolated from the Central American tree *Phyllanthus acuminatus* Vahl. The phyllanthostatins 1 (**2a**), 2 (**2c**), and 3 (**3a**) and phyllanthoside (**2b**) structures were completely assigned by detailed analyses of spectral data (principally by 400-MHz NMR) and have been confirmed by X-ray crystallographic analyses of degradation products. In addition to inhibiting growth of the murine P388 lymphocytic leukemia, both phyllanthostatin 1 and phyllanthoside were found to markedly retard progression of the murine B16 melanoma. One of these unique glycosides, phyllanthoside (**2b**), is in preclinical development at the U.S. National Cancer Institute.

The age-adjusted incidence of melanoma among Caucasians in North America has been increasing[2] and in areas (Texas[2] and Arizona[3]) of the American Southwest has reached an alarming rate.[4] Discovery of new drugs to improve melanoma treatment is an important objective of

(1) (a) For the 103rd part of this series, see: Pettit, G. R.; Goswami, A.; Cragg, G. M.; Schmidt, J. M.; Zou, J.-C. *J. Nat. Prod.*, in press. (b) Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD 20014. (c) Polysciences, Inc., Paul Valley Industrial Park, Warrington, PA 18976. (d) School of Science, University of Costa Rica, San Jose, Costa Rica. (e) Deceased March 25, 1981. (f) Department of Chemistry, University of South Carolina, Columbia, SC 29208.

(2) Scotto, J.; Fears, T. "Cancer Epidemiology in the USA and USSR"; Levin, D. L., Blokhin, N. N., Eds.; NIH Publication No. 80-2044, 1980; p 129.

(3) Private communication, Alberts, D. S. Cancer Center, University of Arizona, Tucson, AZ.

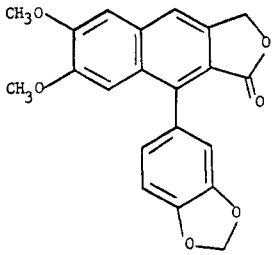
(4) Even with stage 1 melanoma the five-year survival rate can fall to <50% in males with a primary neoplasm thicker than 2 mm (over 4 mm for females[5]). Treatment of human malignant melanoma with combinations of the plant biosynthetic products vincristine, vinblastine, and bleomycin with synthetic agents such as procarbazide has provided overwhelming evidence[6] that this disease will respond to cancer chemotherapy. But the general prognosis for malignant melanoma is still quite poor and the need is great for new and more effective cancer chemotherapeutic drugs.[7]

(5) Veronesi, U.; Cascinelli, N.; Morabito, A.; Bufalino, R.; van der Esch, E. P.; Preda, F.; Vaglini, M.; Rovini, D.; Orefice, S. *Int. J. Cancer* **1980**, *26*, 733–739.

0022-3263/84/1949-4258$01.50/0

the U.S. National Cancer Institute (NCI). And as part of this research we have completed a detailed study[8] of the Costa Rican tree, *Phyllanthus acuminatus* Vahl (Euphorbiaceae).

The roots of *P. acuminatus* have been found to be an especially valuable source of substances that inhibit growth of the NCI murine P388 lymphocytic leukemia (PS system). These range in structure from the PS in vitro[9] active justicidin B (**1**, ED_{50} 3.3 μg/mL) to the PS in vivo active



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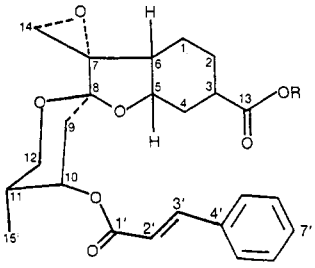
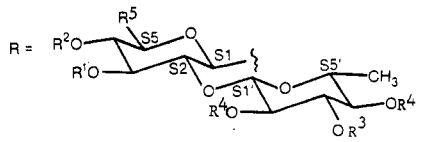
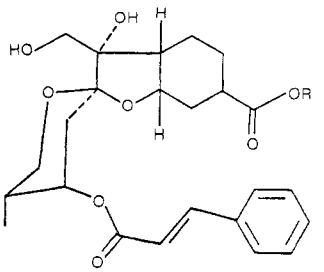
phyllanthostatins (**2**). Most importantly, phyllanthostatin 1 (**2a**, 52% → 90% life extension over the dose range 6 → 48 mg/kg) and phyllanthoside (**2b**, 12% curative level at 8 mg/kg and 62% → 90% life extension at dose levels of 4 → 16 mg/kg) have been found to markedly inhibit growth of the NCI murine B16 melanoma. Furthermore glycosides **2a** and **2b** have been shown to significantly





2a, $R^1 = R^4 = H$; $R^2 = R^3 = Ac$; $R^5 = CH_3$
2b, $R^2 = R^4 = H$; $R^1 = R^3 = Ac$; $R^5 = CH_3$
2c, $R^2 = R^4 = H$; $R^1 = R^3 = Ac$; $R^5 = CH_2OH$
2d, $R^1 = R^2 = R^3 = R^4 = Ac$; $R^5 = CH_3$
2e, $R^2 = R^3 = R^4 = H$, $R^1 = Ac$, $R^5 = CH_3$
2f, $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = CH_3$
2g, $R = CH_3$
2h, $R^1 = R^3 = R^4 = H$; $R^2 = Ac$; $R^5 = CH_3$

inhibit growth of a human myeloma cell line.[3] Also we are pleased to note that phyllanthoside is in preclinical development by the NCI Division of Cancer Treatment. Now follow details of the isolation and structure elucidation of phyllanthostatin 1 (**2a**),[8a] phyllanthoside (**2b**),[8a] and phyllanthostatins 2 (**2c**) and 3 (**3a**).[8b,8c]



3a, R as in **2b**
3b, R = CH_3

Extraction of *P. acuminatus* roots with methanol–methylene chloride (1:1), followed by addition of water and solvent partition (Scheme I)[10,11] of the methylene chloride fraction between aqueous methanol (9:1 → 3:2) with hexane → carbon tetrachloride → methylene chloride, gave PS in vivo active[11] carbon tetrachloride and methylene chloride fractions. Repeated recrystallization of insoluble material isolated during the partitioning process led to justicidin B (**1**).[12] Gel permeation chromatography (Sephadex LH-20) of the combined carbon tetrachloride and methylene chloride fractions, followed by repeated silica gel chromatography, afforded phyllanthostatin 1 (**2a**) and phyllanthoside (**2b**)[13] as amorphous solids. Further chromatographic separation of active fractions on silica gel and reverse-phase silica gel gave phyllanthostatins 2 (**2c**) and 3 (**3a**).

The phyllanthostatins and phyllanthoside proved very difficult to distinguish by thin-layer chromatographic (TLC) or infrared spectral comparison methods. High-pressure liquid chromatographic (HPLC) techniques proved best for establishing purity. Each of the phyllanthostatins and phyllanthoside were obtained as an amorphous solid that resisted crystallization. Indeed all attempts to obtain crystals suitable for X-ray crystal structure determination were unsuccessful. The designation of glycoside **2b** as phyllanthoside was based on comparison of spectral data with that recorded for the phyllanthoside isolated by Kupchan and co-workers.[13] Unfortunately, due to Professor Kupchan's untimely passing in 1976 an authentic specimen of phyllanthoside could not be obtained for an unequivocal comparison. In 1977–1978 Wall[14] and Taylor in the NCI program at Research Triangle Institute (RTI) attempted to reisolate phyllanthoside employing 19 kg of the same root collection employed by the Kupchan group. They obtained a fraction that appeared to correspond to phyllanthoside and, by analytical TLC, to be essentially one component. But by HPLC at RTI this fraction was found to contain at least five constituents. Due to the small quantity of this material, and lack of an authentic specimen, it could not be determined whether the original phyllanthoside was an analogous

(6) (a) Carmo-Pereira, J.; Costa, F. O.; Pimentel, O.; Henriques, E. *Cancer Treat. Rep.* **1980**, *64*, 143–145. (b) Nathanson, L.; Wittenberg, B. K.; *Cancer Treat. Rep.* **1980**, *64*, 133–137. (c) Green, M. R.; Dillman, R. O.; Horton, C. *Cancer Treat. Rep.* **1980**, *64*, 139–142. (d) Banzet, P.; Jacquillat, C.; Civatte, J.; Puissant, A.; Maral, J.; Chastang, C.; Israel, L.; Belaich, S.; Jourdain, J.-C.; Weil, M.; Auclerc, G. *Cancer* **1978**, *41*, 1240–1248. (e) Luce, J. K. *Cancer Treat. Rep.* **1978**, *62*, 2009. (f) Bellet, R. E.; Catalano, R. B.; Mastrangelo, M. J.; Berd, D. *Cancer Treat. Rep.* **1978**, *62*, 2095–2099.

(7) Among the better treatments employing CCNU, procarbazide, and vincristine the complete response rate is only 33% (ref. 6a). The median response lasted eight months and the median survival only twenty months.

(8) Three preliminary communications have been prepared: (a) Pettit, G. R.; Cragg, G. M.; Gust, D.; Brown, P.; Schmidt, J. M. *Can. J. Chem.* **1982**, *60*, 939–941. (b) Pettit, G. R.; Cragg, G. M.; Gust, D.; Brown, P. *Can. J. Chem.* **1982**, *60*, 544–546. (c) Pettit, G. R.; Cragg, G. M.; Niven, M. L.; Nassimbeni, L. R. *Can. J. Chem.* **1983**, *61*, 2630–2632.

(9) Schmidt, J. M.; Pettit, G. R. *Experientia* **1978**, *34*, 659–660.

(10) Leading references to our use of these techniques for initial separation of bilogically active constituents from animal and plant materials have been entered in ref 8a and are based on early studies of marine vertebrae lipid extraction with chloroform–methanol–water systems by Bligh, E. G. and Dyer, W. J. *Can. J. Biochem. Phys.* **1959**, *37*, 911.

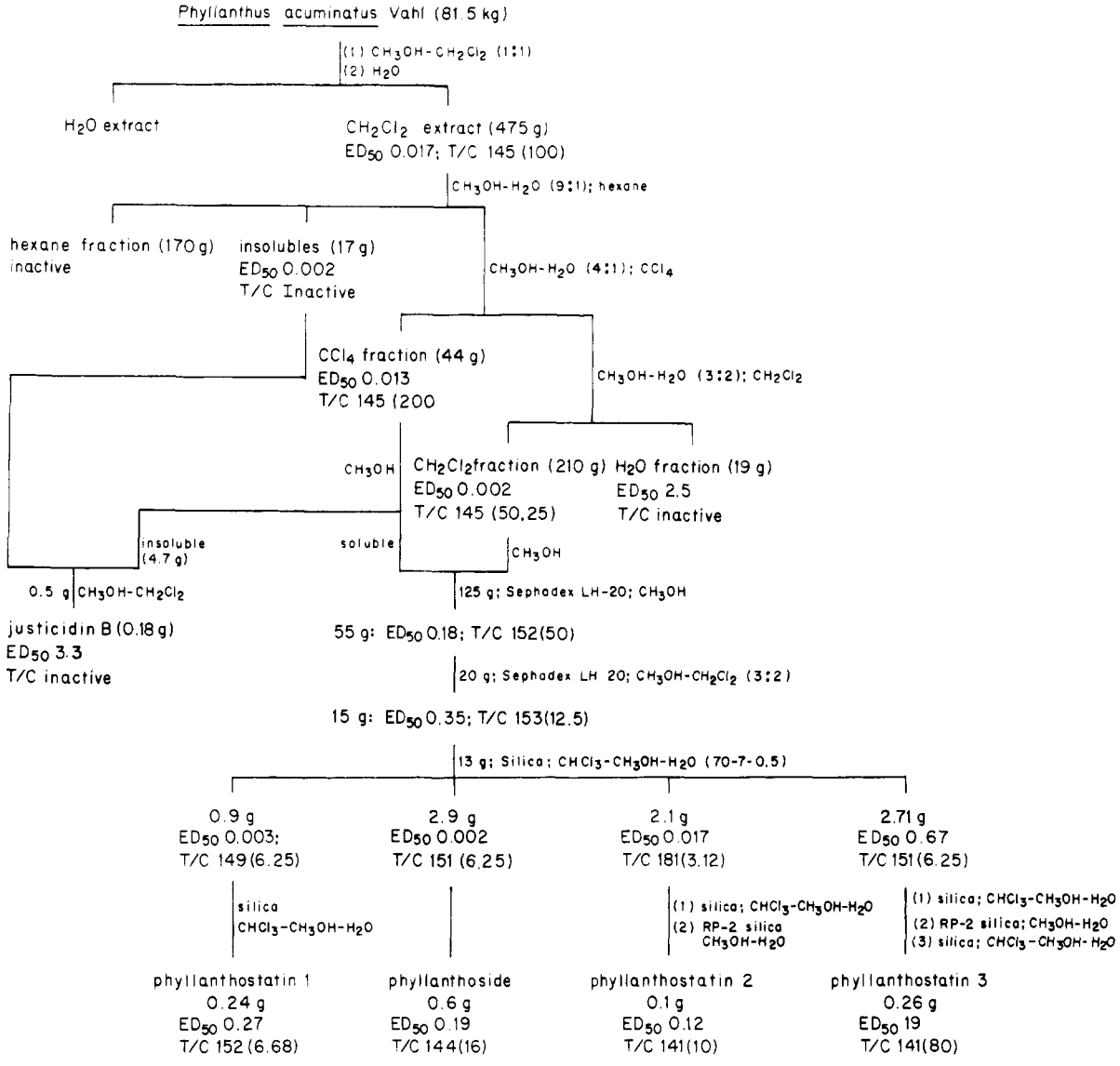
(11) The NCI in vivo and in vitro P-388 lymphocytic leukemia (PS system) bioassays were employed to guide each step of the separation.

(12) Block, E.; Stevenson, R. *J. Org. Chem.* **1971**, *36*, 3453–3455.

(13) Kupchan, S. M.; LaVoie, E. J.; Branfman, A. R.; Fei, B. Y.; Bright, W. M.; Bryan, R. F. *J. Am. Chem. Soc.* **1977**, *99*, 3199–3201.

(14) Unpublished results kindly provided by Dr. M. E. Wall.

Scheme I



mixture of very difficult to separate glycosides or a pure substance. As will become apparent, a mixture of the *Phyllanthus* glycosides containing primarily substances **2a** and **2b** would not affect results and conclusions drawn from the initial structural studies.[13] So the term phyllanthoside has been retained and used to name glycoside **2b**. Methanolysis of phyllanthoside (**2b**) by Kupchan and colleagues[13] gave the aglycone methyl ester phyllanthocin (**2g**) and a disaccharide $C_{12}H_{22}O_9$ which yielded 6-deoxy-D-glucose on acid hydrolysis. Spectral evidence established that the disaccharide portion contained two acetate groups. Therefore glycoside **2b** consisted of an aglycone (**2g**) linked via an ester linkage to a diacetylated 6-deoxy-D-glucose disaccharide. The structure of phyllanthocin was determined by X-ray crystallographic analysis, but a structure for the disaccharide unit was not determined.[13]

In the present study, efforts to establish empirical formulae and obtain structurally important fragments corresponding to the phyllanthostatins and phyllanthoside were undertaken by mass spectrometry. Although some useful fragments (Table I) were obtained by high-resolution electron-impact (HREI) mass spectrometry, molecular ions were not observed. Experiments to obtain the molecular ions by ^{254}Cf plasma desorption mass spectrometry[15] gave data that were subsequently found due to molecular association in the instrument. By both negative and

Table I. Significant HREI Mass Spectral Fragmentation Ions

compd	fragment	weight obsd	weight calcd	interpretation
2b	$C_{32}H_{39}O_{11}$	599.2417	599.2492	M - $C_8H_{13}O_6$: loss of terminal S2-6-deoxyglucose
	$C_{24}H_{28}O_7$	428.1808	428.1835	M - $C_{16}H_{25}O_{10}$ + H: loss of disaccharide
	$C_{16}H_{25}O_{10}$	377.1411	377.1448	disaccharide component
	$C_8H_{13}O_5$	189.0788	189.0763	S2-unit
	$C_8H_{12}O_4$	172.0705	172.0735	S2-unit - H_2O + H
	$C_8H_{11}O_4$	171.0668	171.0657	S2-unit - H_2O
	$C_8H_9O_3$	153.0552	153.0552	S2-unit - $2H_2O$
2a	$C_{24}H_{28}O_7$	428.1835	428.1835	M - $C_{16}H_{25}O_{10}$ + H: loss of disaccharide
	$C_8H_{12}O_4$	172.0719	172.0735	S2-unit - H_2O+H
	$C_8H_{11}O_4$	171.0659	171.0657	S2-unit - H_2O
	$C_8H_9O_3$	153.0565	153.0552	S2-unit - $2H_2O$
2c	$C_{24}H_{28}O_7$	428.1792	428.1835	M - $C_{16}H_{25}O_{11}$: loss of disaccharide
	$C_{12}H_{17}O_7$	273.1077	273.0974	disaccharide component - $2CH_3CO_2H$
	$C_8H_{11}O_4$	171.0679	171.0657	S2-unit - H_2O
	$C_8H_9O_3$	153.0550	153.0552	S2-unit - $2H_2O$

(15) Macfarlane, R. D. *Biomed. Mass Spectrom.* 1981, *8*, 449.

Table II. 400-MHz ^{1}H NMR Spectra of the Phyllanthostatin Glycosides

position no.	phyllantho-statin 1 (2a)	phyllantho-side (2b)	S3′-desacetyl-phyllanthoside (2e)	S3,S3′-didesacetyl-phyllanthoside (2f)	phyllantho-statin 2 (2c)	phyllantho-statin 3 (3a)
			Aglycon			
1	1.94–2.04	1.89–2.03	1.92–1.99	1.85–1.94	1.90–2.01	1.91–2.00
1	1.63 (m)	1.50–1.83	1.56–1.67	1.55–1.77	1.60–1.82	1.74–1.81
2,2	1.37 (m)	1.33 (m)	1.30 (m)	1.28 (m)	1.30 (m)	1.37 (m)
3	2.44 (m)	2.45–2.63	2.34–2.54	2.33–2.49	2.31–2.67	2.17–2.23
4	1.78 (m)	1.50–1.83	1.77 (m)	1.55–1.77	1.60–1.82	1.74–1.81
4	2.44 (m)	2.37 (d, 14.3)	2.34–2.54	2.33–2.49	2.31–2.67	2.17–2.23
5	4.47 (m)	4.43 (q, 3.4)	4.41 (q, 2.9)	4.39 (q, 2.3)	4.43 (q, 3.6)	4.17 (q, 4.0)
6	1.94–2.04	1.89–2.03	1.90 (m)	1.85–1.94	1.90–2.01	1.74–1.81
9	1.68 (m)	1.58–1.83	1.56–1.67	1.55–1.77	1.60–1.82	1.74–1.81
9	1.94–2.04	1.89–2.03	1.92–1.99	1.85–1.94	1.90–2.01	1.91–2.00
10	5.0 (q, 2.7)	5.12 (q, 2.3)	5.14 (q, 2.0)	5.11 (br s)	5.12 (q, 2.4)	5.14 (q, 2.4)
11	1.94–2.04	1.89–2.03	1.92–1.99	1.85–1.94	1.90–2.01	1.91–2.00
12	3.46 (m)	3.43 (m)	3.44 (m)	3.35–3.41	3.42–3.46	3.43 (m)
12	4.01 (dd, 11.5, 11.9)	3.97 (dd, 11.5, 11.9)	3.96 (dd, 11.6, 11.6)	3.95 (dd, 11.5, 11.5)	3.98 (dd, 11.6, 11.6)	4.03 (dd, 11.6, 11.6)
14	2.95, 2.97	2.92, 2.95	2.94 (br s)	2.91 (br s)	2.93, 2.95	4.01 (dd, 11.6, 4.8)
14	(ABq, 5.4)	(ABq, 5.25			(ABq, 5.2)	3.51 (dd, 11.2, 4.8)
15	0.85 (d, 6.8)	0.84 (d, 7.0)	0.85 (d, 7.0)	0.81 (d, 6.6)	0.85 (d, 6.8)	0.86 (d, 6.9)
2′	6.7 (d, 16)	6.59 (d, 16)	6.52 (d, 15.8)	6.51 (d, 16)	6.58 (d, 16)	6.56 (d, 16)
3′	7.81 (d, 16)	7.78 (d, 16)	7.77 (d, 15.8)	7.77 (d, 16)	7.78 (d, 16)	7.75 (d, 16)
ArH	7.50–7.67	7.41–7.61	7.40–7.59	7.36–7.56	7.43–7.60	7.40–7.59
			Disaccharide			
S1	5.37 (d, 8.3)	5.50 (d, 8.1)	5.53 (d, 8.0)	5.43 (d, 8.1)	5.53 (d, 8.1)	5.49 (d, 7.9)
S2	2.51 (dd, 9.4, 8.3)	3.07–3.22	3.15 (m)	2.82–2.87	3.08–3.14	3.12–3.23
S3	3.59 (dd, 9.4, 9.4)	4.90 (dd, 9.5, 9.5)	4.94 (dd, 9.3, 9.3)	3.55 (dd, 8.8, 8.8)	4.99 (dd, 9.4, 9.4)	4.91 (dd, 9.4, 9.4)
S4	4.43 (dd, 9.4, 9.4)	3.07–3.22	3.15 (m)	3.20–3.29	3.19–3.28	3.12–3.23
S5	3.46 (m)	3.43 (m)	3.44 (m)	3.35–3.41	3.42–3.46	3.41–3.46
S6	1.17 (d, 6.1)	1.28 (d, 6.1)	1.29 (d, 6.1)	1.23 (d, 5.5)	3.72 (dd, 13.6, 4.0) 3.84 (dd, 13.6, 3.1)	1.28 (d, 6.1)
$OCOCH_3$	2.20 (s)[a]	2.14 (s)	2.14 (s)		2.14 (s)	2.14 (s)
S1′	3.94 (d, 7.8)	4.00 (d, 7.8)	3.94 (d, 7.6)	4.02 (d, 7.1)	4.02 (d, 7.8)	4.03 (d, 7.8)
S2′	3.38 (dd, 9.4, 7.8)	3.26 (dd, 9.5, 8.0)	3.15 (m)	3.35–3.41	3.19–3.28	3.25 (dd, 10.6, 7.8)
S3′	4.89 (dd, 9.4, 9.4)	4.78 (dd, 9.3, 9.3)	3.04 (dd, 8.9, 8.3)	3.00–3.05	4.78 (dd, 9.4, 9.4)	4.78 (dd, 9.3, 7.8)
S4′	3.17 (dd, 9.4, 9.4)	3.07–3.22	3.33 (dd, 8.3, 7.1)	3.35–3.41	3.08–3.14	3.12–3.23
S5′	3.25 (m)	3.07–3.22	3.15 (m)	3.20–3.29	3.08–3.14	3.12–3.23
S6′	1.25 (d, 6.0)	1.23 (d, 6.0)	1.21 (d, 6.1)	1.18 (d, 5.5)	1.24 (d, 6.0)	1.24 (d, 5.9)
$OCOCH_3$	2.13 (s)[a]	2.14 (s)			2.14 (s)	2.14 (s)

[a] Assignments may be interchanged

positive ion plasma desorption glycosides **2a** and **2b** exhibited bimolecular ions at 1608 along with the monomolecular ions (804 and 827 for M + 23) later found to be correct. The latter conclusion was reached employing field desorption (FD, for glycoside **2b**) and with solution phase secondary ion mass spectrometry using sodium iodide in sulfolane.[16]

Analysis of the HREI mass spectra led to the fragment assignments noted in Table I. A similar fragmentation course was observed with phyllanthostatin 1 (**2a**). With phyllanthostatin 2 (**2c**) the fragment corresponding to $C_{12}H_{17}O_7$ (Table I) confirmed the presence of an additional oxygen atom compared to phyllanthostatin 1 (and **2b**) in the disaccharide moiety. Furthermore, the same $C_8H_{11}O_4$ fragment observed to arise from phyllanthoside and phyllanthostatin 1 indicates loss of a similar S2-carbohydrate unit and places the extra oxygen on the internal S1-carbohydrate unit.

The high-resolution (400 MHz) ^{1}H NMR spectrum (Table II) of phyllanthoside (**2b**) displayed two 6-deoxy-D-glucose ring proton resonances at δ 4.78 and 4.90 approximately 1 ppm downfield from the usual positions for such protons suggesting attachment to carbon atoms bound to ester groups.[17] Analysis of ^{1}H NMR decoupling studies showed that the two ester groups were at S-3 and S-3′ while the ^{1}H and ^{13}C NMR results (Tables II and III, respectively) revealed that the third ester linkage was at S-1 (^{13}C, 92.06; ^{1}H, 5.50 d, J = 8.1 Hz). These values correlated well with 91.3 ppm for C-1 in β-cellobiose octaacetate,[18] and 5.75 ppm (d, J = 6.7 Hz) for the anomeric proton in β-D-glucopyranose pentaacetate. In addition, the S-1′ carbon appeared at ca. 104 ppm correlating well with the value of 102.4 ppm observed for C-1′ in cellobiose.[18] When phyllanthoside was treated with refluxing methanol the resulting didesacetyl derivative **2f** exhibited S-3 and S-3′ proton resonances shifted upfield over 1 ppm. The

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(17) Hall, L. D. *Adv. Carbohydr. Chem.* **1964**, *19*, 51.

(18) Breitmaier, F.; Voelter, W. "^{13}C-NMR Spectroscopy"; Verlag Chemie: Weinheim, 1974; pp 238–239.

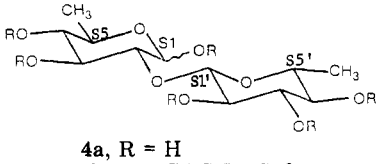
Table III. ^{13}C NMR Spectra of the Phyllanthostatin Glycosides

position no.	phyllanthostatin 1 (2a)	phyllanthoside (2b)	phyllanthoside peracetate (2d)	S3′-desacetylphyllanthoside (2e)	S3,S3′-didesacetylphyllanthoside (2f)	phyllanthocin (2g)	S3′-desacetylphyllanthostatin 1 (2h)	phyllanthostatin 2 (2c)	phyllanthostatin 3 (3a)
					Aglycon				
1	21.71	21.74	21.97	21.80	21.93	22.26	21.80	21.74	20.28
2	25.38	25.74	26.13	25.83	25.90	26.58	25.64	25.77	25.93
3	37.01	37.11	37.30	37.14	37.11	36.95	37.01	37.11	37.04
4	30.51	29.93	29.60	29.80	29.73	30.02	30.02	29.99	29.93
5	72.76	72.66	72.59	72.69	72.79	72.72	72.66	72.66	72.89
6	38.25	38.28	38.44	38.38	38.41	38.70	38.34	38.31	43.48
7	70.64	71.00	71.00	71.06	71.10	71.16	71.10	71.00	85.27
8	102.26	102.16	102.13	102.16	102.13	102.10	102.13	102.23	106.55
9	34.54	34.38	34.44	34.43	34.57	34.44	34.54	34.41	35.84
10	70.06	69.67	69.51	69.57	69.80	69.90	69.77	69.77	69.80
11	33.24	33.18	33.24	33.24	33.27	33.17	33.21	33.21	33.34
12	62.91	62.78	62.81	62.81	62.88	63.07	62.85	62.85	62.98
13	174.11	174.14	173.65	174.50	174.56	176.15	174.34	174.14	174.17
14	50.20	50.01	49.98	50.01	50.30	50.24	50.07	50.07	66.61
15	12.84	12.71	12.71	12.74	12.77	12.80	12.74	12.74	12.64
1′	167.06	166.86	166.68	166.83	167.09	166.73	166.99	166.80	166.83
2′	118.93	118.90	118.87	118.83	118.64	119.06	118.44	119.03	118.90
3′	144.73	144.73	144.77	144.86	145.19	144.51	145.09	144.64	144.70
4′	134.17	134.56	134.63	134.69	134.43	134.79	134.24	134.63	134.56
5′	129.59[a]	129.10[a]	129.10[a]	129.10[a]	129.36[a]	128.84[a]	129.46	129.14[a]	129.10[a]
6′	128.58[a]	128.32[a]	128.13[a]	128.29[a]	128.42[a]	128.06[a]	128.39	128.29[a]	128.32[a]
7′	131.05	130.74	130.50	130.34	130.60	130.05	131.02	130.44	130.31
8′	129.59[a]	129.10[a]	129.10[a]	129.10[a]	129.36[a]	128.84[a]	129.46	129.14[a]	129.10[a]
9′	128.58[a]	128.32[a]	128.13[a]	128.29[a]	128.42[a]	128.06[a]	128.39	128.29[a]	128.32[a]
OCH_3						51.21			
					Disaccharide				
S1	92.12	92.06	91.47	92.09	92.28		92.03	92.28	92.09
S2	83.38	78.47	74.90[b]	79.59	82.73		82.93	78.44	78.44
S3	78.44[b]	77.69	73.37[b]	77.92[b]	76.30[b]		76.36[b]	77.89[b]	77.79
S4	75.32[b]	77.69[b]	73.37[b]	76.23[b]	76.30[b]		75.19[b]	77.47[b]	77.79[b]
S5	74.09[b]	74.51[b]	73.05[b]	75.29[b]	75.00[b]		74.71[b]	76.33[b]	74.54[b]
S6	17.38	17.48	17.29	17.55	17.74		17.32	61.90	17.45
S1′	105.48	103.84	100.73	103.66	104.86		104.89	103.89	103.72
S2′	73.99[b]	72.95[b]	71.46[b]	74.35[b]	74.93[b]		73.89[b]	74.54[b]	72.98[b]
S3′	73.57[b]	74.28[b]	70.45[b]	73.60[b]	72.79[b]		72.27[b]	72.50[b]	74.32
S4′	72.95[b]	72.40[b]	70.03[b]	72.95[b]	72.79[b]		70.74[b]	72.07[b]	72.46[b]
S5′	71.10[b]	72.04[b]	70.03[b]	71.81[b]	72.30[b]		68.34[b]	69.28[b]	72.11[b]
S6′	17.81	17.84	17.68	17.94	17.74		17.68	17.87	17.81
CH_3COO	172.91	172.65	170.34	172.84			170.73	172.58	172.65
			170.05						
			169.88						
CH_3COO	171.15	172.55	169.59					172.43	172.58
			169.40						
CH_3COO	21.06	21.15	20.89 (2 C)	21.28			20.96	21.15	21.15
			20.66 (2 C)						
CH_3COO	20.93	21.06	20.34					21.06	21.06

[a,b] Assignments may be interchanged.

S-1 resonance (^{13}C, 92.28; ^1H, 5.43, d, J = 8.1 Hz) and those associated with the aglycone and other sugar protons remained essentially unchanged. Accordingly, the two acetate groups were assigned the S-3 and S-3′ positions and the disaccharide–aglycone linkage to S-1.

The nature of the disaccharide linkage in glycoside **2b** was determined by the following experiments. Methanolysis of glycoside **2b** gave aglycone methyl ester **2g** and a disaccharide (**4a**) designated phyllanthose.[8c] The latter



4a, R = H
b, R = $COCH_3$, $S1^{\alpha}$

product (**4a**) was converted to peracetate **4b**. The ^1H NMR spectrum of peracetate **4b** exhibited the following resonances: two methyl groups (δ 1.18 and 1.22, d, 6 Hz), six acetate methyl groups (δ 2.00–2.20 ppm), five protons on ring carbons bearing acetate groups (δ 4.55–5.6 ppm; the S-1′ anomeric proton also resonated in this region), a doublet at 6.29 ppm ($^3J_{HH}$ = 3 Hz) due to the S-1 β-anomeric proton, a three proton multiplet at δ 3.40–4.05 attributed to the protons on S-5 and S-5′, and the proton on the carbon atom involved in the disaccharide linkage. Proton decoupling experiments showed that the resonance at δ 6.29 was coupled to a doublet of doublets at δ 3.90 (*J* = 11 and 4 Hz). Therefore, the δ 3.90 signal must arise from the proton at S-2. Since a coupling constant of 7.8 Hz was observed for the S-1′ anomeric proton in phyllanthoside (**2b**) indicating a β-linkage, the disaccharide linkage was established as β-1 → 2α. Definite confirmation of the structure of phyllanthose was provided by an X-ray crystallographic analysis of its peracetate (**4b**).[8c] The structural assignment of phyllanthoside (**2b**) was thereby completed.

Comparison of the ^1H and ^{13}C NMR results (Tables II and III) for phyllanthostatin 1 (**2a**) and phyllanthoside (**2b**)

indicated differences only in the disaccharide portion. The formation of identical acetates (**2d**) and the ready interconversion of **2b** → **2a** via an acetyl shift [19] (on standing in 90% aqueous ethanol) proved that the two glycosides (**2a** and **b**) differed only in the location of one acetate group. Decoupling (^{1}H NMR) studies established the acetate linkages of phyllanthostatin 1 (**2a**) to be at S-4 and S-3′. Thus, the interconversion of phyllanthoside (**2b**) and phyllanthostatin 1 (**2a**) was found to involve an acetyl shift from S-3 to S-4 in the internal 6-deoxy-D-glucose unit. Confirmatory evidence for this assignment was provided by treatment of glycosides **2a** and **2b** with cellulase in an acetate buffer. Two different monodesacetyl derivatives (**2e** and **2h**) were produced. In each case loss of the acetyl group at S-3′ was proved by ^{1}H NMR decoupling experiments. A significant difference between the ^{13}C NMR (Table III) spectra of phyllanthostatin 1 (**2a**) and phyllanthoside (**2b**) appeared in the upfield shift of the S-2 carbon resonance position from 78.47 ppm in phyllanthoside (**2b**) to 83.38 ppm in phyllanthostatin 1 (**2a**, Table III). The shift was ascribed to transfer of the S-3 acetate in glycoside **2b** to S-4 in glycoside **2a**. A similar shift was also observed with the monodesacetyl derivative of **2a** (**2h**) and the didesacetyl derivative **2f**. By means of these careful spectral analyses the structure of phyllanthostatin 1 was unequivocally established as **2a**.

Comparison ^{1}H and ^{13}C NMR data of phyllanthostatin 2 (**2c**) and phyllanthoside (**2b**) indicated that one of the 6-deoxy-D-glucose methyl group signals in glycoside **2b** was replaced in glycoside **2c** by a signal due to a CH_2OH group. When ^{1}H NMR decoupling studies placed the CH_2OH group at position S-5 structure **2c** was assigned to phyllanthostatin 2.

Phyllanthostatin 3 (**3a**) differed significantly from phyllanthoside (**2b**) in the aglycone moiety in that the AB quartet at 2.92 and 2.95 ppm in the ^{1}H NMR spectrum of **2b** due to the epoxide methylene protons (C-14 CH_2, Table II) was replaced by double doublets at 3.51 and 4.01 ppm. Similarly, in the ^{13}C NMR spectra the signals due to C-7 and C-14 in **2b** (71.00 and 50.01 ppm respectively, Table III) were shifted to 85.27 and 66.61 ppm in **3a**. Less pronounced changes were observed in the chemical shift values of C-1, C-6, C-8, and C-9. These observations, together with mass spectral and elemental analyses data, established that the 7,14-epoxide group in **2b** was replaced in **3a** by a vicinal 7,14-diol. Methanolysis of glycoside **3a** gave the aglycone methyl ester named phyllanthocindiol methyl ester (**3b**) whose structure and stereochemistry were unambiguously established by X-ray crystallographic analysis,[8c] thereby confirming the structural assignment based on spectroscopic evidence. Since phyllanthoside (**2b**) and phyllanthostatin 3 were shown to possess identical disaccharide units, structure **3a** was assigned to phyllanthostatin 3.

The very encouraging antineoplastic properties already displayed by phyllanthostatin 1 (**2a**) and phyllanthoside (**2b**) combined with structures reasonably accessible by eventual total syntheses[20] clearly indicate that the phyllanthostatin-type glycosides represent important substances for further biological evaluation and structural modification. A substantial advance in this overall direction toward eventual clinical evaluations was accomplished by development of efficient procedures for large scale isolation of the phyllanthostatins. The very effective method devised for obtaining the phyllanthostatins in quantity has been summarized in the Experimental Section using phyllanthoside for illustration. A study of structure–activity relationships among these potentially important glycosides and a series of related substances is in progress.

(19) A study of the orthoester rearrangement in such glucose systems will be summarized in a future report: Pettit, G. R.; Cragg, G. M.; Gust, D.

(20) The first total synthesis of (±)-phyllanthocin (**2g**) has been completed. McGuirk, P. R.; Collum, D. B *J. Am. Chem. Soc.* **1982**, *104*, 4496. See also, McGuirk, P. R.; Collum, D. B. *J. Org. Chem.* **1984**, *49*, 843.

Experimental Section

All solvents were redistilled and solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Adsorption column chromatography was performed with silica gel 60 (70–230 mesh) or with prepacked silica gel 60 columns sizes A, B, and C (E. Merck, Darmstadt, Germany). Reverse-phase column chromatography was accomplished with RP-2 silanized silica gel 60 (70–230 mesh, from E. Merck, prewashed with methylene chloride and methanol) and steric exclusion chromatography with Sephadex LH-20 (particle size 25–100 μm) supplied by Pharmacia Fine Chemicals, AB, Uppsala, Sweden. Thin-layer chromatography (TLC) was performed with silica gel GHLF Uniplates (layer thickness 0.25 mm) obtained from Analtech, Inc. and pre-coated RP-2 (silanized) silica gel 60 F254 plates (layer thickness 0.25 mm) from E. Merck. Visualization of the TL chromatograms was conducted with anisaldehyde or ceric sulfate spray reagents or by exposure to ultraviolet light. Chromatographic separations were monitored (and partially automated) by using a Gilson Model HM UV-Vis Holochrome with Gilson Model FC-220K and Micro Fractionators. The purity of all products (colorless in each case) was determined by HPLC on a μ-Porasil column (30 cm × 4 mm) with methylene chloride–methanol–water (97:3.0:0.2) as eluent using a Waters Liquid Chromatograph ALC 2000 series with a Model 440 absorbance detector (λ, 254 nm).

Melting points are uncorrected and were determined on a Kofler-type hot-stage apparatus. Optical rotations were measured by using a Perkin-Elmer Model 241 Automatic Polarimeter. Ultraviolet spectra were recorded by employing a Hewlett-Packard Model 8450A UV/vis spectrophotometer and infrared spectra with a Perkin-Elmer Model 299 spectrophotometer. The ^{1}H NMR spectra (deuteriochloroform solution and tetramethylsilane internal standard) were obtained with Bruker WH-400 and Varian XL-100 spectrometers. All ^{13}C NMR spectra were measured at 22.63 MHz with a Bruker WH-90 spectrometer and are reported in ppm downfield from tetramethylsilane. Mass spectra were obtained by using Varian MAT 731 and MAT 312 spectrometers. Elemental analyses were determined by Dr. A. W. Spang of the Spang Microanalytical Laboratory, Eagle Harbor, MI.

Plant Material. Two recollections [81.5 kg (1978) and 150 kg (1980)] of *Phyllanthus acuminatus* Vahl roots were made in Costa Rica. Preliminary extractions with methanol–methylene chloride (1:1) at ambient temperature yielded crude methylene chloride extracts (475 g and 1100 g, respectively). Details of the extraction and fractionation of the 1978 recollection now follows. The large scale recollections (1982) of stem wood (1562 kg) required for preclinical development of phyllanthoside were also made in Costa Rica.

Extraction and Fractionation Procedure. The chipped roots (81 kg) were extracted with methanol–methylene chloride (1:1, 120 L)[10] at ambient temperature for 14 days (Scheme I). The methylene chloride phase which separated upon addition of water (25% by volume) was concentrated in vacuo to give 192 g of extract. The aqueous methanol phase was adjusted by addition of further methanol and methylene chloride to a ratio of 4:1.2:1.8 aqueous phase–methanol–methylene chloride and the plant material was further extracted with this mixture for 7 days. After addition of water (15% by volume) the methylene chloride phase was concentrated to give a second methylene chloride extract (283 g). The combined methylene chloride extracts (475 g) were partitioned between aqueous methanol (1:9, 3 L) and hexane (1 × 2 L and 3 × 1 L) to give a PS[11] inactive hexane soluble fraction (170 g) and insoluble material (17 g, fraction A). The aqueous methanol fraction was diluted to 1:4 with water and extracted with carbon tetrachloride (8 × 1 L) to give a PS active (refer to Scheme I) chlorocarbon soluble fraction (44 g).[11] After further dilution (water) of the aqueous methanol fraciton to 2:3 it was extracted with methylene chloride (7 × 1.5 L) to yield a PS active methylene chloride soluble fraction (210 g).

Treatment of the active carbon tetrachloride soluble fraction (44 g) with methanol (100 mL) led to insoluble material (4.7 g, fraction B) and a soluble fraction which was combined with a solution of the active methylene chloride soluble fraction (210 g) in methanol (700 mL). Half of this solution was applied to a column of Sephadex LH-20 (2 kg, 105 × 10 cm). Elution with methanol gave the PS active components (55 g) between volumes 5100–6100 mL. Two 10-g portions of the active fraction were separately chromatographed on Sephadex LH-20 (700 g, 220 × 4 cm) by using methanol–methylene chloride (3:2) as eluent. Combined volumes 1450–1700 mL contained the next PS active fraction (7.5 g). Careful chromatographic separation of this fraction (13 g, after repeating the procedure) on silica gel-60 (825 g, 84 × 5 cm) using chloroform–methanol–water (70:8:0.5) as eluent afforded the following active fractions (weight, elution volumes): fraction C (0.90 g, 1–1320 mL); fraction D (2.9 g, 1321–1975 mL); fraction E (2.1 g, 2200–2880 mL); fraction F (2.71 g, 2881–4500 mL).

Isolation of Justicidin B (1). The insoluble fraction (fraction A, 17 g) from the hexane–aqueous methanol solvent partition step was treated with hexane–methylene chloride–methanol (1:2:1, 100 mL) at room temperature for 24 h. The insoluble portion (10.9 g) was further treated with methanol–methylene chloride (1:5, 200 mL) for 72 h and collected by filtration. Concentration of the filtrate gave a light brown powder (5.8 g). A portion of the solid (500 mg) was repeatedly recrystallized from methanol–methylene chloride to yield justicidin B (1, PS ED_{50} 3.3 μg/mL, 0.185 g): mp 263–265 °C (lit.[12] mp 237–238 °C from acetone–ether); EI mass spectrum, *m/e* 364 (M^+); IR $\nu_{max}^{CHCl_3}$ 1760, 1625, 1600, 1505, 1482, 1438, 1390, 1340, 1260, 1160, 1043, 1010, 938 and 880 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 3.8 (3 H, s), 4.03 (3 H, s), 5.35 (2 H, s), 6.04 (2 H, d, *J* = 5 Hz), 6.77 (1 H, d, *J* = 7 Hz), 6.82 (1 H, s), 6.94 (1 H, d, *J* = 7 Hz), 7.07 (1 H, s), 7.14 (1 H, s), 7.66 (1 H, s) ppm. The spectroscopic data corresponds to that reported for justicidin B (1).[12]

Similar treatment of the methanol insoluble portion of the carbon tetrachloride extract (fraction B) again gave justicidin B (1, 0.63 g from 2 g).

Isolation of Phyllanthostatin 1 (2a). Fraction C (0.8 g) was chromatographed on silica gel-60 (3 Lobar-B columns in series) using chloroform–methanol–water (98:2.0:0.1) as mobile phase. Elution between volumes 1530–2000 mL gave phyllanthostatin 1 (0.24 g, **2a**) as an amorphous solid: mp 125–126 °C; FD mass spectrum, *m/e* 805 $[M + H]^+$; $[\alpha]^{26}_D$ −3.6° (*c* 0.83, $CHCl_3$); λ_{max}^{MeOH} (log ε) 216 (4.19), 222 (4.12) and 277 (4.29) nm; IR (KBr) ν_{max} 3450, 1755, 1740, 1710, 1640, 1452, 1380, 1310, 1245, 1170, 1075, and 770 cm^{-1}; ^1H and ^{13}C NMR (Tables II and III, respectively).

Anal. Calcd for $C_{40}H_{52}O_{17}$: C, 59.70; H, 6.47. Found: C, 59.40; H, 6.55.

Phyllanthostatin I (**2a**) and the related glycosides described in the sequel were obtained pure by this general procedure. Yet all attempts to uncover a suitable method for crystallization were unsuccessful.

Isolation of Phyllanthoside (2b). Careful chromatographic separation of fraction D (2.9 g) on silica gel-60 (Lobar C column) was performed by using a solvent gradient from chloroform (600 mL) to 1% (500 mL), 2% (500 mL), and 3% methanol–chloroform (350 mL). Elution with 3% methanol–chloroform between volumes 350–620 mL gave a fraction (2.49 g) which was further chromatographed on silica gel-60 (3 Lobar B columns in series) using methylene chloride–methanol–water (97:3.0:0.1). Elution volumes 990–1340 mL provided phyllanthoside (0.60 g; **2b**) as an amorphous solid: mp 125–127 °C (lit.[13] 125–126 °C); solution-phase SIMS mass spectrum,[16] *m/e* 827 $[M + Na]^+$; FD mass spectrum, *m/e* 805 $[M + H]^+$; $[\alpha]^{22}_D$ +16.9° (*c* 0.71, $CHCl_3$) (lit.[13] $[\alpha]^{24}_D$ +19.2°); λ_{max}^{MeOH} (log ε), 216 (4.25), 222 (4.19), 277 (4.34) nm; IR (KBr) ν_{max} 3475, 1750, 1735, 1710, 1640, 1452, 1380, 1311, 1253, 1173, 1080, and 770 cm^{-1}; ^1H and ^{13}C NMR (see Tables II and III).

Anal. Calcd for $C_{40}H_{52}O_{17}$: C, 59.70; H, 6.47. Found: C, 59.35; H, 6.54.

Isolation of Phyllanthostatin 2 (2c). The method used to obtain glycoside **2b** was modified for fraction E (2.1 g) as follows. Chromatographic separation on silica gel-60 (Lobar C column) employing chloroform (500 mL) to 1% (500 mL), 2% (500 mL), 3% (800 mL), and 4% methanol–chloroform (730 mL), followed by elution with 4% methanol–chloroform, led to a 1.43-g fraction from volumes 730–3430 mL. Further chromatography of this fraction (1.43 g) on RP-2 silica gel-60 (100 g) using gradient elution from 70% (800 mL) to 20% aqueous methanol (800 mL) gave (volumes 750–830 mL) phyllanthostatin 2 (0.10 g, **2c**) as an amorphous solid: mp 134–136 °C; FD mass spectrum, *m/e* 821 $[M + H]^+$; $[\alpha]^{24}_D$ + 9.33° (*c* 0.75, $CHCl_3$); λ_{max}^{MeOH} (log ε) 216 (4.28), 222 (4.21), 278 (4.38) nm; IR (KBr) ν_{max} 3470, 1750, 1730, 1710, 1640, 1452, 1380, 1311, 1257, 1171, 1075 and 770 cm^{-1}; ^1H and ^{13}C NMR (refer to Tables II and III).

Anal. Calcd. for $C_{40}H_{52}O_{18}\cdot 2H_2O$: C, 56.07; H, 6.54. Found: C, 56.20; H, 6.19.

Isolation of Phyllanthostatin 3 (3a). Fraction F (2.69 g) was chromatographed on silica gel-60 (Lobar C column). Development of the column with chloroform (500 mL), 1% (500 mL), 2% (500 mL), 3% (500 mL), and 4% methanol–chloroform (650 mL), followed by elution with 4% methanol–chloroform between volumes 650–780 mL, gave an 0.88-g fraction. Further chromatography on RP-2 silica gel-60 (180 g) was performed by using 60% aqueous methanol (100 mL), followed by 60% (900 mL) to 20% aqueous methanol (900 mL) gradient elution. Elution between volumes 540–900 mL gave a fraction (0.5 g) containing a major component. Chromatography of the mixture on silica gel-60 (3 Lobar A columns) and development with chloroform (150 mL), 1% (150 mL), 2% (150 mL), and 3% methanol-chloroform (120 mL), followed by elution with 3% methanol–chloroform between volumes 120–160 mL, gave phyllanthostatin 3 (0.26 g, **3a**) as an amorphous solid: mp 126–130 °C; FD mass spectrum, *m/e* 823 $[M + H]^+$; $[\alpha]^{24}_D$ +15.7° (*c* 0.76, $CHCl_3$); λ_{max}^{MeOH} (log ε) 205 (4.19), 216 (4.25), 222 (4.18), 278 (4.35) nm; IR (KBr) ν_{max} 3450, 1755, 1730, 1700, 1640, 1455, 1380, 1311, 1258, 1175, 1070, 772 cm^{-1}; ^1H and ^{13}C NMR (Tables I and II).

Anal. Calcd for $C_{40}H_{54}O_{18}$: C, 58.39; H, 6.57. Found: C, 58.73; H, 6.60.

Acetylation of Phyllanthostatin 1 (2a) and Phyllanthoside (2b): S-3,4,2′,3′,4′-Pentaacetylphyllanthoside (2d). Phyllanthoside (**2b**, 80 mg) was treated with pyidine (2 mL)–acetic anhydride (2 mL) and the mixture allowed to stand at 3 °C for 48 h. Excess water was added and the flocculent precipitate was collected by centrifuging. After washing (3×) with water, methylene chloride was added, the solution dried, and solvent evaporated to a glassy residue (82 mg). Chromatography on silica gel-60 (Lobar A column) and elution with 1% methanol–methylene chloride gave the pentaacetate (55 mg, **2d**) as an amorphous solid: mp 122–126 °C (lit.[13] 114–117 °C); EI mass spectrum, *m/e* 930 (M^+); $[\alpha]^{30}_D$ + 31.8° (*c* 1.13, $CHCl_3$) (lit.[13] $[\alpha]^{24}_D$ +26.3°); IR (KBr) ν_{max} 1756, 1707, 1636, 1450, 1376, 1246, 1216, 1174, 1124, 1074, 1052, 1034, 950, 905, 767 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 0.85 (3 H, d, *J* = 7 Hz), 1.14 (3 H, d, *J* = 6 Hz), 1.18 (3 H, d, *J* = 6 Hz), 1.98, 2.04, 2.09 (15 H, s), 2.95 (2 H, br s), 5.49 (1 H, d, *J* = 8 Hz), 6.52 (1 H, d, *J* = 16 Hz), 7.36–7.64 (5 H, m), 7.78 (1 H, d, *J* = 16 Hz) ppm; ^{13}C NMR (see Table III). Analogous acetylation of phyllanthostatin 1 (40 mg) gave a pentaacetate (**2d**, 26 mg) which was identical (by HPLC and comparison of infrared spectra) with the pentaacetate formed from phyllanthoside.

Enzymatic Hydrolysis of Phyllanthoside (2b): S3′-Monodesacetylphyllanthoside (2e). Phyllanthoside (**2b**, 0.25 g, 0.31 mmol) was stirred with cellulase (Sigma Chemical Co., 0.37 g, 535 units) in acetate buffer (pH 5.0, 230 mL) for 144 h at room temperature. Extraction with methylene chloride (3 × 50 mL), drying (Na_2SO_4), and evaporation of solvent in vacuo gave a product (0.27 g) which was chromatographed on silica gel-60 (Lobar B column) by using methylene chloride–methanol–water (93:7:0.2) as eluent. Phyllanthoside (10 mg) was recovered from fractions corresponding to volumes 140–150 mL. Further elution and collection of fractions between 280–360 mL gave S3′-monodesacetylphyllanthoside (0.133 g, **2e**) as an amorphous solid: mp 130–135 °C; solution phase SIMS mass spectrum, *m/e* 785 $[M + Na]^+$; $[\alpha]^{26}_D$ +21.98° (*c* 0.91, $CHCl_3$); IR (KBr) ν_{max} 3470, 1750, 1712, 1640, 1452, 1383, 1310, 1255, 1206, 1172, 1122, 1075, 1011, 990, 903, 770, 711, 685 cm^{-1}; ^1H and ^{13}C NMR (Tables II and III).

Anal. Calcd for $C_{38}H_{50}O_{16}\cdot{}^1/_2\,H_2O$: C, 59.14; H, 6.61. Found: C, 59.04; H, 6.57.

Treatment of Phyllanthoside (2b) with Refluxing Methanol: S3,S3′-Didesacetylphyllanthoside (2f). Phyllanthoside (0.20 g) in methanol (20 mL) was heated at reflux for 15 h.

Evaporation of solvent in vacuo gave a 0.198 g residue which was chromatographed on silica gel-60 (Lobar A column) by using methylene chloride–methanol–water (90:10:0.2). Fractions corresponding to volumes 40–60 mL afforded S3,S3′-didesacetylphyllanthoside (0.193 g, **2f**) as an amorphous solid: mp 133–135 °C; solution-phase SIMS mass spectrum, m/e 743 $[M + Na]^+$; $[\alpha]^{26}_D$ +16.28° (c 0.86, $CHCl_3$); IR (KBr) ν_{max} 3410, 1753, 1711, 1640, 1452, 1310, 1280, 1208, 1170, 1123, 1073, 1012, 990, 950, 908, 771, 732 cm^{-1}; ^1H and ^{13}C NMR (Tables II and III).

Anal. Calcd for $C_{36}H_{48}O_{15}\cdot H_2O$: C, 58.54; H, 6.77. Found: C, 58.86; H, 6.62.

Attempted preparation of glycoside **2f** on a larger scale (e.g., 1 g) using only methanol was unsatisfactory. However, the scale-up desacetylation reaction was found to proceed readily by treatment of phyllanthoside with sodium carbonate (0.25 equiv) in methanol at room temperature for 30 min.

Methanolysis of Phyllanthoside (2b): Phyllanthocin (2g) and 2-*O*-(6-Deoxy-β-D-glucopyranosyl)-6-deoxy-α-D-glucopyranoside (Phyllanthose 4a). Phyllanthoside (**2b**, 0.31 g, 0.39 mmol) was stirred at room temperature with 0.1 M sodium methoxide in methanol (15 mL) for 30 min. The mixture was neutralized by the addition (dropwise) of 1 N hydrochloric acid, concentrated in vacuo (to a small volume), and partitioned between water (20 mL) and methylene chloride (3 × 20 mL). After evaporation of the chloroform phase the residue (0.18 g) was chromatographed on silica gel-60 (Lobar A column). Elution with methylene chloride gave phyllanthocin (0.161 g; **2g**). Recrystallization from ether–hexane yielded a pure crystalline specimen: mp 120–121 °C (lit.[13] 125–126 °C); $[\alpha]^{33}_D$ +23.81° (c 1.26, $CHCl_3$) (lit.[13] $[\alpha]^{24}_D$ +25.2°); IR (KBr) ν_{max} 1728, 1708, 1642, 1628, 1497, 1450, 1435, 1385, 1362, 1340, 1325, 1308, 1297, 1278, 1252, 1204, 1170, 1145, 1121, 1072, 1058, 1021, 991, 981, 950, 902, 870, 842, 815, 770, 710, 690, 680, 620 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 0.88 (3 H, d, J. = 7 Hz), 2.91 (1 H, d, J = 5 Hz), 3.00 (1 H, d, J = 5 Hz), 3.30 (3 H, s), 3.47 (1 H, dd, J = 11 and 5 Hz), 4.05 (1 H, t, J = 11 Hz), 4.44 (1 H, m), 5.13 (1 H, m), 6.54 (1 H, d, J = 16 Hz), 7.4–7.7 (5 H, m), 7.82 (1 H, d, J = 16 Hz); ^{13}C NMR (Table III).

The aqueous phase was treated with freshly washed Amberlite MB-3 resin until free of chloride ion. The resin was removed and the aqueous filtrate was lyophilized to provide 2-*O*-(6-deoxy-β-D-glucopyranosyl)-6-deoxy-α-D-glucopyranoside (84 mg, **4a**) as a powder: mp 218–220 °C (lit.[13] 203–204 °C); solution-phase SIMS mass spectrum, m/e 333 $[M + Na]^+$; $[\alpha]^{26}_D$ −3.3° (c 1.51, H_2O) (lit.[13] −3.5°); IR (KBr) ν_{max} 3381, 1445, 1380, 1357, 1248, 1180, 1150, 1125, 1062, 1010, 986, 935, 922, 896, 836, 772 cm^{-1}; ^1H NMR (100 MHz, D_2O) δ 1.27, 1.31 (3 H each, d, J = 6 Hz, 6- and 6′-CH_3), 3.06–4.12 (9 H, m), 4.67 (d, J = 8 Hz), 5.4 (d, J = 4 Hz); ^{13}C NMR (D_2O) δ 104.86 and 103.95 (Cl′), 95.67 (β-Cl), 92.74 (α-Cl), 83.74 (C2), 81.92 (C2), 76.43, 76.17, 76.01, 75.78, 75.00, 74.68, 73.12, 72.95, 72.86, 72.63, 68.14, 17.81 (6- and 6′-CH_3).

Anal. Calcd for $C_{12}H_{22}O_9$: C, 46.45; H, 7.10. Found: C, 45.83; H, 7.13.

Acetylation of the above dissacharide (65 mg) with acetic anhydride (2 mL)–pyridine (2 mL) at room temperature for 48 h, followed by addition of excess water, gave a colorless solid which was collected and washed with water. Recrystallization from acetone–hexane gave 2-*O*-(6-deoxy-β-D-glucopyranosyl)-6-deoxy-α-D-glucopyranose hexaacetate (**4b**): mp 224–226 °C; EI mass spectrum, m/e 562 (M^+), 561 ($M^+ - H$), 503 ($M^+ - O_2CCH_3$); solution-phase SIMS mass spectrum, m/e 585 $[M + Na]^+$; $[\alpha]^{26}_D$ + 71.10 (c 0.83, $CHCl_3$); IR (KBr) ν_{max} 1755, 1435, 1380, 1250, 1222, 1176, 1128, 1067, 1031, 1010, 944, 922, 909, 890 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 1.18 and 1.22 (3 H each, d, J = 6 Hz, 6- and 6′-CH_3), 2.00, 2.02, 2.07, 2.09 (15 H, all s, 5 O_2CCH_3), 2.18 (3 H, s, O_2CCH_3), 3.55 and 3.93 (1 H each, m, 5- and 5′-H), 3.90 (1 H, dd, J = 11 and 4 Hz, 2β-H), 4.55–5.60 (6 H, m, 1′α-H and 5 CHO_2CCH_3), 6.29 (1 H, d, J = 3 Hz, 1β-H); ^{13}C NMR δ 170.37, 169.85 (2 C), 169.53 (2 C), 169.14 (6 × CH_3CO_2), 100.99 (Cl′), 90.47 (Cl), 75.62, 73.54, 73.18, 72.82, 71.62 (2 C), 70.22, 67.30, 20.89 (2 C), 20.83, 20.63 (2 C), 20.41 (6 × CH_3CO_2) and 17.35, 17.22 (6- and 6′-CH_3).

Anal. Calcd for $C_{24}H_{34}O_{15}$: C, 51.24; H, 6.05. Found: C, 50.84; H, 5.96.

Enzymatic Hydrolysis of Phyllanthostatin 1: S3′-Monodesacetylphyllanthostatin 1 (2h). Phyllanthostatin 1 (**2a**, 82 mg, 0.1 mmol) in acetate buffer (pH 5.0, 80 mL) was treated with cellulase (Sigma Chemical Co., 0.10 g, 150 units) as described above with phyllanthoside (**2b**). The product (47 mg) was chromatographed on silica gel-60 (Lobar A column) by using methylene chloride–methanol–water (93:7:0.2). The fractions eluted between volumes 85–100 mL yielded S3′-monodesacetylphyllanthostatin 1 (21 mg, **2h**) as an amorphous solid: mp 127–132 °C; solution-phase SIMS mass spectrum, m/e 785 $[M + Na]^+$, m/e 763 $[M + H]^+$; $[\alpha]^{26}_D$ +20.83° (c 0.72, $CHCl_3$); IR (KBr) ν_{max} 3440, 1750, 1710, 1640, 1452, 1377, 1310, 1280, 1235, 1170, 1075, 1020, 992, 950, 907, 771 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 0.85 (3 H, d, J = 7 Hz), 1.16 (3 H, d, J = 6 Hz), 1.23 (3 H, d, J = 5.5 Hz), 2.11 (3 H, s), 2.96 (2 H, br s), 4.04 (1 H, d, J = 7 Hz), 4.48 (1 H, br s), 4.57 (1 H, t, J = 10 Hz), 5.15 (1 H, br s), 5.44 (1 H, d, J = 8 Hz), 6.54 (1 H, d, J = 16 Hz), 7.40–7.70 (m), 7.82 (1 H, d, J = 16 Hz); ^{13}C NMR (Table III).

Anal. Calcd for $C_{38}H_{50}O_{16}$: C, 59.84; H, 6.56. Found: C, 59.87; H 6.63.

Methanolysis of Phyllanthostatin 3: Phyllanthocindiol Methyl Ester (3b) and Phyllanthose (4a). Treatment of phyllanthostatin 3 (**3a**, 402 mg, 0.49 mmol) with 0.1 M sodium methoxide (10 mL) as described for phyllanthoside, followed by the same isolation procedure, gave (after crystallization from acetone–hexane) phyllanthocindiol methyl ester (150 mg): mp 127–128 °C; $[\alpha]^{25}_D$ +2.5° (c 1.63, $CHCl_3$), λ_{max}^{MeOH} (log ε) 216 (4.24), 221 (4.15), 279 (4.32) nm; IR (KBr) ν_{max} 3550, 3460, 2950, 2880, 1726, 1700, 1625, 1577, 1491, 1437, 1356, 1297, 1277, 1253, 1202, 1110, 1050, 1020, 990, 930, 902, 870, 840, 813, 770, 682, 660, 615 cm^{-1}; ^1H NMR (100 MHz, $CDCl_3$) δ 0.92 (3 H, d, J = 7 Hz), 3.28 (3 H, s), 3.3–3.8 (2 H, m), 3.9–4.3 (3 H, m), 5.17 (1 H, q, J = 2.5 Hz), 6.54 (1 H, d, J = 16 Hz), 7.3–7.7 (5 H, m), 7.81 (1 H, d, J = 16 Hz); ^{13}C NMR (22.63 MHz, $CDCl_3$) δ 176.25, 166.70, 144.51, 134.76, 130.08, 128.84 (2 C), 128.06 (2 C), 119, 106.75, 85.36, 72.95, 69.99, 66.78, 63.27, 51.18, 43.90, 36.88, 35.91, 33.34, 30.03, 26.78, 20.89, 12.71.

Anal. Calcd for $C_{25}H_{32}O_8$: C, 65.22; H, 6.96. Found: C, 65.43; H, 6.93.

Treatment of the aqueous phase as before gave phyllanthose (83 mg), mp 225–229 °C, shown to be identical with the product obtained from methanolysis of phyllanthoside by TLC and IR (KBr) comparisons.

Large Scale Isolation of Phyllanthoside.[21] The chipped stems (1562 kg) of *P. acuminatus* were extracted with methanol–methylene chloride (1:1, 6450 L) by downward percolation over a three-day period. A second extraction was performed in a similar manner using recovered solvent (3330 L). The extracts were treated separately with water (25% by volume) and the combined methylene chloride phases concentrated to 440 L. The concentrate was partitioned in two portions between methanol-water (9:1, 330 L) and hexane (1 × 220 L and 2 × 112 L) to give a hexane soluble fraction (ca. 9 kg in 360 L) and insoluble material (85 g). The methanol–water soluble fraction was concentrated to yield 12.58 kg of a dark tarry residue. Treatment of this residue with acetone–hexane (1:1, 1 × 180 L and 3 × 36 L) gave an upper layer of soluble and a lower layer of insoluble material (3.4 kg) which was separated by decantation and filtration. Concentration of the acetone–hexane soluble fraction under reduced pressure (at 40 °C) gave 6.5 kg of a light colored resin. The resin was chromatographed in three equal amounts on silica gel (Davisil 633 chromatographic grade 200–400 mesh, 67 kg, 305 × 15.2 cm) by using a step-wise gradient from 0–10% methanol in methylene chloride. A flow rate of 60–70 L per hour at a pressure of 100–150 psi was maintained. Development with methylene chloride (28 L), 1% (140 L) and 2% methanol–methylene chloride (260 L), followed by elution with the 2% solvent mixture between volumes 260–540 L, gave fractions enriched in phyllanthoside (1.66 kg). The phyllanthoside fraction was further chromatographed on fresh Davisil 633 silica gel (67 kg, 305 × 15.2 cm). Development was conducted by using a step-wise gradient of acetone in hexane from 25% (40 L) through 26% (200 L), 30% (300 L), and 40% (340 L). Elution with the 40% mixture between volumes 340 and 480 L gave phyllanthoside

(21) For assistance with these scale-up procedures we are pleased to thank Drs. B. D. Halpern and Tara and Messrs. G. Forsyth, B. Epling, and R. Parry.

(345 g). The final purification step involved preparative HPLC using a Waters Prep Pak/500 unit. The phyllanthoside (22 injections of approximately 17 g per 100 mL of methylene chloride) was applied to a system containing two precolumns (30.5 × 5.1 and 61 × 5.1 cm) dry packed with Davisil 633 silica gel and two Waters μ-Porasil cartridges. Elution with methylene chloride–methanol–water (97.7:2.1:0.2) and analysis of fractions by analytical HPLC gave phyllanthoside (214 g, 98% purity). Treatment of this material in 600 mL of HPLC grade methylene chloride with carbon black (Darco G-60, 10 g) followed by filtration through prewashed Celite 545, gave analytically pure phyllanthoside (191.4 g). A further 24.2 g of pure compound was isolated by using similar chromatographic procedures.

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Registry No. 1, 17951-19-8; **2a**, 82209-93-6; **2b**, 63166-73-4; **2c**, 81558-18-1; **2d**, 62955-96-8; **2e**, 91860-53-6; **2f**, 86826-02-0; **2g**, 62948-37-2; **2h**, 86825-99-2; **3a**, 81558-17-0; **3b**, 87925-07-3; **4a**, 86826-01-9; **4b**, 86826-00-8.

Absolute Stereochemistry and Circular Dichroic Properties of Chiral Triptycenes with an Anthracene Chromophore: Application of the CD Exciton Chirality Method to Nondegenerate Systems

Nobuyuki Harada,* Yasufumi Tamai, and Hisashi Uda

Chemical Research Institute of Nonaqueous Solutions, Tohoku University, 2-1-1 Katahira, Sendai 980, Japan

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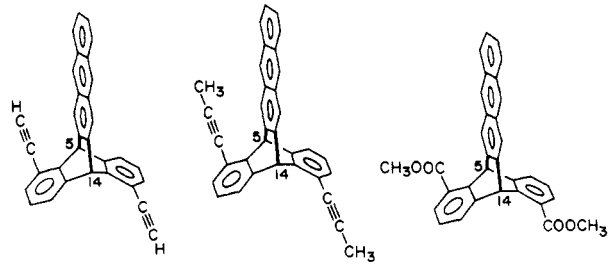
Chiral triptycene compounds with an anthracene chromophore, (5*S*,14*S*)-(+)-1,17-diethynyl-5,14-dihydro-5,14[1′,2′]benzenopentacene (**1**), (5*S*,14*S*)-(+)-5,14-dihydro-1,17-di-1-propynyl-5,14[1′,2′]benzenopentacene (**2**), and (5*S*,14*S*)-(+)-dimethyl 5,14-dihydro-5,14[1′,2′]benzenopentacene-1,17-dicarboxylate (**3**), were synthesized starting from (9*S*,10*S*)-(−)-dimethyl 9,10-dihydro-9,10-ethenoanthracene-1,5-dicarboxylate (**4**) of known absolute configuration, and their absolute configurations were established on the basis of the chemical correlation. The absolute configurations of the chiral triptycenes were also determined by application of the CD exciton chirality method. The CD spectrum of (+)-**1** shows intense positive first and negative second Cotton effects, λ_{ext} 266.4 nm, $\Delta\epsilon$ +203.9, and 228.5 nm, $\Delta\epsilon$ −90.7, which are due to the exciton interaction between the ^1B_b transition of anthracene chromophore and the ^1L_a transitions of two ethynylbenzene chromophores. The positive sign of the first Cotton effect leads to right-handed screw relationships among long axes of the three chromophores. The 5*S*,14*S* absolute configuration of (+)-**1** was thus determined in a nonempirical manner. Similar treatments hold for the case of compounds (+)-**2** and (+)-**3**, establishing their absolute configurations on the basis of a chiral exciton coupling mechanism.

The CD exciton chirality method, a valuable chiroptical tool for determining absolute stereochemistry on the basis of a chiral exciton coupling mechanism, has been extensively applied to various natural and synthetic organic compounds.[1] Recently we applied[2,3] the CD exciton chirality method to synthetic chiral triptycene compounds with three interacting chromophores and achieved chiroptical determination of their absolute configurations in a nonempirical way. In those cases, although the three interacting chromophores were not necessarily identical in structure, the wavelengths of UV absorption maximum of the three chromophores were almost similar to one another; therefore, the compounds were regarded as near-



(5S,14S)-(+)-1 (5S,14S)-(+)-2 (5S,14S)-(+)-3

degenerate triple systems. On the other hand, there are many cases of nondegenerate systems in which component

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