the mechanism of the reaction suggests that it is an S_N2 mechanism, with the RDS being attack of the phosphine nucleophile on the S-S bond.

Experimental Section

General. Tris(2-cyanoethyl)phosphine was obtained from American Cyanamid.[19] Deuterated materials were obtained from Cambridge Isotope Laboratories. Other chemicals were obtained from Aldrich.

Tris(2-carboxyethyl)phosphine Hydrochloride. A slurry of tris(2-cyanoethyl)phosphine (44.6 g, 0.231 mol) in concd aqueous HCl (150 mL) was heated at reflux under an argon atmosphere for 2 h. A clear white precipitate formed when the hot clear solution was cooled to 0 °C. The precipitate was isolated by filtration. Recrystallization from water (200 mL), filtration, and drying in vacuo afforded 28.5 g of white crystals (99.4 mmol, 43%). The combined supernatants were concentrated to 110 mL by boiling. More white crystalline precipitate formed when the mixture was cooled to 0 °C. Filtration, rinsing with 20 mL of water at 0 °C, and drying in vacuo gave 29.5 g of white crystals (88% yield for the combined crops). ^{1}H NMR (D_2O): δ 2.76 (dt, 6 H, $J_{1,P}$ = 18.2 Hz, $J_{1,2}$ = 7.0 Hz), 2.47 (dt, 6 H, $J_{2,P}$ = 13.9 Hz). Mp: 176 °C (lit.[6] mp 175-177 °C). UV: λ_{max} = 218 nm, ϵ = 180 L mol^{-1} cm^{-1}; λ_{max} = 192 nm, ϵ = 150 L mol^{-1} cm^{-1}. Anal. Calcd for $C_9H_{16}O_6PCl$: C, 37.71; H, 5.63; P, 10.81; Cl, 12.37. Found: C, 37.61; H, 5.65; P, 10.99; Cl, 12.38.

Tris(2-carboxyethyl)phosphine Oxide. A crystal of iodine was allowed to react with an aliquot (0.5 mL) of a solution of TCEP·HCl (10 mM) in D_2O. ^{1}H NMR (D_2O): δ 2.66 (dt, 6 H, $J_{1,P}$ = 10.9 Hz, $J_{1,2}$ = 8.0 Hz), 2.20 (dt, 6 H, $J_{2,P}$ = 11.5 Hz).

Competitive Reductions. The competitive reductions were carried out by diluting from stock solutions 1 equiv of each of two disulfides and 1 equiv of 2-butyne-1,4-diol (as an internal standard for ^{1}H NMR spectroscopy) in buffer (20 mM acetate-d_3 in D_2O, pD = 4.5). In mild acid, neither autoxidation nor thiol-disulfide interchange occurs at an appreciable rate. An aliquot of TCEP·HCl stock solution in D_2O (0.8 equiv) was added to the reaction mixture. The reaction mixture was transferred to an NMR tube which was flushed with argon, stoppered, and closed with Parafilm. At the concentrations (0.2-1.0 mM) and temperature (22-25 °C) we used, the reaction was complete within 5 min for all disulfides.

Air Oxidation of Dilute Solutions of TCEP. Deuterated buffer solutions were made by neutralizing solutions of acetic acid-d_4 (0.40 M) or phosphoric acid-d_3 (0.35 M) with 40% NaOD in D_2O to pD 5.0 (acetate), 6.6, 7.4, and 11.6 (phosphate). To 4.5 mL of each solution was added 500 μL (25 mmol) of a solution of TCEP·HCl (49 mM in D_2O). The reaction mixtures were vigorously stirred under air. Aliquots were examined by ^{1}H NMR spectroscopy after 30 min, 23 h, and 72 h.

Acknowledgment. We thank our colleague R. H. Holm for the use of a UV spectrometer. This research was supported by the NIH through Grant GM39589 and by the National Science Foundation under the Engineering Research Center Initiative Biotechnology Process Engineering Center (Cooperative Agreement CDR-88-03014). NMR facilities were provided by the National Science Foundation under grant CHE-84-10774. J.A.B. was a National Science Foundation predoctoral fellow, 1986-1989.

(19) American Cyanamid no longer sells tris(2-cyanoethyl)phosphine. It is available from Strem.

Structure Determination of Natural Epoxycyclopentanes by X-ray Crystallography and NMR Spectroscopy[1]

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Two stereoisomeric epoxycyclopentane cyanohydrin glucosides were isolated, along with several previously described cyclopentene cyanohydrin glucosides, from *Passiflora suberosa* L. (Passifloraceae) and *Kiggelaria africana* L. (Flacourtiaceae); they represent new members of a rare class of natural nonannellated cyclopentane derivatives. The new glucosides were shown, by NMR spectroscopy (including NOE measurements), X-ray crystallography, and enzymatic hydrolysis to the corresponding cyanohydrins, to be (1*R*,2*R*,3*R*,4*R*)- and (1*S*,2*S*,3*S*,4*S*)-1-(β-D-glucopyranosyloxy)-2,3-epoxy-4-hydroxycyclopentane-1-carbonitrile and named suberin A and B, respectively. The crystal structure of suberin A was determined at 110 K and refined to R = 0.036 for 2198 unique reflections; the cyclopentane ring forms an envelope with C5 placed 0.41 Å away from the plane of the remaining four carbon atoms, and to the same side as the three oxygen substituents. In addition to the glucosides, two amides, (1*S*,2*S*,3*R*,4*R*)-2,3-epoxy-1,4-dihydroxycyclopentane-1-carboxamide and (1*S*,4*R*)-1,4-dihydroxy-2-cyclopentene-1-carboxamide, were isolated from *P. suberosa* and characterized; the amides are probably artefacts, and their formation represents a novel enzymatic transformation of plant cyanohydrins.

Introduction

In contrast to the vast abundance of natural products having a cyclopentane ring as a part of a polycyclic system, relatively few naturally occurring nonannellated cyclopentane derivatives are known. Such natural products can be divided into four major groups. These are the prostaglandins,[2] the antibiotics pentenomycins and related mold and bacterial metabolites,[3-5] a few monoterpenes[6] including

(1) Part 13 of the series on natural cyclopentanoid cyanohydrin glycosides. For part 12, see: Olafsdottir, E. S.; Jaroszewski, J. W.; Seigler, D. S. *Phytochemistry* **1991**, *30*, 867.

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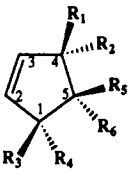
Table I. ^{1}H NMR Spectral Data of Suberin A (2a) and Suberin B (3a) and Their Derivatives[a]

	chemical shifts (δ) and coupling constants (Hz)					
compd	H2[b] ($^3J_{2,3}$)	H3 ($^3J_{3,4}$)	H4[c] ($^3J_{4,5R}$, $^3J_{4,5S}$)	H5$_R$, H5$_S$[c] ($^2J_{5R,5S}$)	H1′ ($^3J_{1,2}$)	remaining sugar resonances
2a	3.98	3.62	4.23	2.45, 1.90	4.70	H2′ 3.25,[d] H6′ 3.86 and 3.69,[e] other 3.3–3.4
	(2.8)	(1.1)	(7.3, 9.1)	(13.2)	(7.7)	
3a	3.99	3.60	4.24	2.48, 1.81	4.69	H2′ 3.27,[d] H6′ 3.89 and 3.68,[e] other 3.3–3.4
	(2.8)	(1.2)	(7.3, 9.0)	(13.0)	(7.7)	
2b[f]	3.84	3.79	5.12[j]	2.61, 1.93	4.94	H3′ 5.26,[g] H5′ 3.79, H6′ 4.24 and 4.16,[h] other 5.0–5.2
	(2.8)	(1.3)	(7.6, 8.9)	(13.4)	(7.9)	
3b[i]	3.85	3.76	5.10[j]	2.63, 1.90	4.94	H3′ 5.25,[g] H5′ 3.83, H6′ 4.25 and 4.21,[h] other 5.0–5.2
	(2.7)	(1.3)	(7.8, 8.9)	(13.4)	(7.9)	
2c	3.84	3.49	4.28	2.38, 1.94	4.61	H6′ 3.76 and 3.63,[j] other 3.2–3.5
	(2.8)	(1.2)	(7.2, 8.9)	(13.3)	(7.2)	
3c	3.98	3.45[k]	4.28	2.37, 1.91	4.63	H6′ 3.80 and 3.61,[l] other 3.2–3.5
	(2.5)		(7.2, 8.8)	(12.8)	(7.2)	
2d, 3d	3.78	3.69	4.36	2.65, 1.68		
	(2.8)	(1.3)	(7.6, 8.5)	(13.4)		
4	3.49	4.00	4.46	2.52, 2.28		
	(2.5)	(2.2)	(6.6, 8.2)	(17.8)		
5	3.39	3.53	4.35	2.11, 1.44		
	(2.9)	(1.4)	(7.9, 8.1)	(12.7)		
6	5.75	6.02	4.85	2.84, 1.66		
	(5.5)	(1.9)	(4.8, 7.2)	(13.5)		

[a] In methanol-d_4 (**2a**, **3a**, **5**, and **6**) or chloroform-d (remaining compounds). [b] $^4J_{2,4}$ 0.5 Hz (1.4 Hz for **5** and 1.3 Hz for **6**). [c] The assignments of the H5$_R$ and H5$_S$ resonances were obtained for **2a** and **3a** from NOE measurements, and the same assignment (H5$_R$ at higher ppm value than H5$_S$) was assumed for the remaining compounds. [d] $^3J_{2,3}$ 9.0 Hz. [e] $^2J_{gem}$ 12.0, $^3J_{5,6}$ 5.4 and 2.0 Hz. [f] Acetyl groups at δ 2.12, 2.08, 2.08, 2.04, and 2.01. [g] $^3J_{2,3}$ and $^3J_{3,4}$ 9.0 Hz. [h] $^2J_{gem}$ 12.3, $^3J_{5,6}$ 2.8 and 5.6 Hz. [i] Acetyl groups at δ 2.11, 2.08, 2.05, 2.04, and 2.02. [j] $^2J_{gem}$ 11.1, $^3J_{5,6}$ 5.7 and 2.2 Hz. [k] Assigned by use of COSY spectra. [l] $^2J_{gem}$ 10.8, $^3J_{5,6}$ 6.8 and 1.8 Hz.

chemical defense compounds of insects,[7] and the natural products derived from 2-cyclopentenyl-L-glycine. The latter group consists of fatty acids[8,9] and cyclopentanoid cyanohydrin glycosides.[10–15] All glycosides derived from 2-cyclopentenyl-L-glycine described thus far retained the double bond in the ring, as exemplified by **1a–g**.[16] The compounds are produced by the tropical plant families Passifloraceae, Flacourtiaceae, and their close allies.[17]



1

a: $R_1 = R_2 = H$, $R_3 = CN$, $R_4 = OGlc$, $R_5 = R_6 = H$
b: $R_1 = R_2 = H$, $R_3 = OGlc$, $R_4 = CN$, $R_5 = R_6 = H$
c: $R_1 = H$, $R_2 = OH$, $R_3 = CN$, $R_4 = OGlc$, $R_5 = R_6 = H$
d: $R_1 = OH$, $R_2 = H$, $R_3 = OGlc$, $R_4 = CN$, $R_5 = R_6 = H$
e: $R_1 = H$, $R_2 = OH$, $R_3 = OGlc$, $R_4 = CN$, $R_5 = R_6 = H$
f: $R_1 = OH$, $R_2 = H$, $R_3 = CN$, $R_4 = OGlc$, $R_5 = R_6 = H$
g: $R_1 = R_6 = H$, $R_2 = R_5 = OH$, $R_3 = CN$, $R_4 = OGlc$

Glc = β-D-glucopyranosyl

Structures and stereochemistry of the epoxy derivatives **2a** and **3a**, which are two new members of this group, are described below; the trivial names suberin A and B, respectively, are suggested for these epoxides.[18] Nonannellated epoxycyclopentanes have so far been isolated only from bacteria and fungi;[5] this is thus the first case of demonstration of such epoxides in plants. Compounds of this kind can be of interest as chiral starting materials for the synthesis of biologically active compounds, for example the prostaglandins.[19]

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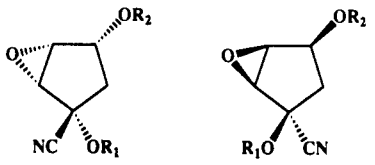
Table II. ^{13}C NMR Spectral Data of Suberin A (2a) and Suberin B (3a) and Their Derivatives[a]

compd	chemical shifts (δ)						
	C1	C2 and C3	C4	C5	CN	C1′	remaining glucose carbons
2a	75.6	55.6, 55.8	67.9	36.2	116.8	99.8	60.6, 69.3, 72.8, 76.0, 76.4
3a	75.9	56.8, 57.0	67.2	36.0	116.3	100.1	60.6, 69.4, 72.7, 76.0, 76.4
2b[b]	75.9	54.6, 56.0	70.1	34.4	116.2	98.0	61.8, 68.2, 70.9, 72.6, 72.6
3b[c]	76.0	55.4, 57.2	69.7	34.5	115.8	98.2	61.8, 68.2, 70.8, 72.6, 72.6
2c	75.4	56.2, 56.4	69.2	38.1	117.3	100.4	61.9, 71.4, 75.0, 77.3, 78.1
3c	76.0	57.5, 57.7	68.3	37.1	116.8	100.7	62.2, 71.7, 74.9, 77.3, 78.3
5[d]	80.8	60.0, 60.6	72.1	39.9			
6[e]	86.3	136.5, 139.7	76.5	49.2			

[a] In methanol-d_4 (**2a**, **3a**, **5**, and **6**) or chloroform-*d* (remaining compounds). [b] Acetyl groups: δ 20.5 (5 CH_3), 169.3, 169.3, 170.2 170.3, 170.5. [c] Acetyl groups: δ 20.6 (3 CH_3), 20.7 (2 CH_3), the carbonyl carbons were not assigned. [d] Amide carbon: δ 179.1. [e] Amide carbon: δ 180.6.

Results and Discussion

Extraction of *Passiflora suberosa* L. (Passifloraceae), a vine common in tropical America and Southeast Asia, and fractionation of the extract by column chromatography gave three cyanogenic fractions, which were further purified by HPLC as described in the Experimental Section. The two minor fractions were known glucosides: tetraphyllin A (**1b**)[11,12] and a mixture of epivolkenin (**1c**) and taraktophyllin (**1d**).[15] The main cyanogenic fraction was the epoxide **2a**.



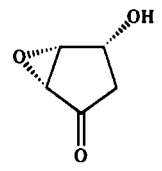
2 3

a: R_1 = β-D-glucopyranosyl, R_2 = H

b: R_1 = tetra-O-acetyl-β-D-glucopyranosyl, R_2 = $COCH_3$

c: R_1 = tetra-O-trimethylsilyl-β-D-glucopyranosyl, R_2 = $Si(CH_3)_3$

d: R_1 = R_2 = H



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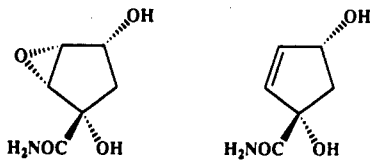

5 6

The NMR spectra of the major glucoside, in contrast to all previously isolated cyclopentanoids, showed no olefinic signals; instead, ^1H resonances at 3.62 and 3.98 ppm, with a characteristic splitting of about 3 Hz (Table I), and ^{13}C resonances at 55.6 and 55.8 ppm (Table II), corresponding to epoxide protons and carbons,[20] were observed. Other resonances in the ^1H and ^{13}C NMR spectra, including the glucopyranosyl group signals, were similar to those reported before for **1c–f**.[14,15]

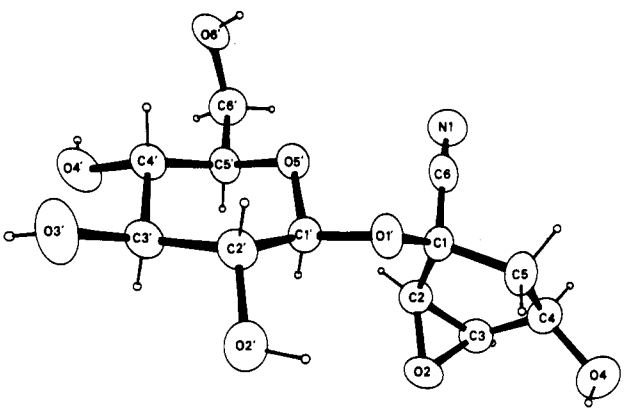

Figure 1. ORTEP drawing[32] of the molecule of **2a**, showing the numbering system of atoms.

The aglucon of 1-(β-D-glucopyranosyloxy)-2,3-epoxy-4-hydroxycyclopentane-1-carbonitrile has four chiral centers and eight possible stereoisomers. The relative configuration of C3 and C4 was established on the basis of NOE difference spectra. Irradiation of the H4 resonance at δ 4.23 ppm caused 5% enhancement of the H3 signal at δ 3.62 ppm, 4% enhancement of the $H5_R$ signal at δ 2.45 ppm, and had no effect on the intensity of the $H5_S$ signal at δ 1.90 ppm. However, the configuration of the remaining asymmetric center (C1) could not be inferred from the NMR data alone.[21] Therefore, an X-ray analysis of **2a** was carried out.

The structure was determined at 110 K and refined to R = 0.036 as described in the Experimental Section. The configuration of the aglucon was found to be 1*R*,2*R*,3*R*,4*R* by reference to the β-D-glucopyranosyl group. The conformation of **2a** found in the crystal is shown in Figure 1. The cyclopentane ring adopts an envelope conformation with C5 placed 0.41 Å away from the plane formed by the remaining carbons and to the same side as the three attached oxygens. The geometry of the glucopyranosyl group is in agreement with that normally found.[22]

The packing of the molecules in the crystal is dominated by the five hydroxy groups, which form hydrogen bonds to neighboring molecules, as shown in Figure 2. Three

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(21) For cyclopentene cyanohydrin glucosides with enantiomeric aglycons (**1a–f**), the chemical shift of the anomeric proton of the β-D-glucopyranose moiety has a lower value for the 1*S* configuration than for the 1*R* configuration.[12–15] However, the chemical shifts of the anomeric protons in **2a** and **3a** are practically the same (Table I) and hence give no information about the configuration of C1. Also, we were not able to observe NOE's between the glucopyranosyl group and the protons attached to C5.

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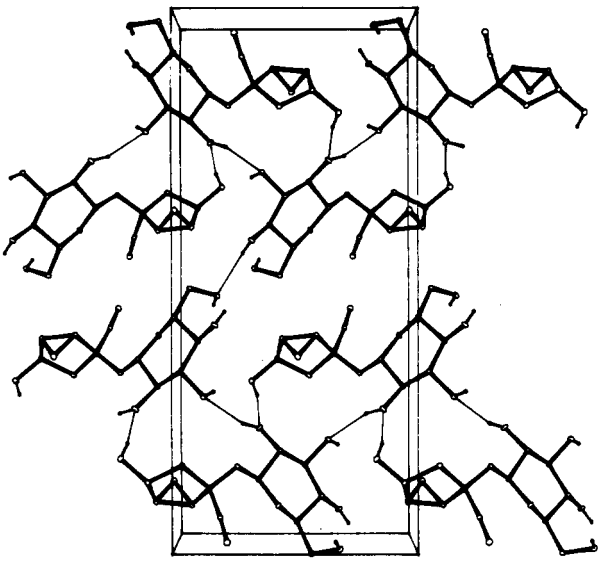

Figure 2. The packing of the molecules of **2a** in the crystal. Hydrogen bonds are represented by thin lines. The x-axis points into the paper, the y-axis points from left to right, and the z-axis downward.

of the hydroxy groups (4-OH, 2′-OH and 3′-OH) connect the molecules in a helix along the x-axis, assisted by 6′-OH forming a hydrogen bond to O4′ in the adjacent molecule along the x-axis. These hydrogen bonds are the principal forces that link the molecules in the x- and y-axis directions, building double layers of molecules. The outer surface of the molecules of these layers (at $z = 0$, $z = 0.5$, etc.) is apolar, apart from a single hydrogen bond from 4′-OH to O6′. Therefore, only weak forces hold the molecules in the z-axis direction. The extreme difficulties in growing suitable crystals for the structure determination are thus understood, as the crystals grew as very thin needles in the x-axis direction, showing more or less disorder in the packing in the z-axis direction.

The two oxygens at C1 and C4 in the crystals of **2a** are in a quasiequatorial position, as shown in Figure 1. By examining the values of the $^3J_{H4,H5}$ coupling constants,[23] the conformation of the epoxycyclopentane ring of **2a** and **3a** in solution can be concluded to be identical with that in the crystal. The $^3J_{H4,H5R}$ is about the same for the cyclopentenes **1c** and **1d**, which have quasi-axial oxygens at C1 and C4 (7.2 Hz), and the epoxides **2a** and **3a**, which have quasi-equatorial oxygens at C1 and C4 (7.3 Hz), whereas the $^3J_{H4,H5S}$ is considerably larger for the quasi-diequatorial conformation (9.0 Hz) than for the quasi-diaxial one (4.8 Hz).[23]

An epoxide similar to **2a** was isolated from *Kiggelaria africana* L. (Flacourtiaceae), which produces gynocardin (**1g**) as the major cyclopentanoid.[10,24] Other minor glucosides isolated were deidaclin (**1a**), tetraphyllin A (**1b**), epivolkenin (**1c**), and taraktophyllin (**1d**).[12,15]

The ^{1}H and ^{13}C NMR spectra of the second epoxide were only slightly different from those of **2a** (Tables I and II). The only significant difference in the ^{1}H NMR spectra was in the chemical shifts of the geminal H5 protons, which were 1.81 and 2.48 ppm ($\Delta\delta$ 0.67 ppm), as compared to 1.90 and 2.45 ppm for **2a** ($\Delta\delta$ 0.55 ppm). In the ^{13}C NMR spectra of **2a** and the new epoxide, all chemical shifts differed by less than 1.5 ppm, indicating that the compounds are closely related stereoisomers. NOE spectra with irradiation of H4 at 4.24 ppm gave results, which were identical to those obtained with **2a**, i.e., about 5% enhancement of H3 and $H5_R$ signals at 3.60 and 2.48 ppm, respectively. Thus, the two epoxides have the same relative stereochemistry of C3 and C4, and hence also of C2. The configuration of the last asymmetric center (C1) was established as follows.

The two $^3J_{H4,H5}$ coupling constants in cyclopentenes **1c** and **1d** (the cis-1,4-dioxygenated pair) are identical, i.e., 7.2 Hz ($^3J_{cis}$) and 4.8 Hz ($^3J_{trans}$).[15] For **1e** and **1f** (the trans-1,4-dioxygenated pair) the coupling constants are again identical, i.e., 6.5 Hz ($^3J_{cis}$) and 4.0 Hz ($^3J_{trans}$),[14] but significantly different from the former values. The same pattern is observed for the acetates and TMS derivatives of these stereoisomeric pairs.[14,15] These relationships reflect the dependence of the coupling constants on the geometry of the coupling path and orientations of electronegative substituents relative to the coupled nuclei. The ring geometry can hence be concluded to be identical for the glucosides with enantiomeric aglucons (**1c** and **1d**, **1e** and **1f**), irrespective of the presence of the same β-D-glucopyranosyl group in each case. The same can be assumed to be valid for epoxy derivatives of these cyclopentenes. For the epoxides, as well as their acetates and TMS derivatives, the two $^3J_{H4,H5}$ couplings (cis and trans) are identical for each pair, i.e., **2a** and **3a**, **2b** and **3b**, and **2c** and **3c** (Table I). This indicates, that the configuration of C1 relative to that of C4 is the same in both epoxides.

Corroborative evidence for the aglucon structure of **3a** is obtained by considering the chemical shifts of the geminal protons attached to C5. The chemical shift differences between the geminal protons of the cis-1,4-dioxygenated cyclopentenes, **1c** and **1d**, and their derivatives ($\Delta\delta$ 0.69–0.98 ppm),[15] are greater than the same differences in the trans-1,4-dioxygenated isomers, **1e** and **1f**, and their derivatives ($\Delta\delta$ 0.18–0.69 ppm).[14] This is due to deshielding by different number of cis oxygens (one oxygen cis to each of the geminal protons in **1e** and **1f**; two cis oxygens to one, and none to the other geminal proton in **1c** and **1d**). Since the differences in the chemical shifts of the geminal protons in **2a–c** and **3a–c** are quite similar, especially for the acetates and TMS ethers (Table I), where interferences from the β-D-glucopyranosyl group and from solvation effects can be assumed to be small, it can be concluded that the number of oxygens cis to each of the geminal protons in **2a–c** and **3a–c** is the same. In other words, the two epoxides have enantiomeric aglucons.

Final proof of the stereochemistry of the epoxides was obtained from their enzymatic hydrolysis to the corresponding cyanohydrins. The ^{1}H NMR spectra of the cyanohydrins **2d** and **3d**, obtained from **2a** and **3a**, respectively, were identical, showing that they indeed are enantiomers. An identity of ^{1}H NMR spectra of two cyanohydrins of 2,3-epoxy-4-hydroxycyclopentan-1-one, which are epimeric at C1, can be safely excluded. Thus, the change of stereochemistry at C1 would affect coupling constants, by changing the conformation of the cyclopentane ring, as well as alter chemical shifts of protons vicinal to the 1-hydroxy group, as discussed above. The configuration of the *Kiggelaria* epoxide is thus 1*S*,2*S*,3*S*,4*S*, as shown in **3a**. Besides the cyanohydrins, the hydrolysis products contained ketone **4**, formed by dissociation of the latter.

Along with **2a**, two amides, **5** and **6**, were isolated from *P. suberosa*, when the plant material was dried prior to extraction, and hence they are probably artefacts of iso-

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lation. NOE measurements were performed on both amides and gave results similar to those obtained with **2a** and **3a**. In addition, irradiation of the high-field H5 resonance gave 7% enhancement of the protons of the two hydroxy groups (DMSO as solvent). Thus, amides **5** and **6** have the same relative configuration at the chiral centers as the aglycons of epoxide **2a** and the cyclopentene **1c**. The absolute configuration of the amides follows from their molecular rotations, dominated by strongly dextrorotatory interaction of the amide group with the double bond or the epoxy group.[14,25] The amides can be logically considered to be degradation products of **2a** and **1c**, respectively; although hydrolysis of cyanohydrin glucosides to the corresponding cyanohydrins and carbonyl compounds is well known,[26] we are not aware of any previous report of efficient hydrolysis to amides by plant enzymes.

It is noteworthy that the epoxides **2a** and **3a** described in this work are present in the two plants as single isomers, in contrast to the corresponding cyclopentenes **1a–f**, which practically always occur as pairs with enantiomeric aglucons.[12-15,17] A plausible explanation of the latter phenomenon is that the enzymes, hydroxylating 2-cyclopentenecarbonitrile (the presumed intermediate[27] in the biosynthesis of **1a–f**) at C1, do not recognize the position of the double bond in the cyclopentene ring. Thus, *both* enantiomers of 2-cyclopentenecarbonitrile are hydroxylated at C1, giving racemic cyanohydrin, which leads to the cooccurrence of **1a** and **1b**. Similarly, further hydroxylation of the latter cyanohydrin at C4 is regioselective, the 4-hydroxy group being introduced either cis or trans to the hydroxy group at C1, but not enantioselective. This leads to cosynthesis of **1c** and **1d** or **1e** and **1f**.

On the other hand, epoxide synthetase involved in the biosynthesis of **2a** or **3a** apparently recognizes the position of the double bond relative to other functional groups in the molecule. This results in selective epoxidation of only one enantiomer and formation of either **2a** or **3a**, *but not both at the same time*, in spite of the fact that **1c**, as well as **1d**, is present in the plants producing these epoxides.

Experimental Section

General Methods. Elemental analyses were performed at the Institute of Chemistry, University of Copenhagen. Melting points were determined in capillaries and are corrected.

Thin-layer chromatographic and column chromatographic separations were carried out using Merck silica gel 60 precoated aluminium plates, and Merck silica gel 60, 0.066–0.2 mm, respectively. HPLC separations were performed using 1.6 × 25 cm columns of Lichrosorb RP-18 (5 μm) or Lichrosorb Si60 (7 μm) and refractive index detection.

(1*R*,2*R*,3*R*,4*R*)-1-(β-D-Glucopyranosyloxy)-2,3-epoxy-4-hydroxycyclopentane-1-carbonitrile (2a). Fresh leaves and stems of *Passiflora suberosa* L. were collected in September in a greenhouse of the Botanical Garden, University of Copenhagen, and were extracted immediately. The material (1200 g) was divided into several portions, and each portion added to 1000 mL of boiling methanol–water (4:1). The mixture was boiled for 3 min, and then the hot mixture was homogenized while hot, boiled for additional 2 min, and filtered. The filter cake was extracted again for 5 min with a fresh portion of boiling solvent. The combined extracts were evaporated almost to dryness. The residue (20 g) was redissolved in methanol, adsorbed on silica gel by evaporation, and chromatographed on a 8 × 30 cm column of silica gel, with mobile phase consisting of ethyl acetate–acetone–dichloromethane–methanol–water (20:15:6:5:4). The fractions (25 mL) were monitored by TLC with the same solvent, using cyanide-specific (sandwich picrate assay[28]) as well as sugar-specific (naphthoresorcinol reagent) method of visualizing the spots. Fractions 65–79 were evaporated and further purified by reverse-phase HPLC using methanol–water (3:7), to give about 4 mg of tetraphyllin A (**1b**), identified by ^{1}H NMR spectra in CD_3OD.[12,29] Fractions 80–237 were evaporated, and the residue (5.1 g) was chromatographed again, on a 4 × 75 cm column of silica gel eluted with ethyl acetate–methanol–water (78:20:2). The crude cyanogenic isolate (4.7 g) was further purified by reverse-phase HPLC using methanol–water (1:9) to give 1380 mg of epoxide **2a** and ca. 100 mg of a mixture of epivolkenin (**1c**) and taraktophyllin (**1d**) (ratio 15:1); the latter mixture was not resolved, but the glucosides were identified and their ratio measured by means of ^{1}H NMR spectra in CD_3OD.[15] The epoxide **2a** was recrystallized from acetone–ethyl acetate: mp 197–198 °C; $[\alpha]^{24}_D$ +12.1° (*c* 0.5, methanol); IR (KBr) 3390 (br, s), 1425 (m), 1380 (w), 1340 (w), 1320 (w), 1295 (m), 1260 (m), 1230 (w), 1150 (m), 1110 (m), 1075 (s), 1035 (s), 985 (m), 910 (s) cm^{-1}.

Anal. Calcd for $C_{12}H_{17}NO_8$: C, 47.53; H, 5.65; N, 4.62. Found: C, 47.14; H, 5.82; N, 4.44.

(1*S*,2*S*,3*S*,4*S*)-1-(β-D-Glucopyranosyloxy)-2,3-epoxy-4-hydroxycyclopentane-1-carbonitrile (3a). Leaves and stems of *Kiggelaria africana* L. obtained from the Botanical Garden, University of Copenhagen, were freeze-dried and pulverized, and the material (48 g) was extracted with 2 × 1.5 L of boiling methanol–water (4:1 v/v). The extract (11.6 g) was fractionated on a 5 × 50 cm silica gel column, similarly as described above. Fractions 40–44 were further purified by reverse-phase HPLC using methanol–water (3:7) to give 6 mg of a mixture of deidaclin (**1a**) and tetraphyllin A (**1b**) (ratio 1:2). Fractions 45–90 were purified in the same way, but using methanol–water in a ratio of 1:9. This yielded 80 mg of a mixture of epivolkenin (**1c**) and taraktophyllin (**1d**) (ratio 3:4), 240 mg of gynocardin (**1g**), and 42 mg of a mixture of the latter and epoxide **3a**, which was separated by normal-phase HPLC with ethyl acetate–acetone–dichloromethane–methanol–water (20:15:6:5:4), to give 35 mg of pure **3a**. Another isolation from 167 g of fresh plant material gave 48 mg of **3a**, recrystallized from acetone–ethyl acetate: mp 130–133 °C; $[\alpha]^{21}_D$ −49° (*c* 0.3, methanol); IR (KBr) 3390 (br, s), 1410 (m), 1340 (m), 1315 (m), 1260 (m), 1240 (w), 1210 (w), 1150 (m), 1070 (s), 1030 (s), 985 (m), 905 (m) cm^{-1}.

Anal. Calcd for $C_{12}H_{17}NO_8$: C, 47.53; H, 5.65; N, 4.62. Found: C, 47.52; H, 5.55; H, 4.25.

Enzymatic Hydrolysis of 2a and 3a. The epoxide (25 mg of **2a** or 15 mg of **3a**) was dissolved in 2 mL of 50 mM phosphate buffer (pH 5.0), and 0.5 mL of *Helix pomatia* enzyme preparation[28,30] (Sigma, cat. no. G 0876, β-glucuronidase activity 10^4 units/mL) was added. After 35 min at room temperature, the solution was extracted with ethyl acetate (3 × 2 mL), the extract was evaporated, and the residue was dissolved in chloroform-*d* and immediately examined by ^{1}H NMR. The cyanohydrins present in both cases gave identical spectra (Table I); besides the cyanohydrins, the spectra showed the presence of the respective ketones (4 and its enantiomer).

(1*R*,2*R*,3*R*,4*R*)-1-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-2,3-epoxy-4-hydroxycyclopentane-1-carbonitrile (2b). The epoxide **2a** was treated overnight with a 1:1 mixture of acetic anhydride and pyridine, the mixture was evaporated, and the resulting acetate was recrystallized from ether–petroleum ether: mp 184.5–185.5 °C; $[\alpha]^{24}_D$ +11.6° (*c* 0.5, chloroform); IR (KBr) 2960 (w), 1740 (s), 1370 (m), 1220 (s), 1065 (s), 900 (m) cm^{-1}.

Anal. Calcd for $C_{22}H_{27}NO_{13}$: C, 51.46; H, 5.30; N, 2.73. Found: C, 51.66; H, 5.35; N, 2.65.

(1*S*,2*S*,3*S*,4*S*)-1-(Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-2,3-epoxy-4-hydroxycyclopentane-1-carbonitrile (3b). The acetate **3b**, obtained as described for **2b**, was recrystallized

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Table III. Fractional Coordinates and Isotropic Equivalent Thermal Parameters for Non-hydrogen Atoms (esd's in Parentheses)

$$B_{eq} = 4/3\sum_i\sum_j \beta_{ij} a_i a_j$$

atom	x	y	z	B_{eq}
C1	0.0811 (3)	0.3358 (2)	0.13537 (9)	0.79 (3)
C2	0.2671 (4)	0.4112 (2)	0.0995 (1)	0.87 (3)
C3	0.2241 (4)	0.5541 (2)	0.1068 (1)	0.98 (3)
C4	0.0119 (4)	0.5750 (2)	0.1472 (1)	0.94 (3)
C5	−0.0247 (4)	0.4401 (2)	0.1782 (1)	0.94 (3)
C6	−0.0924 (4)	0.2889 (2)	0.0894 (1)	0.84 (3)
N1	−0.2206 (3)	0.2602 (2)	0.05170 (9)	1.20 (3)
O2	0.4172 (3)	0.4888 (1)	0.13762 (7)	1.16 (2)
O4	0.0357 (3)	0.6820 (2)	0.18862 (7)	1.28 (3)
C1′	0.3294 (3)	0.1461 (2)	0.14389 (9)	0.71 (3)
C2′	0.4444 (3)	0.0611 (2)	0.19239 (9)	0.76 (3)
C3′	0.6096 (3)	−0.0399 (2)	0.16268 (9)	0.74 (3)
C4′	0.4920 (3)	−0.1073 (2)	0.1083 (1)	0.73 (3)
C5′	0.3785 (3)	−0.0059 (2)	0.06589 (9)	0.76 (3)
C6′	0.2522 (4)	−0.0647 (2)	0.01206 (9)	0.98 (3)
O1′	0.1640 (2)	0.2287 (1)	0.17160 (7)	0.78 (2)
O2′	0.5800 (3)	0.1447 (2)	0.23111 (7)	1.29 (2)
O3′	0.6710 (3)	−0.1358 (1)	0.20708 (7)	1.03 (2)
O4′	0.6723 (2)	−0.1798 (2)	0.07782 (7)	1.01 (2)
O5′	0.2106 (2)	0.0671 (1)	0.10057 (7)	0.83 (2)
O6′	0.1065 (2)	−0.1751 (1)	0.02592 (7)	0.92 (2)

from ether–petroleum ether: mp 155–156 °C; $[\alpha]^{24}_D$ −9.0° (*c* 0.3, chloroform); IR (KBr) 2960 (w), 2920 (m), 1750 (s), 1370 (m), 1220 (s), 1070 (s), 900 (m) cm^{-1}.

Anal. Calcd for $C_{22}H_{27}NO_{13}$: C, 51.46; H, 5.30; N, 2.73. Found: C, 51.56; H, 5.28; N, 2.68.

Penta-*O*-trimethylsilyl Derivatives 2c and 3c. These derivatives were obtained by heating **2a** or **3a** for 20 min at 40 °C in a 1:1:1 mixture of trimethylchlorosilane, hexamethyldisilazane, and pyridine, followed by evaporation. The derivatives were used for ^1H and ^{13}C NMR analysis without purification.

(1*S*,2*S*,3*R*,4*R*)-1,4-Dihydroxy-2,3-epoxycyclopentane-1-carboxamide (5). When oven-dried, rather than fresh, plant material of *P. suberosa* was used for isolation of **2a**, 17 g of the extract gave, along with **1b–d** and **2a**, 258 mg of a crystalline mixture of amides **5** and **6** (ratio 10:1). From this mixture, 149 mg of **5** was isolated by reverse-phase HPLC with methanol–water (1:99). The amide was recrystallized from methanol–ether: mp 164–165 °C; $[\alpha]^{24}_D$ +91° (*c* 0.2, methanol); IR (KBr) 3430 (s), 3370 (s), 3320 (s), 3280 (s), 3050 (w), 2925 (m), 1670 (s), 1650 (s), 1257 (m), 1140 (s), 900 (s) cm^{-1}.

Anal. Calcd for $C_6H_9NO_4$: C, 45.28; H, 5.70; N, 8.80. Found: C, 45.30; H, 5.70; N, 8.76.

(1*S*,4*R*)-1,4-Dihydroxy-2-cyclopentene-1-carboxamide (6). This amide was obtained along with **5** as described above. Reverse-phase HPLC gave 14 mg of pure **6**, recrystallized from methanol–ether: mp 143–144 °C; $[\alpha]^{24}_D$ +275° (*c* 0.2, methanol); IR (KBr) 3440 (s), 3380 (s), 3280 (s), 2990 (w), 2950 (w), 2920 (w), 1675 (s), 1650 (s), 1615 (m) cm^{-1}.

Anal. Calcd for $C_6H_9NO_3$: C, 50.35; H, 6.34; N, 9.78. Found: C, 50.36; H, 6.18; N, 9.79.

Crystal Structure Determination of 2a. Crystals were grown from a solution of the epoxide in a mixture of water and butanol, by allowing the solvent to evaporate very slowly (several months) at room temperature. Very thin, needle-shaped crystals were formed, which exposed to X-ray radiation appeared as multiple crystals. A crystal having the dimensions 0.03 × 0.08 × 0.27 mm was used for the space group determination and data collection at 110 K using Cu Kα radiation (λ = 1.541 78 Å).

Crystal data are as follows: $C_{12}H_{17}O_8N$, M = 303.3, orthorhombic, space group $P2_12_12_1$ (no. 19), a = 5.6856 (6) Å, b = 10.086 (1) Å, c = 22.099 (2) Å, V = 1267.3 Å^3, Z = 4, D_c = 1.589 g/cm^3. Although the crystal consisted of two fragments, it was possible to collect the data exclusively from the principal fragment. Intensities of 3948 reflections ($0 \le h \le 7, 0 \le k \le 12, -27 \le l \le 27$) were measured using $\omega - 2\theta$ scan mode. Intensities of three reflections measured every 10^4 s showed no unusual variation during the experiment. The 3948 reflections were merged resulting in 2642 reflections, of which 2198 with $|F_o|^2 \ge 3\sigma(|F_o|^2)$ were used in the structure determination.

The structure was solved by use of MULTAN80[31] and refined by full-matrix least-squares methods. All non-hydrogen atoms were refined anisotropically. No corrections for absorption was used. The locations of the 17 hydrogen atoms were determined from succesive difference Fourier syntheses; the positions of the five hydroxylic hydrogen atoms were refined, whereas the remaining hydrogen atoms were kept in calculated positions. A final R of 0.036 and R_w of 0.049 were obtained, minimizing $\sum w(|F_o| - k|F_c|)^2$ with $w = 1/\sigma^2(|F_o|)$, where $\sigma(|F_o|) = \sigma(|F_o|^2)/(2|F_o|)$. During the last cycle of refinement the maximum shift was 0.02σ. Atomic scattering factors were those incorporated in the SDP program package, which was used for all calculations. The final fractional coordinates and isotropic equivalent temperature factors for non-hydrogen atoms are given in Table III.

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Supplementary Material Available: Positional parameters, bond distances and angles, torsion angles, and anisotropic displacement parameters for compound **2a** (7 pages). Ordering information is given on any current masthead page.

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