ratio of 41 to 42 by NMR). **2-Butyl-5-[3-[[3-(ethylthio)-propyl]thio]propyl]-2-cyclopentenone (41)**: 500-MHz ^{1}H NMR δ 0.89 (t, J = 7.3, 3 H, $CCH_2CH_2CH_2CH_3$), 1.24 (t, J = 7.3, 3 H, SCH_2CH_3), 1.31 (q, t, J = 7.3, 6.9, 2 H, $CCH_2CH_2CH_2CH_3$), 1.43 (m, 2 H, $CCH_2CH_2CH_2CH_3$), 1.63 (obscured, m, 1 H, $CCHHCH_2CH_2S$), 1.64 (partly obscured, t, t, J = 7.3, 6.9, 2 H, $CCH_2CH_2CH_2S$), 1.85 (quintet, J = 6.9, 2 H, $SCH_2CH_2CH_2S$), 1.87 (obscured, m, 1 H, $CCHHCH_2CH_2S$), 2.15 (d, d, d, d, d, J = 9.2, 7.3, 3.2, 1.8, 1.8, 2 H, $CCH_2CH_2CH_2CH_3$), 2.21 (d, d, d, d, d, J = 18.8, 3.2, 3.2, 2.3, 2.3, 1 H, 4-H), 2.53 (q, J = 7.3, 2 H, SCH_2CH_3), 2.54 (t, J = 7.3, 2 H, $CCH_2CH_2CH_2S$), 2.60 (t, J = 6.9, 2 H, $SCH_2CH_2CH_2S$), 2.62 (t, J = 6.9, 2 H, $SCH_2CH_2CH_2S$), 2.74 (d, d, d, d, d, J = 18.8, 9.2, 4.6, 2.3, 2.3, 2 H, 4-H), 7.23 (d, d, t, J = 3.2, 2.3, 1.8, 1 H, vinyl-H); 75-MHz ^{13}C NMR δ 13.5, 14.5, 22.2, 24.3, 25.7, 27.2, 29.2, 29.7, 30.3, 30.6, 30.8, 31.9, 33.3, 44.9, 146.1, 155.9, 211.8; IR (cm^{-1}) 2949, 1683 (CO), 1445; mass spectrum (EI) m/z 314 (M$^+$), 179 (100).

Acknowledgment. We are grateful to the National Science Foundation, the National Institutes of Health, the Sloan Foundation, and the Camille and Henry Dreyfus Foundation for partial support of this work.

Supplementary Material Available: Preparation of cobalt complexes 1 and 2, preparation of thioacetate 47, preparation and spectral data for sulfide 13, and NMR data for mesylates 45, 49, 50, 52, 53 and 55 (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Structures of Breynins A and B, Architecturally Complex, Orally Active Hypocholesterolemic Spiroketal Glycosides

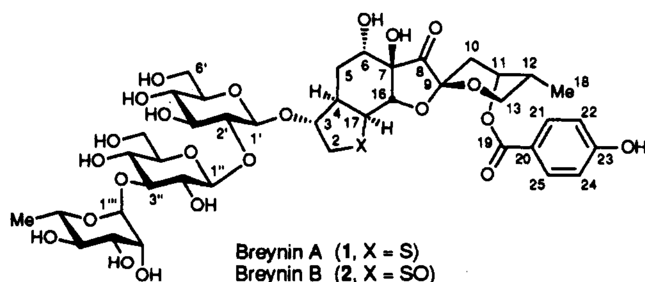
Amos B. Smith, III,* Terence P. Keenan, Rex T. Gallagher, George T. Furst, and Peter G. Dormer

Department of Chemistry, the Monell Chemical Senses Center, and the Laboratory for Research on the Structure of Matter, University of Pennsylvania, Philadelphia, Pennsylvania 19104

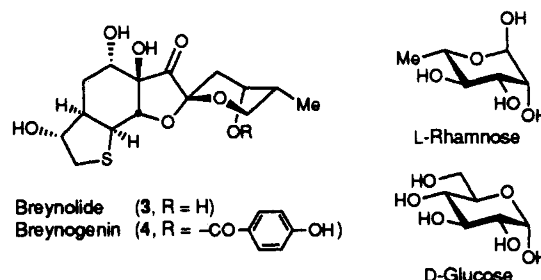
Received May 5, 1992

Breynin A and its oxy congener, breynin B, were reisolated from the woody portion of the Taiwanese shrub *Breynia officinalis* Hemsl and formulated as 1 and 2, respectively. An arsenal of NMR techniques including DEPT, heteronuclear chemical shift correlation, ^{1}H–^{1}H COSY, and inverse long-range ^{1}H–^{13}C experiments were employed. Of particular importance for the NMR study was the preparation of breynin A undecaacetate (11). The analysis independently generated structure 1, confirming the assignment for breynin A recently reported by Ohkuma et al. However, spectral data and direct oxidation of breynin A to B demonstrated that the latter is not the hemithioacetal 8 as suggested by Ohkuma, but rather the isomeric sulfoxide 2. Improved purification of the *Breynia* glycosides via droplet counter-current distribution and HPLC is also described.

In 1973, Hirata at Nagoya University, in collaboration with Sasaki at the Bristol-Myers Institute (Tokyo), reported the isolation of two sulfur-containing glycosides, breynins A and B (1 and 2), from the Taiwanese woody shrub *Breynia officinalis* Hemsl.[1] Initial screening demonstrated that both 1 and 2 are potent hypocholesterolemic agents,[2] reducing serum cholesterol in rats by 20–35% upon interperitoneal injection of 0.005–0.025 mg/kg/d. More recently, Trost discovered that the breynins are orally active, lowering rat serum cholesterol by 30–60% after ten daily doses of 10–20 mg/kg.[3]



Exhaustive hydrolysis of breynin A afforded breynolide (3) along with D-glucose (2 equiv), L-rhamnose, and *p*-hydroxybenzoic acid.[1,4] The structure of 3 was secured via single-crystal X-ray analysis.[1,5] Also isolated was the parent aglycon breynogenin (4) which embodied the *p*-hydroxybenzoate unit.[4] Degradation and NMR studies employing the tri- and tetraacetate derivatives of 4 then demonstrated that the benzoate moiety was incorporated as a C(11) ester.[4] The critical connectivity of the saccharide units, however, remained unknown; only the C(7) tertiary hydroxyl was eliminated as a point of attachment via the observation of the hydroxyl proton as a singlet in the NMR spectrum of 1 in DMSO-d_6.

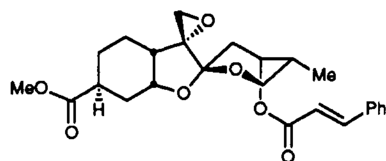


Our interest in the breynins was initially stimulated by the structural similarity of breynolide (3) to phyllanthocin (5),[6] the aglycon methyl ester of the phyllanthoside anti-

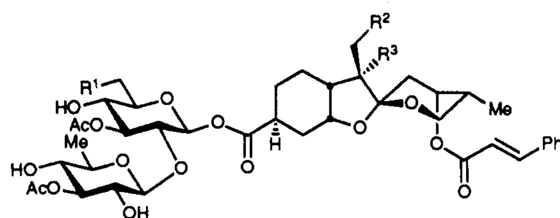
(1) Sasaki, K.; Hirata, Y. *Tetrahedron Lett.* 1973, *14*, 2439.
(2) Koshiyama, H.; Hatori, M.; Ohkuma, H.; Fumihide, S.; Imanishi, H.; Ohbayashi, M.; Kawaguchi, H. *Chem. Pharm. Bull.* 1976, *24*, 169.
(3) Trost, W. *IRCS Med. Sci.* 1986, *14*, 905.
(4) Sakai, F.; Ohkuma, H.; Koshiyama, H.; Naito, T.; Kawaguchi, H. *Chem. Pharm. Bull.* 1976, *24*, 114.
(5) Sasaki, K.; Hirata, Y. *Acta Crystallogr.* 1974, *B30*, 1347.
(6) Kupchan, S. M.; La Voie, E. J.; Branfman, A. R.; Fei, B. Y.; Bright, W. M.; Bryan, R. F. *J. Am. Chem. Soc.* 1977, *99*, 3199.

0022-3263/92/1957-5115$03.00/0 © 1992 American Chemical Society

tumor agents [e.g., phyllanthoside (6) and phyllanthostatin 3 (7)].[7] Not surprisingly, others in the synthetic com-

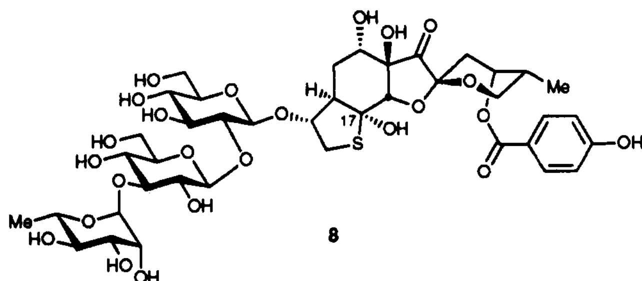


Phyllanthocin (5)



Phyllanthoside (6, R^1 = H, R^2, R^3 = epoxide)
Phyllanthostatin 3 (7, R^1 = R^2 = R^3 = OH)

munity have also been attracted to this arena;[8] in 1990, Williams et al. reported the total synthesis of (+)-breynolide,[9] and shortly thereafter we disclosed a second sucessful approach.[10] In this full account, we describe studies undertaken as prelude to the synthesis of breynins A and B, whereby we have reisolated the breynins from *Breynia officinalis* Hemsl, developed an oxidative procedure for the conversion of A to B, and assigned their complete structures.[11] After completion of our work, we received a paper by Ohkuma et al.[12] who independently formulated breynin A as 1. Ohkuma also proposed the hemithioacetal structure 8 for B, but we have established that breynin B is in fact the sulfoxide derivative of A (i.e., 2), and not the isomeric hemithioacetal.



Reisolation of Breynins A and B. In an effort to streamline the 1976 extraction protocol of Koshiyama and co-workers,[2] only the woody portion of *Breynia officinalis* Hemsl was utilized; TLC analysis indicated no significant amounts of either breynin in the leaf or twig material[13] Thus, 5 kg of finely ground wood chips were extracted

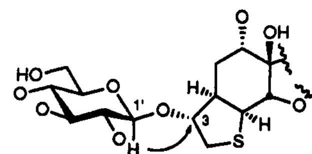


Figure 1. Anomeric linkage in 1 revealed by long-range heteronuclear correlation.

three times with methanol at reflux. The extract was filtered and concentrated in vacuo, affording a paste which was partitioned between *n*-butanol and water. Concentration of the organic phase and precipitation by slow addition of ethyl acetate then furnished a crude solid; the latter was shown by TLC to be rich in breynins A and B, whereas the filtrate contained only less polar material. In the published procedure, the solid was further purified via counter-current distribution. We employed the recently developed droplet counter-current distribution technique[14] in the descending mode; this tactic proved remarkably effective for the separation of 1 and 2. Final refinement to analytical purity, via a combination of silica chromatography and C-18 reversed-phase HPLC, furnished 40 mg of breynin A and 10 mg of breynin B. A subsequent isolation from 5 kg of freshly cut *Breynia officinalis* Hemsl furnished an additional 500 mg of A and 20 mg of B. In conjunction with the ease of oxidation of B to A (vide infra), the latter result suggested that breynin B may be an artifact of the isolation process.

The samples of breynin A (1) were readily identified via their characteristic melting points (195–197 °C (lit.[2] mp 195–197 °C)), optical rotations [$[\alpha]^{27}_D$ +12° (*c* 1.3, water) (lit.[2] $[\alpha]^{22}_D$ +13° (*c* 0.5, water))], and R_f values and by high-resolution mass spectrometry. Breynin B (2) was characterized in similar fashion. Importantly, the mass spectrum of 2 indicated that the additional oxygen resides within the aglycon moiety rather than the saccharide residues.

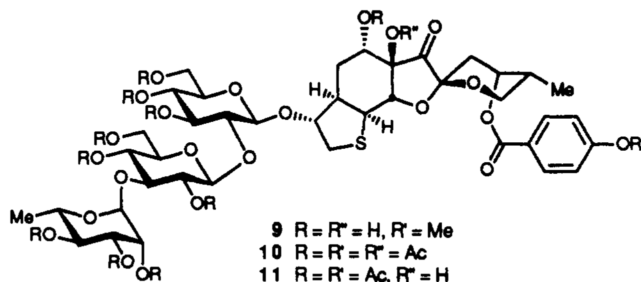
Analysis of Breynin A via NMR and Mass Spectrometry. The ^{1}H NMR spectrum of breynin A was observed in a number of solvents; deuteriomethanol afforded the greatest dispersion.[11] The resonances associated with the aglycon portion of 1—essentially the structure of breynogenin (4)—were readily identifiable via a combination of ^{1}H–^{1}H COSY and ^{1}H–^{13}C heteronuclear correlations. In contrast, analysis of the saccharide contributions was complicated by the highly convoluted envelope observed between 3.2 and 3.7 ppm. Total correlation spectroscopy (TOCSY)[15] did permit assignment of the glucose H(6′) and H(6″) resonances. Correlation with the corresponding carbon signals at 62.5 and 61.6 ppm, respectively, eliminated the possibility of glycosidic linkages at these positions as methyl β-D-glucopyranosides exhibit downfield shifts of ca. 10 ppm upon alkylation of the C(2), C(3), or C(6) hydroxyls.[16] Further ^{1}H–^{13}C correlations were obscured by the highly convoluted envelope between 69 and 78 ppm in the ^{13}C NMR spectrum, hindering the unequivocal assignment of other carbon signals. However, one additional long-range ^{1}H–^{13}C correlation between C(3) of the aglycon (91.4 ppm) and the anomeric proton of a glucose unit at 4.43 ppm served to define the first anomeric linkage in 1 (Figure 1).

(7) (a) Pettit, G. R.; Cragg, G. M.; Gust, D.; Brown, P. *Can. J. Chem.* **1982**, *60*, 544. (b) Pettit, G. R.; Cragg, G. M.; Gust, D.; Brown, P.; Schmidt, J. M. *Can. J. Chem.* **1982**, *60*, 939. (c) Pettit, G. R.; Cragg, G. M.; Suffness, M. I.; Gust, D.; Boettner, F. E.; Williams, M.; Saenz-Renauld, J. A.; Brown, P.; Schmidt, J. M.; Ellis, P. D. *J. Org. Chem.* **1984**, *49*, 4258. (d) Pettit, G. R.; Cragg, G. M.; Niven, M. L.; Nassimbeni, L. R. *Can. J. Chem.* **1983**, *61*, 2630. (e) Pettit, G. R.; Cragg, G. M.; Suffness, M. I. *J. Org. Chem.* **1985**, *50*, 5060.
(8) Nishiyama, S.; Ikeda, Y.; Yoshida, S.; Yamamura, S. *Tetrahedron Lett.* **1989**, *30*, 105.
(9) Williams, D. R.; Jass, P. A.; Allen Tse, H.-L.; Gaston, R. D. *J. Am. Chem. Soc.* **1990**, *112*, 4552.
(10) Smith, A. B., III; Empfield, J. R.; Rivero, R. A.; Vaccaro, H. A. *J. Am. Chem. Soc.* **1991**, *113*, 4037.
(11) Preliminary communication: Smith, A. B., III; Gallagher, R. T.; Keenan, T. P.; Furst, G. T.; Dormer, P. G. *Tetrahedron Lett.* **1991**, *32*, 6847. This paper contains the ^{1}H and ^{13}C NMR chemical shifts and assignments for 2 and 11 in tabular format.
(12) Ohkuma, H.; Tsuno, T.; Konishi, M.; Naito, T.; Kawaguchi, H. *Chem. Pharm. Bull.* **1991**, *39*, 942.
(13) *n*-Butanol-acetic acid-water (63:10:27) was used as eluant.
(14) Hostettmann, K. In *Advances in Chromatography*; Giddings, J. C., Keller, R. A., Eds.; Dekker: New York, 1983; Vol. 21, pp 165–186.
(15) (a) Homans, S. W.; Dwek, R. A.; Boyd, J.; Soffe, N.; Rademacher, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1202. (b) Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521. (c) Bazzo, R.; Boyd, J. *J. Magn. Reson.* **1987**, *75*, 452.
(16) Haque, M. E.; Kikuchi, T.; Yoshimoto, K.; Tsuda, Y. *Chem. Pharm. Bull.* **1985**, *33*, 2243.

Analysis of the $^1H-^1H$ and $^1H-^{13}C$ coupling constants elucidated the anomeric configurations of the saccharide moieties. The β configurations of both glucose residues derived from the $J_{H(1)-H(2)}$ values of 7.7 and 7.5 Hz for the anomeric protons at 4.43 and 4.08 ppm, respectively,[17] as well as the $J_{C(1)-H(1)}$ couplings, determined via coupled INEPT, of 159.7 and 160.4 Hz for the anomeric carbons at 103.8 and 106.6 ppm,[18] respectively. The anomeric configuration for the rhamnose unit proved to be α, as indicated by the $J_{C(1)-H(1)}$ value of 173.2 Hz for the carbon resonance at 102.6 ppm,[18] again determined via coupled INEPT. Unfortunately the $J_{H(1)-H(2)}$ couplings for rhamnopyranoses have little diagnostic significance, but the anomeric proton chemical shift (5.11 ppm) is typical of α-L-rhamnosides (α anomers: 5.02–5.92 ppm; β anomers: 4.55–4.93 ppm).[18]

Mass spectral studies of 1 suggested a linear arrangement of saccharides containing a terminal deoxyhexose (i.e., rhamnose). Positive-ion fast atom bombardment (FAB-MS) showed an $[M + Na]^+$ ion at m/z 959 and an $[M + H]^+$ ion at m/z 937. Importantly, negative-ion FAB-MS displayed a molecular-ion-minus-one peak $[M - H]^-$ at m/z 935 and fragment ions corresponding to sequential loss of deoxyhexose followed by two hexose (i.e., glucose) units, at m/z 789, 627, and 465.[19] The NMR and mass spectral data together implicated a linear trisaccharide moiety with a terminal rhamnopyranose residue α-linked to a glucopyranose unit. The latter in turn is β-linked to the second glucopyranose, which is likewise connected via a β glycosidic bond to the C(3) oxygen of the aglycon.

Derivatization of Breynin A. With strong evidence in hand for the trisaccharide connectivity outlined above, we envisioned that derivatives of 1 might afford better resolution of the ^1H and ^{13}C saccharide resonances and also definitively eliminate the C(6) and C(23) oxygens of the aglycon as possible points of connectivity. To this end, treatment of 1 with ethereal diazomethane at 0 °C afforded anisole derivative 9 in 60% yield.[20] This protocol selec-



tively methylates phenols in the presence of alcohols such as pyranose hydroxyls.[21] Although the dispersion did not markedly improve in the NMR spectra of 9, the possibility of a glycosidic linkage involving the C(23) position was clearly excluded. The latter conclusion also derived independently from the mass spectrum of 2, which revealed fragmentation via loss of hydroxybenzoate.[19]

In an effort to completely rule out the C(6) hydroxyl as a point of connectivity and clarify the unresolved features of the ^1H spectrum, we attempted to peracetylate breynin A. Treatment with acetic anhydride and pyridine in the presence of DMAP catalyst[22] afforded the desired dodecaacetate 10 in 7% yield; the major product (71%) was undecaacetate 11, the C(7) tertiary hydroxyl resisted acylation under these conditions. The undecaacetate proved to be ideal for further spectroscopic studies.

NMR Analysis of Undecaacetate 11. We observed NMR spectra of 11 in a number of solvents; in deuteriobenzene essentially all of the ^1H and ^{13}C resonances were resolved.[11] The most critical findings were the chemical shifts of the glucose H(2′) and H(3″) protons at 3.64 and 3.57 ppm, respectively. In conjunction with the downfield absorptions of the numerous α-acetoxy methine protons, these resonances confirmed the presence of a (1‴ → 3″)-O-(1″→2′)-O-linked linear trisaccharide. The significant downfield shift of the aglycon C(6) proton (5.4 ppm) eliminated this position as a possible point of glycosidic connectivity.

Heteronuclear shift experiments established correlations of the glucose H(2′) and H(3″) protons with the carbon resonances at 77.9 and 82.3 ppm; as expected the latter appear at lower field than C(2) and C(3) of methyl β-D-glucopyranoside (74.9 and 78.2 ppm, respectively).[16] Long-range heteronuclear shift correlation linked C(1‴) carbon of the terminal rhamnose at 100.0 ppm with the glucose H(3″) proton at 3.57 ppm as well as the corresponding linkage for the H(1‴) proton at 4.85 ppm with the C(3″) carbon at 82.3 ppm (Figures 2 and 3). Other significant long-range heteronuclear shift correlations linked the C(1″) carbon of the central glucose unit (101.3

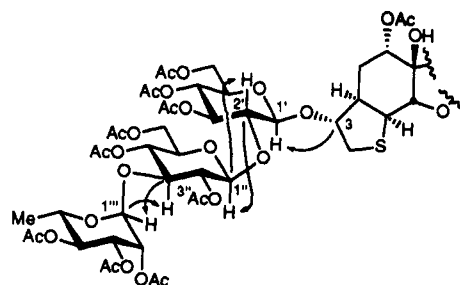


Figure 2. Long-range heteronuclear shift correlation involving the saccharide portion of 11.

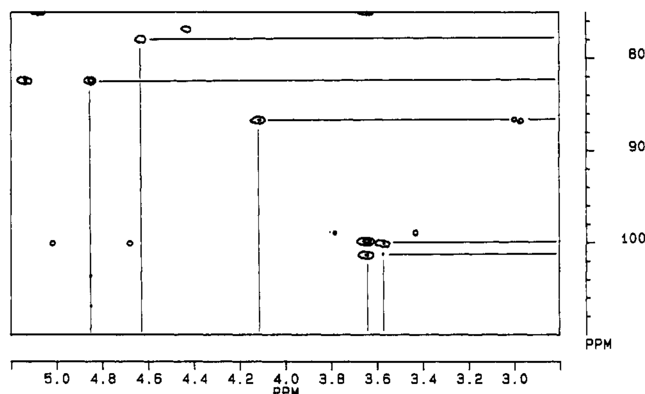


Figure 3. 2D long-range HETCOR of 11.

(17) (a) van der Veen, J. M. *J. Org. Chem.* **1963**, *28*, 564. (b) Casu, B.; Reggiani, M.; Gallo, G. G.; Vigevani, A. *Tetrahedron* **1966**, *22*, 3061. (c) Casu, B.; Reggiani, M.; Gallo, G. G.; Vigevani, A. *Tetrahedron* **1968**, *24*, 803. (d) Casu, B.; Reggiani, M.; Gallo, G. G.; Vigevani, A. *Carbohydr. Res.* **1970**, *12*, 157. (e) DeBygn, A.; Anteunis, M.; Verhegge, G. *Bull. Soc. Chim. Belg.* **1975**, *84*, 721.

(18) (a) Bock, K.; Lundt, I.; Pedersen, C. *Tetrahedron Lett.* **1973**, *14*, 1037. (b) Bock, K.; Pedersen, C. *Acta Chem. Scand.* **1975**, *B29*, 258. (c) Kasai, R.; Okihara, M.; Asakawa, J.; Mizutani, K.; Tanaka, O. *Tetrahedron* **1979**, *35*, 1427.

(19) Positive-ion FAB-MS of 2 showed a protonated molecular ion peak $[M + H]^+$ at m/z 953 and fragment ions for sequential loss of a deoxyhexose unit $[M + H - 146]^+$ (m/z 807), two hexose units [$[M + H - 146 - 162]^+$ (m/z 645) and $[M + H - 146 - (2\times162)]^+$ (m/z 483)] and p-hydroxybenzoic acid $[M + H - 146 - (2\times162) - 138]^+$ (m/z 345).

(20) Black, T. H. *Aldrichim. Acta* **1983**, *16*, 3.

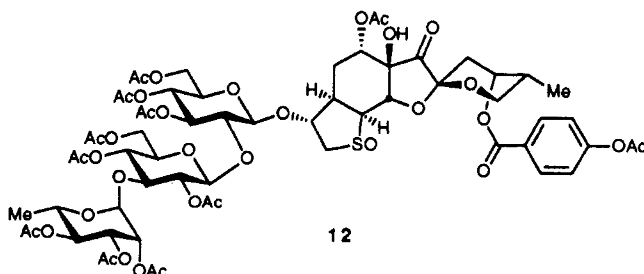
(21) Neeman, M.; Hashimoto, Y. *J. Am. Chem. Soc.* **1962**, *84*, 2972.

(22) Hölfe, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569.

ppm) to H(2′) of the first glucose residue (3.64 ppm); conversely, a correlation was observed between the H(1″) proton (4.63 ppm) and the C(2′) carbon (77.9 ppm). The latter moiety had shown a long-range correlation to the aglycon in the spectrum of 1 (vide supra); for 11, the H(1′) proton at 4.11 ppm also displayed a strong correlation to the aglycon C(3) carbon at 86.7. All of this data uniquely support structure 1 for breynin A.

Oxidation of Breynin A to Breynin B. In the course of our isolation work, we noted the facile air oxidation of breynin A in aqueous solution; this observation led us to speculate that breynin B was in fact a sulfoxide derivative of A. Similar adventitious oxidation of sulfur had complicated our synthesis of breynolide (**3**).[10] Oxidation of breynin A with the Davis phenyloxaziridine, which selectively converts sulfides to sulfoxides,[23] efficiently furnished two diastereomers in a 7:1 ratio. The major sulfoxide proved to be identical in all respects [melting and mixed melting points, 500-MHz ^{1}H and 125-MHz ^{13}C NMR spectra, and optical rotations] with a sample of natural breynin B.

NMR Analysis of Breynin B. Our conclusion that breynin B is the sulfoxide **2**, rather than the isomeric hemithioacetal **8** as proposed by Ohkuma et al., [12] is also derived from persuasive spectroscopic evidence. As in the case of **1**, deuteriomethanol solvent afforded the greatest dispersion in the ^{1}H NMR spectrum of breynin B. The Tokyo group deduced the hemithioacetal formulation from the apparent absence of a C(17) methine proton resonance for the aglycon and the downfield shifts of the adjacent H(4) and H(16) protons (0.24 and 0.36 ppm, relative to breynin A, respectively) in dimethyl sulfoxide-d_6.[12] Our 125-MHz ^{13}C chemical shifts for semisynthetic breynin B match those recently published (within 0.2 ppm);[11] as assigned, the ^{1}H NMR data differ only with respect to the H(2) resonances.[24] Analysis of semisynthetic **2** by polarization transfer (DEPT), heteronuclear shift correlation, COSY, and 2D TOCSY (600 MHz) in dimethyl sulfoxide-d_6 revealed that the C(17) carbon at 70.5 ppm is in fact a methine, not a hydroxylated quaternary carbon. Furthermore, the large 21.0 ppm downfield shift of the C(2) carbon resonance at 56.0 ppm is of the expected magnitude for a sulfide-to-sulfoxide transformation.[25] We prepared the undecaacetate derivative of breynin B (**12**) via the protocol employed for **11**; in the ^{1}H NMR spectrum of **12**, the H(17) proton appears at 4.04 ppm. Both chemical correlation and spectroscopic analysis clearly define the revised structure **2** for breynin B.



We tentatively assign the α configuration to the major sulfoxide diastereomer based on related results from our breynolide synthetic venture. Oxidation of advanced intermediates containing the aglycon skeleton of **1** with the Davis phenyloxaziridine furnished predominantly the α-sulfoxides as demonstrated by single-crystal X-ray analysis.[26] Moreover, these α oxidations typically induced 0.30–0.35 ppm upfield shifts of the H(5) axial proton, an effect not observed for the β sulfoxides.[26] The shift of the H(5) axial proton upon conversion **1** to **2** was 0.5 ppm downfield.

Experimental Section[27]

Extraction and Purification of Breynins A and B. The plant material, *Breynia officinalis* Hemsl (Euphorbiaceae), was obtained from the Brion Research Institute of Taiwan in Taipei, Taiwan. One plant was obtained in 1984 and stored and a second freshly harvested in 1991.

The woody portion was milled into finely divided chips to afford ca. 5.0 kg of material per plant. Each sample was divided into 10 0.5-kg batches. Each was extracted three times with 3.0-L portions of methanol; the extractions were continued for 24 h at a gentle reflux maintained via a steam bath, with intermittent agitation of the plant material. Gravity filtration of the combined extracts followed by concentration in vacuo afforded a crude extract paste for each plant. The paste was taken up in water (5.0 L) and the mixture vigorously shaken for 1 h. The resultant aqueous extract was gravity filtered and extracted with n-butanol (3 × 500 mL). The n-butanol extracts were then combined, concentrated in vacuo to a volume of 200 mL, and gravity filtered to afford a clear solution. Addition of ethyl acetate (2.0 L) induced deposition of a fine precipitate after ca. 10 min. The mixture was allowed to stand for 3 h and then filtered under vacuum to afford ca. 10 g of solid material. The latter was initially purified via a droplet counter-current distribution apparatus (DCCD) in descending mode with an n-butanol–methanol–3% aqueous NaCl solvent mixture. The lower aqueous phase was used as the mobile solvent and the n-butanol upper layer as the stationary phase.

(23) Davis, F. A.; Jenkins, R., Jr.; Yocklovich, S. G. *Tetrahedron Lett.* **1978**, *19*, 5171.

(24) We recognize the unlikely possibility that we and Ohkuma et al. have isolated and characterized different compounds.

(25) Eliel, E. L.; Pietrusiewicz, K. M. In *Topics in Carbon-13 NMR Spectroscopy*; Levy, G. C., Ed.; Wiley-Interscience: New York, 1979; Vol. 3, p 171.

(26) Empfield, J. R. Ph.D. Dissertation, University of Pennsylvania, 1991.

(27) **Materials and Methods.** Diethyl ether was distilled from sodium and benzophenone; other solvents were HPLC grade. Precoated silica gel plates (250 μm) with a fluorescent indicator (E. Merck) were used for analytical thin-layer chromatography. Melting points were determined with a Bristoline micro hot stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. UV–vis spectra were recorded on an IBM Model 9420 spectrophotometer. Infrared spectra were recorded on an IBM Model IR/97 FTIR spectrometer at 4 cm^{-1} resolution. High-resolution mass spectra were measured by the University of Pennsylvania Mass Spectrometry Facilities in positive or negative FAB mode, with a Cs ion source and DTE/DTT (1:5, v/v) matrix, on a VG ZAB-E spectrometer interfaced with a VG 11-250 data system. Additional mass spectra were measured in a similar fashion at SmithKline Beecham Pharmaceuticals with a VG-705 mass spectrometer. ^{1}H and ^{13}C spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AM500 spectrometer (University of Pennsylvania) with a 5-mm ^{1}H/^{13}C or 5-mm inverse ^{1}H/BB probe or at 600 and 150 MHz, respectively, on a Bruker AMX600 (Bruker Instruments Inc.) with a 5-mm triple-resonance ^{1}H/^{13}C/^{15}N probe. ^{13}C-NMR multiplicities were determined via the distortionless enhancement by polarization transfer (DEPT) method. ^{1}H COSY, ^{1}HDQF-COSY, ^{1}H TOCSY, ^{1}H–^{13}C Hetcor, ^{1}H–^{13}C HMQC, and ^{1}H–^{13}C HMBC two-dimensional spectra were acquired using standard Bruker pulse sequences (AM-DISR90; AMX-UXNMR vs 910901). Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane with the solvent signals as internal reference (CHD_2OD, 3.30 and 49.0 ppm; (CHD_2)SO(CD_3), 2.49 and 29.0 ppm; C_6HD_5, 7.17 and 128.0 ppm). Coupling constants are given in Hz. Microanalyses were performed by Robertson Microlit Laboratories Inc., Madison, NJ. Droplet counter-current chromatography was performed on a Tokyo Rikakikai Co., Ltd. Model D. C.C.-A chromatograph equipped with 214 40-cm × 3.4-mm-i.d. tubes and an Isco automated fraction collector. Reverse-phase high-pressure liquid chromatography (HPLC) analysis was performed with a Varian Model 5000 chromatograph equipped with a Rheodyne injector and a Perkin-Elmer LC-235 diode array detector. Chromatograms were recorded on a Perkin-Elmer GP-100 graphics printer. A 10-mm × 25-cm Rainin Dynamax-A60 analytical column with 8-μm Microsorb C-18 packing and a similar 21.4-mm × 25-cm preparative column were used. Preparative HPLC separations on normal-phase silica gel were performed on a Rainin system equipped with a Dynamax Method Manager, a Rabbit MPX solvent delivery system, Rheodyne injector, and Gilson Model 115 variable-wavelength UV detector. A 10-mm × 25-cm Rainin Dynamax-A60 column with 8-μm packing was employed.

Ca. 3 g of solid material was applied for each run in a 1:1 mixture of stationary and mobile phases. With a flow rate of 0.35 mL/min, fractions (i.e., 40-cm × 3.4-mm-i.d. tubes) were collected at 20-min intervals. Typically, fractions rich in **2** were obtained in tubes 60–90 and fractions rich in **1** in tubes 100–150. Concentration in vacuo furnished solid material which was desalinated by rapid chromatography on silica gel with 30% methanol–dichloromethane as eluant. After concentration the breynins were purified further by HPLC on C-18 reversed-phase silica gel, using a 21.4-mm × 25-cm Dynamax-A60 column with methanol–water (12:13) as eluant and a diode array detector monitoring the chromophores of **1** and **2** at 258 nm; the retention times of **1** and **2** were 11.4 and 8.4 min, respectively, using an analytical 10-mm × 25-cm Dynamax-A60 column and a flow rate of 3.0 mL/min. Concentration afforded 40 and 500 mg of **1** and 10 and 20 mg of **2**, respectively, for the older and freshly harvested plant samples.

Breynin A (1): mp 195–197 °C (lit.[2] mp 195–197 °C); TLC on Si gel R_f 0.29 [lit.[2] R_f 0.24 [*n*-butanol–acetic acid–water (63:10:27)]]; $[\alpha]^{27}_D$ +12° (*c* 1.3, H_2O) [lit.[2] $[\alpha]^{22}_D$ +13° (*c* 0.5, H_2O)]; UV (H_2O) λ_{max} 258 nm (ϵ 10600); IR (KBr) 3430 (br, s), 2935 (m), 1780 (w), 1695 (m), 1610 (m), 1515 (w), 1280 (s), 1170 (m), 1115 (s), 1075 (s), 1040 (s), 1010 (s), 775 (w) cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 8.03 (d, *J* = 8.8, 2 H, H-21/25), 6.92 (d, *J* = 8.8, 2 H, H-22/24), 5.39 (m, 1 H, H-11), 5.11 (d, *J* = 1.6, 1 H, H-1‴), 4.43 (d, *J* = 7.7, 1 H, H-1′), 4.37 (s, 1 H, H-16), 4.35 (d, *J* = 3.6, 1 H, H-3), 4.18 (d, *J* = 5.1, 1 H, H-17), 4.08 (d, *J* = 7.5, 1 H, H-1″), 4.00–3.90 (m, 4 H), 3.86 (dd, *J* = 12.0 and 2.4, 1 H, H-6a′), 3.73 (dd, *J* = 12.1 and 5.5, 1 H, H-6b′), 3.67 (dd, *J* = 9.5 and 3.4, 1 H, H-6a″), 3.63-3.59 (m, 2 H), 3.52–3.20 (m, 10 H), 3.05 (dd, *J* = 12.0 and 4.0, 1 H, H-2a), 2.86 (dt, *J* = 13.0 and 4.4, 1 H, H-4), 2.41 (dt, *J* = 6.3 and 2.7, 1 H), 2.14 (m, 1 H, H-12), 2.06 (t, *J* = 3.5, 2 H, H-10a,b), 1.67 (td, *J* = 13.8 and 2.4, 1 H, H-5a), 1.49 (dt, *J* = 13.7 and 3.6, 1 H, H-5b), 1.27 (d, *J* = 6.2, 3 H, H-6‴), 0.88 (d, *J* = 6.9, 3 H, H-18); ^{13}C NMR (125 MHz, CD_3OD) δ 213.38 (C-8), 167.74 (C-19), 163.69 (C-23), 133.35 (C-21/25), 122.64 (C-20), 116.74 (C-22/24), 106.64 (d, *J* = 160.4, C-1″), 103.82 (d, *J* = 159.7, C-1′), 102.57 (d, *J* = 173.2, C-1‴), 99.83 (C-9), 91.37 (C-3), 85.71, 84.29, 78.04, 77.84, 77.24, 76.96, 76.75 (C-16), 75.71, 73.98 (C-7), 72.31, 72.21, 70.85 (2 C), 70.14, 69.90 (C-11), 69.04, 63.82 (C-13), 62.53 (C-6′), 61.56 (C-6″), 47.04 (C-17), 39.38 (C-4), 36.11 (C-2), 34.37 (C-12), 32.68 (C-10), 27.33 (C-5), 17.93 (C-6‴), 12.86 (C-18); ^{13}C NMR (125 MHz, DMSO-d_6) δ 212.25, 165.02, 161.95, 131.64 (2 C), 120.67, 115.32 (2 C), 104.82, 102.10, 100.29, 97.73, 88.66, 83.18, 80.42, 76.83, 76.57, 76.23, 75.97, 74.07, 72.12, 70.60, 70.53, 69.66, 68.68, 68.11, 67.84, 67.23, 62.72, 60.85, 60.00, 48.60, 45.30, 37.96, 35.09, 32.22, 30.56, 26.47, 17.86, 11.85; high-resolution mass spectrum (FAB) *m/z* 959.2721 [(M + Na)$^+$, calcd for $C_{40}H_{56}O_{23}SNa$ 959.2831].

Breynin B (2): mp 208–210 °C (lit.[2] mp 208–210 °C); TLC on Si gel R_f 0.21 (lit.[2] R_f 0.20 [*n*-butanol–acetic acid–water (63:10:27)]]; $[\alpha]^{27}_D$ −3° (*c* 1.1, H_2O) [lit.[2] $[\alpha]^{22}_D$ +2° (*c* 0.5, H_2O)]; UV (H_2O) λ_{max} 285 nm (ϵ 16200); IR (KBr) 3430 (br, s), 2935 (m), 1785 (m), 1695 (m), 1610 (m), 1515 (w), 1275 (s), 1170 (m), 1120 (s), 1075 (s), 1035 (s), 775 (w) cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 7.99 (d, *J* = 8.6, 2 H), 6.92 (d, *J* = 8.6, 2 H), 5.36 (d, *J* = 3.0, 1 H), 5.10 (s, 1 H), 4.87 (s, 1 H), 4.49–4.47 (m, 2 H), 4.05 (d, *J* = 7.7, 1 H), 3.99–3.88 (m, 5 H), 3.80 (dd, *J* = 12.2 and 1.9, 1 H), 3.71–3.61 (m, 4 H), 3.57 (dd, *J* = 12.3 and 2.0, 1 H), 3.50–3.41 (m, 3 H), 3.38–3.19 (m, 7 H), 3.09–3.06 (m, 1 H), 2.48 (br s, 1 H), 2.14–2.10 (m, 1 H), 2.07 (dd, *J* = 11.8 and 4.0, 1 H), 2.04 (dd, *J* = 14.6 and 3.4, 1 H), 1.65 (m, 1 H), 1.24 (d, *J* = 6.2, 3 H), 1.16 (td, *J* = 12.7 and 1.4, 1 H), 0.87 (d, *J* = 6.9, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 212.40, 167.74, 163.68, 133.28 (2 C), 122.63, 116.83 (2 C), 106.47, 103.77, 102.51, 100.64, 88.21, 85.09, 83.93, 77.98, 77.76, 77.37, 76.73, 75.17, 74.96, 73.99, 72.30, 72.21, 71.57, 70.73, 70.29, 70.10, 69.87, 69.16, 64.09, 62.47, 61.71, 56.55, 38.64, 34.29, 32.57, 29.10, 17.93, 12.87; high-resolution mass spectrum (FAB) *m/z* 975.2837 [(M + Na)$^+$, calcd for $C_{40}H_{56}O_{24}SNa$ 975.2780].

Oxidation of Breynin A (1). A solution of breynin A (**1**) (60.0 mg, 0.064 mmol) in methanol (2.0 mL) was treated with (*E*)-2-(phenylsulfonyl)-3-phenyloxaziridine (25.2 mg, 0.097 mmol) at room temperature. The reaction mixture was stirred for 30 min and then concentrated via a stream of nitrogen. Flash chromatography with methanol–dichloromethane (1:4 → 1:1) as eluant and further purification by C-18 reversed-phase HPLC afforded breynin B (**2**) (43.6 mg, 71% yield) and a minor sulfoxide (6.5 mg, 10% yield). In addition to those given above, the following data were obtained for **2**: mixed mp with authentic **2**, 208–210 °C; $[\alpha]^{27}_D$ −2° (*c* 0.60, H_2O); ^{13}C NMR (125 MHz, DMSO-d_6) δ 211.44, 165.02, 162.28 (br), 131.55 (2 C), 120.29 (br); 115.50 (2 C), 104.07, 100.92, 100.22, 98.25, 84.63, 81.93, 80.31, 76.74 (2 C), 76.57, 76.07, 75.54, 73.71, 73.66, 72.14, 70.59, 70.54, 69.64, 68.24, 68.00, 67.72 (2 C), 63.21, 60.96, 60.44, 56.02, 36.16, 32.07, 30.29, 28.01, 17.82, 11.64; high-resolution mass spectrum (FAB) *m/z* 975.2712 [(M + Na)$^+$, calcd for $C_{40}H_{56}O_{24}SNa$ 975.2780].

Minor sulfoxide (epibreynin B): mp 190–192 °C; UV (H_2O) λ_{max} 258 nm (ϵ 12000); $[\alpha]^{24}_D$ +0.2° (*c* 0.41, H_2O); IR (KBr) 3475 (br, s), 2935 (m), 1785 (m), 1700 (m), 1610 (m), 1515 (w), 1280 (s), 1170 (m), 1080 (s), 1040 (s), 1010 (s), 910 (w), 605 (w) cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 8.01 (d, *J* = 8.7, 2 H), 6.87 (d, *J* = 8.7, 2 H), 5.38 (m, 1 H), 5.12 (d, *J* = 1.5, 1 H), 4.90 (s, 1 H), 4.71 (d, *J* = 4.4, 1 H), 4.43 (d, *J* = 7.7, 1 H), 4.40 (d, *J* = 15.0, 1 H), 4.09–4.04 (m, 2 H), 3.99–3.87 (m, 5 H), 3.76–3.65 (m, 7 H), 3.60 (dd, *J* = 12.2 and 2.4, 1 H), 3.52 (dd, *J* = 8.7 and 1.8, 1 H), 3.48 (d, *J* = 9.1, 1 H), 3.41–3.37 (m, 2 H), 3.24–3.15 (m, 2 H), 3.11 (dd, *J* = 15.1 and 4.7, 1 H), 2.47–2.45 (m, 1 H), 2.19–2.15 (m, 2 H), 2.09 (dd, *J* = 14.7 and 3.3, 1 H), 1.86–1.76 (m, 2 H), 1.27 (d, *J* = 6.2, 3 H), 0.90 (d, *J* = 6.9, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 210.02, 167.70, 163.66, 133.31 (2 C), 122.67, 116.70 (2 C), 106.63, 103.50, 102.53, 101.17, 88.13, 85.40, 84.04, 78.13, 77.75, 77.34, 76.75, 74.43, 73.98, 73.34, 72.31, 72.20, 71.55, 70.87, 70.12, 69.92, 69.07, 64.02, 62.52, 62.47, 61.95, 61.66, 40.02, 34.30, 32.73, 28.73, 17.94, 12.94; high-resolution mass spectrum (FAB) *m/z* 975.2854 [(M + Na)$^+$, calcd for $C_{40}H_{56}O_{24}SNa$ 975.2780].

C(23)-Methyl Ether of Breynin A (9). A solution of breynin A (**1**) (10.0 mg, 0.011 mmol) in methanol (0.5 mL) was cooled to 0 °C and treated with a freshly prepared ethereal solution of diazomethane (1.0 mL, ca. 0.3 mM). The reaction mixture was stirred at 0 °C until the yellow color dissipated. Concentration in vacuo and purification by C-18 reversed-phase HPLC afforded **9** (6 mg, 60% yield) as a colorless solid: mp 161–163 °C; $[\alpha]^{27}_D$ +8.1° (*c* 0.21, H_2O); UV (H_2O) λ_{max} 259 nm (ϵ 12200); IR (KBr) 3420 (br, s), 2935 (m), 1780 (w), 1695 (w), 1605 (m), 1510 (w), 1385 (m), 1285 (m), 1260 (s), 1175 (m), 1115 (m), 1075 (s), 1040 (s), 770 (w) cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 8.15 (d, *J* = 8.9, 2 H), 7.10 (d, *J* = 8.9, 2 H), 5.38 (m, 1 H), 5.14 (d, *J* = 1.6, 1 H), 4.43 (d, *J* = 7.7, 1 H), 4.37 (s, 1 H), 4.34 (d, *J* = 3.4, 1 H), 4.19 (d, *J* = 5.0, 1 H), 4.01–3.95 (m, 2 H), 3.92 (s, 3 H), 3.88–3.86 (m, 2 H), 3.73–3.57 (m, 5 H), 3.52–3.38 (m, 4 H), 3.31–3.24 (m, 5 H), 3.20–3.18 (m, 2 H), 3.04 (dd, *J* = 12.0 and 4.0, 1 H), 2.82 (dt, *J* = 13.1 and 4.0, 1 H), 2.39 (dt, *J* = 9.6 and 3.2, 1 H), 2.17–2.11 (m, 1 H), 2.10 (dd, *J* = 14.8 and 3.9, 1 H), 2.04 (dd, *J* = 14.6 and 3.3, 1 H), 1.67 (td, *J* = 13.7 and 2.2, 1 H), 1.48 (dt, *J* = 13.8 and 3.6, 1 H), 1.29 (d, *J* = 6.2, 3 H), 0.87 (d, *J* = 6.9, 3 H); ^{13}C NMR (125 MHz, CD_3OD) δ 213.48, 167.56, 165.11, 133.19 (2 C), 124.17, 115.33 (2 C), 106.72, 103.96, 102.46, 99.87, 91.44, 85.66, 83.71, 78.00, 77.56, 77.45, 76.96, 76.83, 75.76, 74.01, 72.32, 72.23, 71.05, 70.86, 70.19, 70.10, 69.15, 63.66, 62.55, 61.76, 56.30 (phenoxy methyl), 47.24, 39.49, 36.03, 34.41, 32.89, 27.26, 17.97, 12.98; high-resolution mass spectrum (FAB) *m/z* 973.3116 [(M + Na)$^+$, calcd for $C_{41}H_{58}O_{23}SNa$ 973.2987].

Acetylation of Breynin A. At room temperature a solution of breynin A (**1**) (30.0 mg, 0.032 mmol) in pyridine (2.0 mL) was treated with a solution of acetic anhydride (392 mg. 3.84 mmol) in pyridine (0.5 mL) and a catalytic amount (ca. 1 mg) of DMAP. The reaction was allowed to stir for 48 h. Following concentration in vacuo, purification by HPLC on silica gel with 2% methanol/dichloromethane as eluant afforded the slower eluting undecaacetate **11** (32 mg, 71% yield) and the faster eluting dodecaacetate **10** (3.4 mg, 7% yield).

11: mp 138–140 °C; $[\alpha]^{27}_D$ +20° (*c* 0.73, CH_3OH); UV (CH_3OH) λ_{max} 236 nm (ϵ 14100); IR ($CHCl_3$) 3020 (w), 2940 (w), 1750 (s), 1370 (m), 1220 (s), 1040 (m) cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6) δ 8.26 (d, *J* = 8.6, 2 H, H-21/25), 7.12 (d, *J* = 8.6, 2 H, H-22/24), 5.46–5.36 (m, 5 H, H-6, H-11, H-2‴, H-3‴, and H-4‴), 5.31 (t, *J* = 9.5, 1 H, H-3′), 5.25 (t, *J* = 8.4, 1 H, H-2″), 5.14 (t, *J* = 9.4, 1 H, H-4″), 5.08 (t, *J* = 9.8, 1 H, H-4′), 4.85 (br s, 1 H, H-1‴), 4.73 (br s, 1 H, H-16), 4.63 (d, *J* = 8.1, 1 H, H-1″), 4.41 (dd, *J* = 5.4 and 1.5, 1 H, H-17), 4.33 (dd, *J* = 12.4 and 4.4, 1 H, H-6a′), 4.20–4.08 (m, 4 H, H-3, H-1′ and H-6a,b″), 3.98–3.94 (m, 1 H, H-5‴), 3.89 (dd, *J* = 12.3 and 1.9, 1 H, H-6b′), 3.77 (t, *J* = 10.8, 1 H, H-13a), 3.64 (dd, *J* = 9.1 and 7.9, 1 H, H-2′), 3.57 (t, *J* =

9.4, 1 H, H-3″), 3.43–3.36 (m, 2 H, H-5″ and H-13b), 3.25–3.21 (m, 1 H, H-5′), 2.96 (d, J = 12.1, 1 H, H-2a), 2.76 (dd, J = 12.1 and 4.3, 1 H, H-2b), 2.73–2.70 (m, 1 H, H-4), 2.42 (s, 3 H, phenolic acetate), 2.39 (dd, J = 14.8 and 4.3, 1 H, H-10a), 2.21 (dd, J = 14.4 and 3.5, 1 H, H-10b), 2.09 (s, 3 H, acetate), 1.97 (s, 3 H, acetate), 1.93 (s, 3 H, acetate), 1.85 (s, 3 H, acetate), 1.81 (s, 3 H, acetate), 1.79 (s, 3 H, acetate), 1.78–1.74 (m, 3 H, H-12 and H-5a,b), 1.72 (s, 3 H, acetate), 1.66 (s, 3 H, acetate), 1.63 (s, 3 H, acetate), 1.60 (s, 3 H, acetate), 1.21 (d, J = 6.2, 3 H, H-6‴), 0.70 (d, J = 6.9, 3 H, H-18); ^{13}C NMR (125 MHz, C_6D_6) δ 209.19 (C-8) [170.15, 170.09, 170.03, 169.77, 169.69 (2 C), 169.59, 169.43 (2 C), 169.22, 169.00 (acetate carbonyls)], 165.24 (C-19), 154.78 (C-23), 131.58 (C-21/25), 128.88 (C-20), 122.22 (C-22/24), 101.31 (C-1″), 100.03 (C-1‴), 99.82 (C-1′), 98.88 (C-9), 86.66 (C-3), 82.30 (C-3″), 77.86 (C-2′), 76.77 (C-16), 74.88 (C-3′), 74.18 (C-7), 72.74, 72.03 (C-2″), 71.79 (C-5′ and C-5″), 70.87 (C-4″), 70.80, 70.43, 69.55 (C-4‴), 69.37 (C-11), 68.73 (C-4′), 68.05 (C-5‴), 63.13 (C-13), 62.98 (C-6″), 61.79 (C-6′), 45.48 (C-17), 39.26 (C-4), 35.04 (C-2), 33.08 (C-12), 31.76 (C-10), 24.08 (C-5) [20.85 (3 C), 20.79, 20.64, 20.44, 20.26 (3 C), 20.21 (2 C) (acetate methyls)], 17.27 (C-6‴), 12.37 (C-18); high-resolution mass spectrum (FAB) m/z 1421.3822 [(M + Na)$^+$, calcd for $C_{62}H_{78}O_{34}SNa$ 1421.3993].

Anal. Calcd for $C_{62}H_{78}O_{34}S$: C, 53.22; H, 5.62. Found: C, 53.33; H, 5.90.

10: mp 133–135 °C; $[\alpha]^{27}_D$ +7.1° (c 0.49, CH_3OH); UV (CH_3OH) λ_{max} 236 nm (ϵ 11 700); IR ($CHCl_3$) 3020 (w), 2940 (w), 1750 (s), 1370 (m), 1220 (s), 1040 (m) cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6) δ 8.20 (d, J = 8.7, 2 H), 7.01 (d, J = 8.7, 2 H), 5.67–5.64 (m, 1 H) 5.60 (br s, 1 H), 5.48 (s, 1 H), 5.45–5.27 (m, 5 H), 5.20 (t, J = 9.5, 1 H), 5.08 (t, J = 9.8, 1 H), 4.87 (d, J = 1.7, 1 H), 4.63 (d, J = 4.7, 1 H), 4.55 (d, J = 8.1, 1 H), 4.34 (dd, J = 12.4 and 4.1, 1 H), 4.30 (dd, J = 12.3 and 3.8, 1 H), 4.15 (dd, J = 12.1 and 3.5, 1 H), 4.11 (d, J = 7.7, 1 H), 4.00–3.94 (m, 2 H), 3.90–3.85 (m, 2 H), 3.82 (dd, J = 11.8 and 3.9, 1 H), 3.60–3.53 (m, 2 H), 3.31 (dt, J = 9.7 and 3.5, 1 H), 3.23–3.19 (m, 1 H), 3.01 (d, J = 12.1, 1 H), 2.77 (dt, J = 13.2 and 4.1, 1 H), 2.71 (dd, J = 12.2 and 4.3, 1 H), 2.52 (dd, J = 13.9 and 7.0, 1 H), 2.44–2.42 (m, 1 H), 2.40 (s, 3 H), 2.07 (s, 3 H), 1.97 (s, 3 H), 1.94 (s, 3 H), 1.92 (s, 3 H), 1.89–1.85 (m, 1 H), 1.84 (s, 3 H), 1.78 (s, 3 H), 1.74–1.72 (m, 2 H), 1.72 (s, 3 H), 1.64 (s, 3 H), 1.63 (s, 3 H), 1.62 (s, 3 H), 1.59 (s, 3 H), 1.20 (d, J = 6.2, 3 H), 0.91 (d, J = 6.9, 3 H); ^{13}C NMR (125 MHz, C_6D_6) δ 204.61, 170.33, 170.03, 169.91, 169.73, 169.55, 169.52, 169.31 (2 C), 169.18, 169.01, 168.68, 167.67, 165.16, 154.62, 131.44 (2 C), 129.04, 122.01 (2 C), 101.54, 100.20, 99.94, 99.31, 86.23, 82.35, 77.46, 75.01, 72.22, 71.80, 71.03, 70.99, 70.81, 70.45, 70.13, 70.06, 69.47, 68.82, 68.53, 68.02, 64.52, 62.31, 61.63, 45.30, 38.62, 35.23, 32.73, 31.73, 24.28, 20.83 (2 C), 20.76, 20.70, 20.60, 20.51, 20.23 (3 C), 20.19 (2 C), 20.11, 17.27, 11.94; high-resolution mass spectrum (FAB) m/z 1463.4182 [(M + Na)$^+$, calcd for $C_{64}H_{80}O_{35}SNa$ 1463.4099].

Anal. Calcd for $C_{64}H_{80}O_{35}S$: C, 53.33; H, 5.59. Found: C, 53.20; H, 5.75.

Acetylation of Breynin B. A solution of breynin B (**2**) (26.0 mg, 0.027 mmol) in pyridine (5 mL) was treated with a solution of acetic anhydride (669 mg, 6.55 mmol) in pyridine (2 mL) and a catalytic amount (ca. 1 mg) DMAP at room temperature. The reaction mixture was stirred for 16 h. Concentration in vacuo followed by HPLC on silica gel with 2% methanol–dichloromethane as eluant afforded **12** (10.8 mg, 28% yield) as a solid: mp 145–146 °C; $[\alpha]^{27}_D$ −2° (c 0.2, CH_3OH); UV (CH_3OH) λ_{max} 235 nm (ϵ 13 300); IR ($CHCl_3$) 1750 (s), 1370 (m), 1220 (s), 1040 (m) cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6) δ 8.30 (d, J = 8.7, 2 H, H-21/25), 7.25 (d, J = 8.7, 2 H, H-22/24), 5.46–5.39 (m, 5 H, H-6, H-11, H-2‴, H-3‴, and H-4‴), 5.32 (t, J = 9.4, 1 H, H-3′), 5.28–5.22 (m, 2 H, H-2″ and H-4″), 5.10 (t, J = 9.8, 1 H, H-4′), 5.04 (d, J = 2.9, 1 H, H-16), 4.84 (d, J = 1.7, 1 H, H-1‴), 4.71 (d, J = 8.0, 1 H, H-1″), 4.38–4.32 (m, 2 H, H-6′a and H-6″a), 4.25 (d, J = 7.6, 1 H, H-1′), 4.19 (dd, J = 12.3 and 3.2, 1 H, H-6″b), 4.16–4.14 (m, 1 H, H-3), 4.08–4.00 (m, 2 H, H-17 and H-5‴), 3.93 (dd, J = 12.4 and 2.1, 1 H, H-6′b), 3.90–3.86 (m, 1 H, H-13a), 3.68 (dd, J = 9.2 and 7.6, 1 H, H-2′), 3.61 (t, J = 9.4, 1 H, H-3″), 3.59–3.56 (m, 1 H, H-5″), 3.46 (dd, J = 11.4 and 4.4, 1 H, H-13b), 3.24–3.18 (m, 2 H, H-2a and H-5′), 3.10–3.07 (m, 1 H, H-4), 3.01 (br d, J = 14.9, 1 H, H-2a), 2.39 (s, 3 H, phenolic acetate), 2.31 (dd, J = 14.5 and 4.1, 1 H, H-10a), 2.20 (dd, J = 14.5 and 3.5, 1 H, H-10b), 2.01 (s, 3 H, acetate), 1.95 (s, 3 H, acetate), 1.94 (s, 3 H, acetate), 2.00–1.85 (m, 2 H, H-5a and H-12), 1.84 (s, 3 H, acetate), 1.83 (s, 3 H, acetate), 1.76 (s, 3 H, acetate), 1.72 (s, 3 H, acetate), 1.66 (s, 3 H, acetate), 1.63 (s, 3 H, acetate), 1.61 (s, 3 H, acetate), 1.43–1.34 (m, 1 H, H-5b), 1.22 (d, J = 6.2, 3 H, H-6‴), 0.72 (d, J = 6.9, 3 H, H-18); ^{13}C NMR (125 MHz, C_6D_6) δ 208.90, 170.71, 170.21, 170.06, 169.83, 169.63, 169.57, 169.57, 169.54 (2 C), 169.45, 169.23, 168.95, 165.55, 155.01, 131.49 (2 C), 128.68, 122.52 (2 C), 101.25, 100.11, 99.99, 99.35, 83.00, 82.48, 77.47, 74.75, 74.02, 73.72, 72.32, 72.08, 71.87, 71.79, 70.82, 70.41, 70.14, 69.61, 69.26, 68.65, 68.06, 63.20, 62.31, 61.72, 56.57, 37.47, 33.05, 31.87, 25.73, 20.89, 20.86, 20.70, 20.67, 20.63, 20.39, 20.29 (2 C), 20.24 (3 C), 17.30, 12.46; high-resolution mass spectrum (FAB) m/z 1437.3991 [(M + Na)$^+$, calcd for $C_{62}H_{78}O_{35}SNa$ 1437.3942].

Anal. Calcd for $C_{62}H_{78}O_{35}S$: C, 52.62; H, 5.55. Found: C, 52.42; H, 5.39.

Acknowledgment. We are pleased to acknowledge support of this investigation by the National Institutes of Health (National Cancer Institute) through Grant CA19033 to A.B.S. and a Postdoctoral Fellowship to T.P.K. and by a Fulbright travel fellowship to R.T.G. We also thank Mr. John Dykins (University of Pennsylvania) and Dr. Mark Hemling (SmithKline Beecham Pharmaceuticals) for providing mass spectra and Dr. Clemens Anklin (Bruker Instruments, Inc.) for measuring 600-MHz NMR spectra. Dr. Christopher S. Shiner provided helpful suggestions and critical comments.

Supplementary Material Available: ^{1}H (500-MHz) and ^{13}C (125-MHz) NMR spectra of breynin A (**1**), breynin B (**2**), C-(23)-methyl ether of breynin A (**9**), and the minor sulfoxide (i.e., epibreynin B) derived from breynin A (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.