COMMUNICATION

Structure of the principal antineoplastic glycosides of *Phyllanthus acuminatus* Vahl[1]

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A series of degradative and X-ray crystallographic studies has led to unequivocal evidence for the structure (**3***a*) assigned to the unique disaccharide phyllanthose. In turn, the X-ray crystal structure of phyllanthose confirms structures previously assigned to the potentially important antineoplastic plant glycosides phyllanthostatins 1 and 2 (**1***a*, *c*) and phyllanthoside (**1***b*). The chemical degradation and crystallographic analyses allowed assignment of structure **2***a* to phyllanthostatin 3.

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Une série d'études impliquant des dégradations et des diffractions de rayons-X a permis d'établir la structure du disaccharide phyllanthose (**3***a*). Cette démonstration de la structure du phyllanthose permet de confirmer les structures attribuées antérieurement aux glycosides phyllanthostatines 1 et 2 (**1***a* et **1***c*) et phyllanthoside (**1***b*), des produits antinéoplastiques potentiellement importants extraits de plantes. Les dégradations chimiques et les analyses cristallographiques permettent d'attribuer la structure **2***a* à la phyllanthostatine 3.

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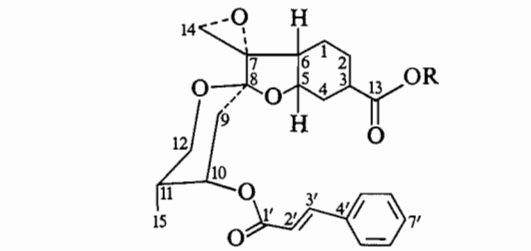
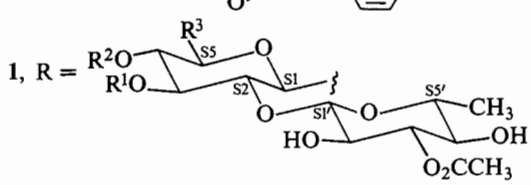
While only a relatively small number (<5%) of higher plants have received even a superficial examination for anticancer constituents, a series of such promising candidates bearing novel structures is beginning to reach clinical trial in the U.S. National Cancer Institutes (NCI) programs. Several are already established drugs for human cancer treatment (1). Analogous chemical and medical progress continues to be made with lower plant biosynthetic products for cancer chemotherapy. New advances in the chemistry of both higher (2) and lower (3) plant antineoplastic components combined with increasing successes in the total syntheses (4, 5) of these very important substances provide an instructive overview.

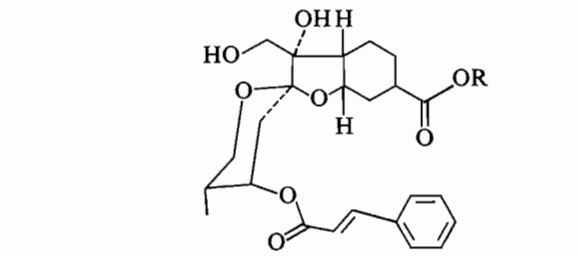
Recently we summarized the isolation and antineoplastic activity of phyllanthostatins 1–3 (**1***a*, *c*, **2***a*) and phyllanthoside (**1***b*) from roots of the Central American tree *Phyllanthus acuminatus* Vahl (2*a*, *b*). Glycosides **1***a* and **1***b* are two of the higher plant constituents presently being considered by the NCI for eventual clinical trial. The structural assignments for these principal *P. acuminatus* glycosides were based upon detailed interpretation of their high resolution (400 MHz) ^{1}H nmr, ^{13}C nmr, and mass spectra. Assignment (2*a*) of the aglycone unit of glycosides **1***a*–*c* resided upon an earlier X-ray crystallographic study of phyllanthocin (**1***d*) (6), but the unique 1 → 2 linked disaccharide (**3***a*) herein named phyllanthose, as well as definition of the phyllanthostatin 3 (**2***a*) stereochemistry at position C-7, could not be established with absolute certainty. We now report that further chemical degradation, and X-ray crystallographic studies, have culminated in unequivocal





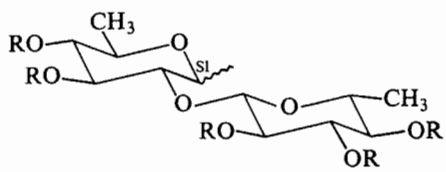
1, R = R^2O, R^1O, R^3

a, R^1 = H; R^2 = $COCH_3$; R^3 = CH_3
b, R^1 = $COCH_3$; R^2 = H; R^3 = CH_3
c, R^1 = $COCH_3$; R^2 = H; R^3 = CH_2OH
d, R = CH_3



2*a*, R = CH_3CO_2

2*b*, R = CH_3



3*a*, = H
b, = $COCH_3$, Slα

[1]Component 94 of Antineoplastic Agents; for the preceding part, see ref. 8.

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evidence for the structure (**3***a*) previously assigned phyllanthose and thereby for the very important glycosides **1***a*–*c*. We also report completion of the phyllanthostatin 3 (**2***a*) assignments by determining the crystal structure of an aglycone segment termed phyllanthocindiol methyl ester (**2***b*).

Selective methanolysis (0.1 *N* sodium methoxide in methanol, 30 min, room temperature) of phyllanthostatin 3 (**2***a*) afforded the aglycone methyl ester **2***b*: crystals from acetone–hexane, mp 127–128°C; $[\alpha]_D^{25}$ +2.45° (*c* 1.63, chloroform).[4] The neutralized (1 *N* hydrochloric acid) aqueous fraction from isolation of ester **2***b* was treated with Amberlite MB-3 and lyophilized to yield phyllanthose (**3***a*) (6); mp 225–229°C; $[\alpha]_D^{26}$ −3.3°; FAB ms *m/e*: 333 $(M + Na)^+$.[4] Phyllanthose (**3***a*) was found identical (ir in KBr, tlc) with the disaccharide (**3***a*) obtained by methanolysis of phyllanthostatin 1 (**1***a*) and phyllanthoside (**1***b*). Peracetylation (acetic anhydride – pyridine, 1:1, room temperature, 2 days) of phyllanthose yielded 2-*O*-(6-deoxy-β-D-glucopyranosyl)-6-deoxy-α-D-glucopyranose hexaacetate (**3***b*) as crystals from acetone–hexane; mp 224–226°C; $[\alpha]_D^{26}$ +71.1° (*c* 0.83, chloroform); and EI ms *m/e*: 562 (M^+), 561 $(M^+ - H)$ and 503 $(M^+ - O_2CCH_3)$.[4]

A single crystal (0.75 × 0.5 × 0.5 mm) of phyllanthocindiol methyl ester (**2***b*) recrystallized from acetone–hexane was analyzed using a Philips PW1100 four-circle diffractometer with CuKα radiation (λ = 1.5418 Å). The crystals were found to exhibit monoclinic space group $P2_1$ with cell constants a = 9.891 (5), b = 24.84 (1), and c = 9.687 (5) Å; β = 94.82 (2) Å by least squares from the setting of 25 high-order reflections. Intensities were collected in the range $8 < 2\theta < 136$ by the $\omega-2\theta$ scan technique and were corrected for Lorentz polarization but not for absorption. Of the 4011 reflections obtained, 3692 (with $I_{rel} > 2\sigma I_{rel}$) were employed in the solution. The crystal density (ρ = 1.21 Mg m^{-3}) gave $Z = 4$ corresponding to two molecules of $C_{25}H_{32}O_8$, M_r = 460.52, per asymetric unit.

The structure (**2***b*) was solved by direct methods using the multisolution tangent refinement procedures of the program SHELX (7). A starting set of eight reflections (with three used for origin definition) generated 256 permutation. Twelve *E*-maps were obtained and the first (reliability index $R_A = 0.138$), which gave 36 of the 66 non-hydrogen atoms, yielded sufficient phase information to locate all the non-hydrogen atoms in subsequent weighted difference syntheses. The hydrogens of CH_2 and CH units were placed at 1.00 Å from their parent carbons and their positions were dictated by the geometry of the molecule. Methyl hydrogens were treated as rigid groups. Hydroxyl hydrogens (on 07 and 014) were not revealed in the final difference maps, and are not included in the final model. However, there is strong evidence for both intra- and intermolecular H-bonding involving the 07 and 014 atoms. Because of the large number of independent atoms, final refinements were accomplished using the blocked-matrix technique with the non-hydrogen atoms treated anisotropically and the hydrogen atoms isotropically to provide a final $R = 0.072$ (R_w = 0.085, $w = (\sigma^2 F + 0.002F^2)^{-1}$.

By the same general X-ray crystallographic techniques, a single crystal (0.15 × 0.25 × 0.50 mm) of phyllanthose peracetate (**3***b*) led to the following summary data: space group $P2_1$ with a = 13.077 (7), b = 9.306 (5), and c = 12.009 (6) Å with β = 92.33 (2) Å. As above, intensities were collected in the range $10° < 2\theta < 120°$ by the $\omega-2\theta$ scan technique. A total of 2299 reflections was obtained and 1930 (with $I_{rel} > 2\sigma I_{rel}$) were employed in the solution. Density (ρ = 1.24 Mg m^{-3}) of the crystal gave $Z = 2$, allowing one molecule of $C_{24}H_{34}O_{15}$ with M_r = 562.52 for each asymmetric unit.

The program SHELX (7) was employed as outlined above, using a starting set of eleven reflections to generate 256 permutations. Of the twelve *E*-maps obtained, the fifth (reliability index $R_A = 0.129$) gave, as a recognizable molecular fragment, 21 of the 39 non-hydrogen atoms in the asymmetric unit. Weighted difference syntheses yielded the remaining non-hydrogen atoms. Hydrogen atoms were placed at 1.00 Å from their respective carbon atoms and their positions were based on the geometry of the molecule. Again, methyl hydrogens were handled as rigid groups. Because of the large number of independent atoms, final refinements were performed using the blocked-matrix technique with the non-hydrogen atoms treated anisotropically and the hydrogen atoms isotropically to yield $R = 0.053$ (unit weights).

Completion of the X-ray crystal structure determinations of phyllanthocindiol methyl ester (**2***b*) and phyllanthose acetate (**3***b*) clearly substantiates the assignment of structure **2***a* to phyllanthostatin 3 and confirms the previous (**2***a*, *b*) assignments of phyllanthostatins 1 and 2 (**1***a*, *c*) and of phyllanthoside (**1***b*). Presently, the results of these structural elucidations are being employed in establishing structures for related glycosides in this potentially important series of biosynthetic products.

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