Cyanogenesis of *Adenia volkensii* Harms and *Tetrapathaea tetrandra* Cheeseman (Passifloraceae) Revisited: Tetraphyllin B and Volkenin. Optical Rotatory Power of Cyclopentenoid Cyanohydrin Glucosides*

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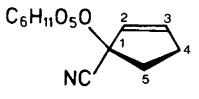
The cyclopentenoid cyanohydrin glucosides tetraphyllin B and volkenin, (1*S*,4*S*)- and (1*R*,4*R*)-1-(β-D-glucopyranosyloxy)-4-hydroxy-2-cyclopentene-1-carbonitrile, were isolated from *Adenia volkensii* Harms and *Tetrapathaea tetrandra* Cheeseman (Passifloraceae). Enzymatic hydrolysis of the glucosides yielded enantiomeric 1,4-dihydroxy-2-cyclopentene-1-carbonitriles along with the enantiomeric 4-hydroxy-2-cyclopenten-1-ones, establishing the relative stereochemistry of C1 and C4. Molecular rotations of alkyl β-D-glucopyranosides and simple allylically substituted cyclopentenes have been surveyed. The sign and the magnitude of the optical rotations of cyclopentenoid cyanohydrin glucosides at 589 nm were demonstrated to be suitable for immediate determination of the stereochemistry of the allylic hydroxy group. Tetraphyllin B and volkenin are easily converted into the corresponding amides, (1*S*,4*S*)- and (1*R*,4*R*)-1-(β-D-glucopyranosyloxy)-4-hydroxy-2-cyclopentene-1-carboxamides; the reaction probably involves assistance by the allylic hydroxy group. The (1*S*,4*S*) amide was synthesized from tetraphyllin B by alkaline hydrolysis, esterification of the resulting carboxylic acid, and ammonolysis. On standing in methanol, the carboxylic acid undergoes spontaneous lactonization involving the hydroxy group at C2 of the glucose moiety.

Stereochemical aspects of the biosynthesis of natural products are of outstanding interest. The pantropical plant family Passifloraceae, adjacent tribes of Flacourtiaceae, and related families produce a group of stereochemically diversified cyclopentenoid cyanohydrin glycosides,[2–4] the structures of which demonstrate unique stereospecificity of the biosynthetic pathways involved. According to the known biosynthetic schemes for natural cyanohydrins,[3–5] the cyclopentenoids should arise from 2-cyclopentene-L-glycine,[6,7] a non-protein amino acid also capable of furnishing the primer in the biosynthesis of cyclopentenoid fatty acids.[8,9] 2-Cyclopentene-L-glycine[10–12] is an inhibitory analogue of valine and isoleucine,[13,14] protein amino acids which are largely indiscriminately converted to the cyanohydrins linamarin and lotaustralin in numerous taxa of flowering plants,[4] as well as in insects.[15–17] Linamarin and lotaustralin have recently been demonstrated to occur in some Passifloraceae,[18–20] alone or together with the cyclopentenes, emphasizing the complexity of evolutionary relationships and biosynthetic patterns within this group of plants.[6]

Earlier studies on deidaclin (1) and tetraphyllin

*Part III of the series on natural cyclopentenoid cyanohydrin glycosides. For part II see Ref. 1.



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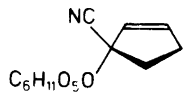

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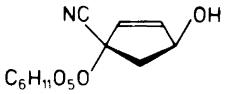

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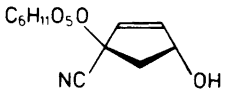

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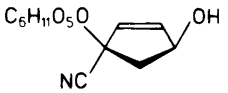

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A (**2**) ($C_6H_{11}O_5$ = β-D-glucopyranosyl), the simplest representatives of the group, indicate that these glucosides often, if not always, co-occur in plants.[2] Control mechanisms underlying formation of the epimers in a particular ratio are not known. The allylically hydroxylated derivatives, tetraphyllin B (**3**) and volkenin (**4**),[1] are the subject of the present work.

Tetraphyllin B (**3**), the glucoside of (1*S*,4*S*)-1,4-dihydroxy-2-cyclopentene-1-carbonitrile,[21] was isolated originally[22] from the New Zealand passion fruit *Tetrapathaea tetrandra* Cheeseman[23] (*Passiflora tetrandra* Banks and Sol. ex DC.[24]). The glucoside **3** was subsequently detected[25] in *Adenia volkensii* Harms[26] (Passifloraceae) along with another glucoside, which was

Table 1. ^{1}H NMR spectra of volkenin (**4**) and tetraphyllin B (**3**) and their derivatives.[a]

Compound	H2 and H3[b]	H4[c]	H5[d]	H1′ (anomeric)[e]	H2′	H3′	H4′	H5′	H6′
Volkenin	6.12, 6.23	4.98	2.30, 2.81	4.60	3.18[f]	[g]	[g]	[g]	3.66, 3.85[h]
Tetraphyllin B[i]	6.15, 6.31	4.98	2.23, 2.91	4.49	3.18[f]	[g]	[g]	[g]	3.69, 3.87[h]
Volkenin pentaacetate[j]	6.13, 6.24	5.71	2.50, 2.68	4.92	4.94[k]	5.22[k]	5.01[k]	3.77[l]	4.18[m]
Tetraphyllin B pentaacetate[n]	6.07, 6.33	5.73	2.46, 2.87	4.88	4.99[k]	5.24[k]	5.04[k]	3.79	4.18, 4.25[o]
Penta-*O*-trimethylsilyl-volkenin[p]	6.06, 6.18	4.99	2.37, 2.70	4.63	[r]	[r]	[r]	[r]	3.60, 3.78[s]
Penta-*O*-trimethylsilyl-tetraphyllin B[t]	6.03, 6.20	4.99	2.24, 2.93	4.49	[r]	[r]	[r]	[r]	3.67, 3.80[s]

[a]270 or 500 MHz spectra in methanol-d_4 (free glucosides; spectra in deuterium oxide are reported in Ref. 1) or chloroform-*d* (the derivatives); in the footnotes below the letters A and B designate high- and low-field proton, respectively. [b]Doublets of doublets, $^3J_{AB}$ 5.5 Hz, J_{AX} 1.5 Hz (1.0 Hz for the acetates), J_{BX} 2.0 Hz. [c]Unresolved multiplet. [d]Doublets of doublets, $^2J_{AB}$ −15.0 Hz (−14.5 Hz for the TMS-ethers), $^3J_{AX}$ 4.0 Hz (3.0 Hz for the acetates, 4.5 Hz for the TMS-ethers), $^3J_{BX}$ 6.5 Hz. [e]Doublet, $^3J_{1,2}$ 7.7 Hz (7.4 Hz for the TMS-ethers). [f]Double doublet, $^3J_{1,2}$ 7.7 Hz, $^3J_{2,3}$ 9.2 Hz. [g]Complex pattern at δ 3.3–3.4. [h]Doublets of doublets, $^2J_{AB}$ −12.0 Hz, $^3J_{AX}$ 5.0 Hz, $^3J_{BX}$ 2.0 Hz. [i]Spectra in deuterium oxide are reported in Refs. 18, 22, 27, and 35; the δ value reported by Seigler *et al.* for the high-field olefinic resonance (Refs. 27 and 35) is incorrect. [j]Acetyl groups at δ 1.98, 2.01, 2.04, 2.05 and 2.07. [k]Tentatively assigned by comparison with **1** and **2** (Ref. 2); $^3J_{2,3} \sim {}^3J_{3,4} \sim {}^3J_{4,5} \sim 9.2$ Hz. [l]Doublet of triplets at 270 MHz, normal double quartet at 500 MHz. [m]Two peaks at δ 4.18 separated by 4 Hz at 270 MHz; the AB(X) spectrum is not resolved even at 500 MHz. [n]Acetyl groups at δ 2.00, 2.03, 2.04, 2.06 and 2.10; cf. Refs. 22, 27 and 35. [o]Doublets of doublets, $^2J_{AB}$ −12.3 Hz, $^3J_{AX}$ 2.5 Hz, $^3J_{BX}$ 5.5 Hz. [p]Cf. Refs. 25 and 28. [r]Complex pattern at δ 3.2–3.5. [s]Doublets of doublets, $^2J_{AB}$ −11.0 Hz, $^3J_{AX}$ 5.5 Hz, $^3J_{BX}$ 2.0 Hz. [t]Cf. Refs. 25, 27 and 28.

not isolated in a pure condition but was characterized by ^{1}H NMR spectroscopy in a mixture with **3**. The new glucoside was claimed to differ from **3** in the stereochemistry of the cyanohydrin centre rather than of the allylic hydroxy group, and was named epitetraphyllin B (**5**).[25] The possibility that **3** and the new glucoside might differ in the configuration of both asymmetric centres in the cyclopentene ring was not considered.[25] The new glucoside, which we named volkenin, has in fact the structure **4**.[1] At present, tetraphyllin B and volkenin appear to be the most widely distributed cyanohydrin glucosides with cyclopentenoid aglucones.[27–35]

Results and discussion

We have reisolated cyclopentenoids of *T. tetrandra* and *A. volkensii* and characterized the glucosides fully for the first time. Tubers of *A. volkensii* contained tetraphyllin B and volkenin[1] in a ratio of 1:1. Fruits of *T. tetrandra* contained the two in a ratio of 30:1, along with **1** and **2** in a ratio of 1:20.[21,22,30] As for **1** and **2**,[2] the co-occurrence of **3** with **4** may be more usual than currently recognized. A summary of ^{1}H and ^{13}C NMR spectroscopic properties of **3** and **4** and their derivatives is given in Tables 1 and 2.

The glucosides and the derivatives show differences between resonances of anomeric protons within the isomeric pairs (Table 1) which parallel the differences observed[2] for **1** and **2**. Tetraphyllin B and volkenin thus have opposite configurations at C1,[25] viz. (1*S*)[21] and (1*R*), respectively. Differences in chemical shifts of the anomeric carbons are small (Table 2), as they are for **1** and **2**[2] and other epimeric pairs of natural cyanohydrin glycosides,[36–38] anomeric carbon signals of the glycosides with (1*R*) configuration appearing at a slightly lower field. Furthermore, differences between the chemical shifts of the olefinic protons and carbons and the cyanohydrin carbons of volkenin (**4**) and tetraphyllin B (**3**) closely parallel those[2] of deidaclin (**1**) and tetraphyllin A (**2**). The differences between chemical shifts of the isomers (Tables 1 and 2) were confirmed by recording spectra of the respective mixtures.

Chemical shifts of H4 in volkenin and tetraphyllin B are the same (Table 1). The values are also identical for the per-*O*-trimethylsilyl derivatives[25] and very close for the acetates (Table 1). This suggests that H4 in both glucosides is *cis* to the same substituent on C1 (i.e., that the allylic oxygens in both cases are *trans*), rather than that the configuration of C4 is unchanged, as claimed.[25] In simple 1,4-disubstituted 2-cyclopentenes the change of stereochemistry at one allylic carbon atom causes distinctive changes in the chemical shift of the proton attached to the other.[39–45] Thus, the transannular deshielding caused by a *cis* oxygen atom tends to be larger than the effect of the cyano group.

Proof that volkenin and tetraphyllin B differ in absolute configuration of C4 is provided by cir-

Table 2. ^{13}C NMR spectra of volkenin (**4**) and tetraphyllin B (**3**) and their derivatives.[a]

Compound	C1	C2, C3	C4	C5	CN	C1′ (anomeric)	Remaining glucose carbons
Volkenin	83.4	133.4, 143.4	75.7	48.6	120.4	101.9	62.7, 71.5, 75.0, 78.1, 78.4
Tetraphyllin B[b]	82.6	132.1, 144.9	76.0	48.9	121.0	101.5	62.9, 71.6, 75.0, 78.2, 78.4
Volkenin pentaacetate[c]	81.6	134.6, 137.8	76.4	44.7	117.3	98.2	61.9, 68.2, 70.9, 72.4, 72.6
Tetraphyllin B pentaacetate[d]	81.4	133.7, 138.6	77.0	44.3	117.5	98.2	61.8, 68.2, 71.0, 72.4, 72.6
Penta-*O*-trimethylsilylvolkenin	81.4	132.8, 140.6	74.5[e]	47.8	118.2	100.7	62.1, 71.6, 75.1,[e] 77.1, 78.2
Penta-*O*-trimethylsilyltetraphyllin B	80.5	130.4, 143.1	75.0	47.8	118.7	100.2	61.8, 71.3, 75.0, 77.1, 78.2

[a]125.7 MHz spectra in methanol-d_4 (free glucosides) or chloroform-*d* (the derivatives). [b]Spectrum in deuterium oxide is reported in Refs. 18 and 29. [c] Acetyl groups: δ 20.6 (3 CH_3), 20.7, 20.9, 169.3, 169.5, 170.1, 170.5, 170.6. [d]Acetyl groups: δ 20.6 (3 CH_3), 20.7, 20.9, 169.2, 169.4, 170.1, 170.5, 170.6. [e]The assignment may be reversed.

cular dichroism spectra of ketones obtained by enzymatic hydrolysis of the glucosides.[1] The freshly prepared hydrolyzates also contained small amounts of undissociated cyanohydrins. The cyanohydrins obtained from volkenin and tetraphyllin B had identical ^{1}H NMR spectra (see Experimental), confirming that they are enantiomers.

Even without degradation to cyclopentenones, the optical rotations of volkenin and tetraphyllin B reveal the stereochemistry of the allylic hydroxy group. In the absence of Cotton effect data, the values of molecular rotations at a single wavelength (sodium D line, 589 nm) can be used. Thus, substituents on the cyclopentene ring, viewed as components of screw patterns of electron polarizability,[46–51] give predictable and additive[52] contributions to the observed rotations.

The molecular rotations of the cyclopentenoid glucosides may be regarded approximately as being made up of contributions from the β-D-glucopyranosyloxy unit, from the chiral conformation [46–51] about the bond from the glucosidic oxygen to C1, and from the cyano and oxygen substituents at C1 and C4 interacting with the double bond.[48] In order to discuss optical rotations of the glucosides, it is necessary to consider the rotatory contributions from the sugar part. Molecular rotations of model β-D-glucopyranosides are collected in Table 3.[53–60] Except for the methyl and ethyl β-D-glucopyranosides, which have appreciably lower rotations, the rotation of primary and secondary alkyl β-D-glucopyranosides is fairly constant at about −90°. β-D-Glucopyranosides of cyclic secondary alcohols have appreciably higher rotation. The presence of the allylic double bond in 1-(2-propenyl) β-D-glucopyranoside does not alter the rotation significantly as compared with the saturated analogue. Molecular rotations of β-D-glucopyranosides of sterically hindered alcohols, constant at −45°, are appreciably lower than those of β-D-glucopyranosides of primary and secondary alcohols, the difference being presumably due to altered geometry of the glucosidic bond.[61] The data suggest that replacement of a methyl group with a cyano group increases levorotation by 20–25°.

Table 3. Molecular rotations of alkyl β-D-glucopyranosides and model cyanohydrin β-D-glucopyranosides.[a]

β-D-Glucopyranoside	[*M*]D
Methyl[b]	−63°
Ethyl[b]	−79°
1-Propyl[b]	−87°
1-Butyl[c]	−87°
1-Pentyl[d]	−91°
1-Hexyl[d]	−91°
1-Decyl[d]	−91°
1-Hexadecyl[e,f]	−89°
1-(2-Propenyl)[g]	−93°
Cyanomethyl[h]	−101°
2-Propyl[b]	−91°
(*S*)-2-Butyl[c]	−76°
(*R*)-2-Butyl[c]	−105°
Cyclopentyl[i]	−125°
Cyclohexyl[f]	−109°
Linamarin[j]	−70°
Lotaustralin[j] (*R*)	−51°
Epilotaustralin[j] (*S*)	−90°
2-(2-Methylpropyl)[c]	−45°
2-(2-Methylbutyl)[c]	−45°
3-(3-Methylpentyl)[c]	−45°

[a]In water unless stated otherwise. [b]From Ref. 53. [c]From Ref. 54. [d]From Ref. 55. [e]In ethanol. [f]From Ref. 56. [g] From Ref. 57. [h]From Ref. 58. [i]From Ref. 59. [j]From Ref. 60.

Rotatory contributions from the glucose and aglucone units seem to be fairly independent. Thus, the molecular rotations of the two epimeric 2-butyl β-D-glucopyranosides average to the rotation of the 2-propyl compound (Table 3). The same relation holds between the rotations of lotaustralin, epilotaustralin and linamarin.[60]

Molecular rotations of natural cyclopentenoid glucosides, their derivatives, and model cyclopentenes[62–69] are collected in Table 4. In simple cyclopentenes, the rotation is largely determined by the polarizability of the allylic substituent.[46,48] In tetraphyllin A (**2**) and B (**3**) and gynocardin (**6**)[70,71] the glucosidic oxygen and the cyano group are expected[48] to make a negative and a positive contribution to the overall rotation, respectively, the reverse contributions being expected for the remaining glucosides, (**1**) and (**4**). However, deidaclin and tetraphyllin A have similar rotations (Table 4), which are within the range observed for β-D-glucopyranosides with achiral aglucones (Table 3). The overall contributions from the C1−O1 bond and from the C1−CN bond appear, therefore, largely to cancel each other, and consequently the optical rotations of these cyclopen-

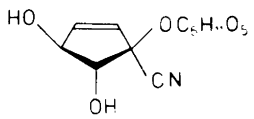
Table 4. Molecular rotations of cyclopentenes.

Compound	$[M]_D$
3-Methylcyclopentene[a]	±145°
2-Cyclopenten-1-ol[b]	±165°
2-Cyclopenten-1-ol acetate[c]	~±210°
2-Cyclopentene-1-carboxylic acid[d]	±294°
Deidaclin (**1**)[e] (1*R*)	−68°
Tetraphyllin A (**2**)[e] (1*S*)	−54°
Volkenin (**4**)[f,g] (1*R*,4*R*)	+77°
Tetraphyllin B (**3**)[f,h] (1*S*,4*S*)	−215°
Volkenin pentaacetate[f]	+206°
Tetraphyllin B pentaacetate[f,h]	−266°
Gynocardin (**6**)[i] (1*S*,4*S*,5*R*)	+220°
Gynocardin hexaacetate[i]	+223°

[a]Neat; from Ref. 62; for a discussion of earlier, incorrect values (cf. Ref. 48), see Ref. 63. [b]Calculated from specific rotation of 2-cyclopentene-1-acetic acid (cf. Ref. 64), obtained (Ref. 65) by a stereospecific pathway from partially resolved 2-cyclopenten-1-ol (in carbon tetrachloride); the value derivable from data in Ref. 66 is apparently too low. [c]Estimated: half of the molecular rotation of the diacetate of optically active *trans*-4-cyclopentene-1,3-diol (from Ref. 67; in methanol); the molecular rotation of the *trans*-monoacetate (362° in methanol; from Ref. 67) minus the rotation of 2-cyclopenten-1-ol gives 200°; a similar calculation for *cis*-monoacetate (Ref. 68 and references therein) gives a higher value, possibly owing to interaction between the substituents. [d]From Ref. 69 (in chloroform); the value cited in Ref. 48 is too low. [e]In ethanol; from Ref. 2. [f]This work; in methanol. [g]Preliminary value obtained with non-crystalline material; the value reported in Ref. 1 is +57°. [h]The figures reported in Refs. 21 and 22 are substantially in error. [i]In water (free glucoside) or chloroform (hexaacetate); from Ref. 70.

tenes give no information about the configuration of C1.[6]

Introduction of the allylic hydroxy group to give **3** and **4** is expected to increase levo- or dextrorotation, respectively, by a value similar to the rotation of 2-cyclopenten-1-ol. Indeed, the difference between the molecular rotations of tetraphyllin A (**2**) and B (**3**) is 161° (Table 4). The introduction of the allylic hydroxy group into deidaclin (**1**) to give volkenin (**4**) increases the dextrorotation by 145°. The observed span between the molecular rotations of the acetates of tetraphyllin B and volkenin (472°) slightly exceeds the estimated (Table 4) rotation of 2-cyclopenten-1-ol acetate multipled by two (420°). The sign and the magnitude of optical rotations at the sodium D line must thus be regarded as an important aid to structure determination of allylically hydroxylated cyclopentenone cyanohydrin glucosides.



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Gynocardin (**6**),[70,71] the dihydroxy derivative of tetraphyllin A (**2**), is 274° more dextrorotatory than the latter (Table 4). The largest part (ca. 165°) of this difference is evidently due to the presence of the allylic hydroxy group. The rest can be explained in terms of dextrorotatory skew interaction between the cyano group and the 5-hydroxy group,[46–51] and an increased positive rotatory contribution from the cyano group. The free energy difference between axial and equatorial conformes of cyclohexanecarbonitrile is several times lower than for cyclohexanol,[72–74] reflecting negligible steric effects of the cyano group. Accordingly, the presence of the additional hydroxy group in **6** is expected to enhance semi-axial orientation of the cyano group as compared to **3** or **4**, and hence, if the magnitude of the rotatory contribution varies with the sine of the torsion angle,[47,49,50] to increase the rotatory power. Comparison of the molecular structures of gynocardin [as a 6′-*O*-(4-bromobenzenesulfonyl) derivative[71]] and of tetraphyllin B (**3**)[21] (Table 5; deidaclin tetraacetate[2] included for comparison) in the crystalline state shows that the cyclopentene ring in the former case is considerably more puckered, with semi-equatorial C–O bonds and a semi-axial cyano group. In **3**, the ring is flattened (Table 5), with C3 and C5 eclipsed and the cyano group and the glucosidic oxygen atom placed almost symmetrically with respect to the ring. The torsion angle between the cyano group and the 5-hydroxy group in gynocardin (Table 5) is about 25°, giving a dextrorotatory effect. The semiquantitative explanation of the dextrorotation of gynocardin is thus also satisfactory.[75] In methyl dihydrogynocardinate[70] the double bond of **6** has been reduced and the cyano group converted into methoxycarbonyl. Effects of the interactions with the double bond have disappeared, and the rotatory contributions of the

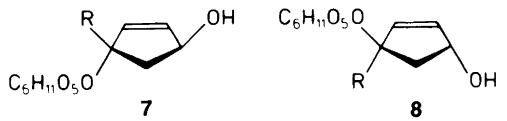
Table 5. Conformation of the cyclopentene moieties and glucosidic linkages of cyclopentenoid cyanohydrin glucosides in the crystalline state.

Torsion angle[a]	Deidaclin (**1**) tetraacetate[b] (1*R*)	Tetraphyllin B (**3**)[c] (1*S*,4*S*)	Gynocardin (**6**) 6′-*O*-(4-bromobenzenesulfonate)[d] (1*S*,4*S*,5*R*)
C1-C2-C3-C4	−3°	−3°	7°
C2-C3-C4-C5	−10°	8°	4°
C3-C4-C5-C1	18°	−9°	−13°
C4-C5-C1-C2	−20°	8°	17°
C5-C1-C2-C3	15°	−3°	−15°
C6-C1-C2-C3	−105°	118°	109°
C6-C1-C5-C4	97°	−114°	−108°
O1-C1-C2-C3	133°	−125°	−139°
O1-C1-C5-C4	−145°	132°	140°
O4-C4-C3-C2	–	−112°	133°
O4-C4-C5-C1	–	107°	−144°
O5-C5-C4-C3	–	–	−147°
O5-C5-C1-C2	–	–	150°
O5-C5-C4-O4	–	–	82°
O5-C5-C1-O1	–	–	−88°
O5-C5-C1-C6	–	–	25°
O5′-C1′-O1-C1	−89°	−102°	−96°
C2′-C1′-O1-C1	154°	138°	147°
C1′-O1-C1-C2	52°	37°	33°
C1′-O1-C1-C5	168°	−81°	−85°
C1′-O1-C1-C6	−71°	159°	155°
Distance of C5 from the least-squares plane through C1, C2, C3 and C4	0.32 Å	0.14 Å	0.24 Å

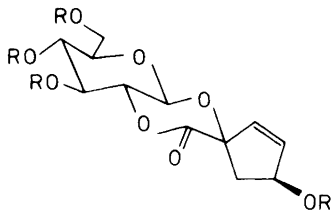
[a]Oxygen atoms have the numbers of the carbon atoms to which they are attached; C6 is the nitrile carbon; atoms of the glucose moiety are primed. [b]Cf. Ref. 2. [c]Cf. Ref. 21. [d]Cf. Ref. 71.

skew interactions reduced [46–48] owing to greater conformational flexibility[76–78] of the saturated ring. The molecular rotation falls from +220° to −85°,[70] a value within the normal range for β-D-glucopyranosides without extra chirality.[75]

During purification of **3** and **4** from *A. volkensii* by HPLC, we isolated small amounts of the corresponding amides **7a** and **8a**. The accumulation of the latter amide was observed on storing syrupy concentrates of **4**; the amides must thus be artefacts of isolation. Only minute amounts of the amides have been isolated, and crystalline samples were not obtained. The structural assignments rest on spectroscopic data (^{1}H and ^{13}C NMR, IR) and on the synthesis of **7a** from **3** via **7b** and **7c**. Working with **7b**, we found that methanolic solutions of this acid accumulate the ester **7c** along with a new compound, to which the structure **9a** is assigned. Acetylation of **9a** afforded the crystalline tetraacetate **9b**, identified by spectroscopic (IR, ^{1}H and ^{13}C NMR, M + 1 ion in the FAB mass spectrum) and analytical data. In particular, a COSY spectrum of **9b** (Fig. 1) identified all vicinal couplings in the glucose moiety, confirming the absence of an acetyl



a R = $CONH_2$
b R = COOH
c R = $COOCH_3$



9a R = H

b R = $COCH_3$

group at the oxygen bound to C2′ (δ H2′ 4.35); acetylation of the remaining hydroxy groups caused expected (see e.g. Table 1) shifts for protons attached to C4, C3′, C4′, and C6′. The vicinal coupling constants for **9b** (see Experimental) confirm that the glucose ring is in the $^4C_1$ conformation. The lactones **9a** and **9b** are dextrorotatory; the change of the sign of the rotation as compared with **3** and **7b** must reflect conformational locking of the molecule upon lactonization. Since formation of amides similar to **7a** and **8a** was not observed when handling **1** and **2**,[2] the hydration of the nitrile group in **3** and **4** may well involve delivery of a water molecule assisted by the allylic hydroxy group.

Experimental

General. Unripe fruits of *T. tetrandra* were collected in May near Christchurch, New Zealand. Tubers of *A. volkensii* were harvested in October near Nairobi, Kenya. The plant material was air-dried slowly before dispatch to Copenhagen.

NMR spectra were recorded on a Bruker HX 270, AM 250 or AM 500 spectrometer. CD and UV spectra were recorded on a CNRS Roussel-

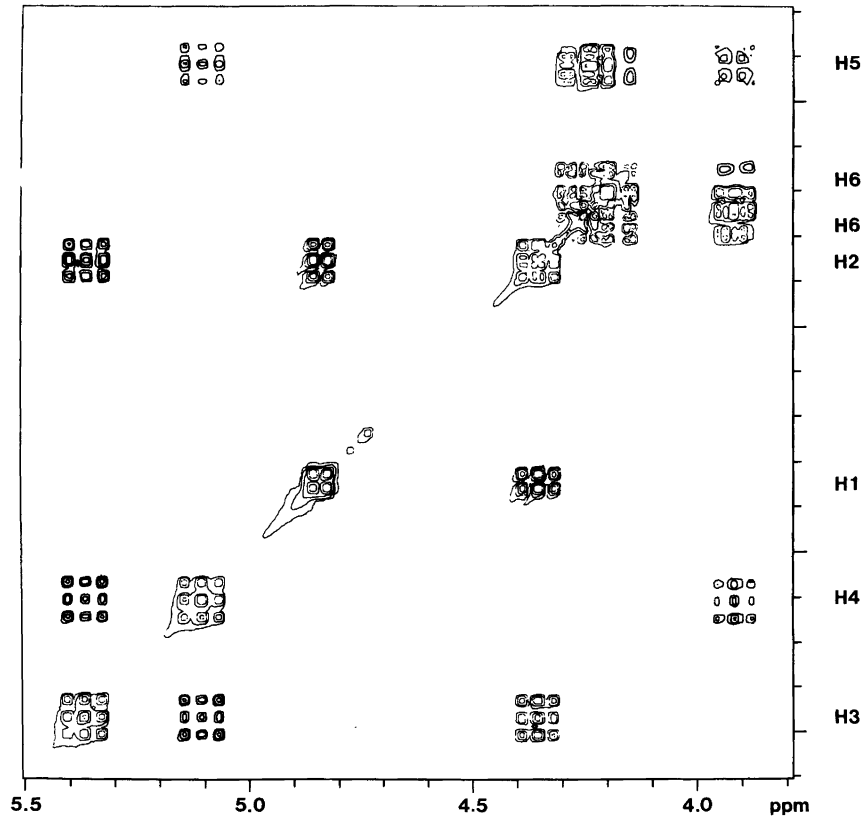


Fig. 1. Fragment of a $^1H-^1H$ correlated NMR spectrum of **9b** in chloroform-*d*, showing the signals of the glucopyranose moiety (250 MHz).

Jouan Dichrographe III and a Beckman Acta CIII spectrophotometer, respectively. IR spectra were recorded on a Perkin Elmer model 781 spectrophotometer or a Bruker FT-IR model 113V instrument. Mass spectra were obtained with a VG 20–240 spectrometer at 70 eV (EI), or using an ION TECH FAB gun (Xenon, 8 keV). Optical rotations were determined with a Perkin Elmer model 241 polarimeter.

Column-chromatographic and thin-layer-chromatographic separations were carried out using Merck Kieselgel 60, 0.066–0.2 mm, and Merck Kieselgel 60 precoated plates. HPLC separations were performed on a chromatograph consisting of a Waters model 590 solvent supply unit, 0.5 ml Rheodyne injector, a refractive index monitor and a recorder, using 1.6 × 25 cm prepacked Knauer columns. Melting points were determined in capillaries and are corrected. Microanalyses were performed by Mr. P. Hansen, Chemical Laboratory II, University of Copenhagen.

Isolation of cyanohydrin glucosides from T. tetrandra. The dried, finely ground fruits (33 g) were added in small portions to 500 ml of boiling methanol/water (4:1 *v/v*) in order to deactivate hydrolytic enzymes. The mixture was boiled for 5 min, chilled and filtered, and the filter cake repeatedly washed with the boiling solvent. The combined extracts were evaporated almost to dryness, redissolved in methanol, adsorbed on 50 g of silica gel by evaporation, and chromatographed on 250 g of silica gel with ethyl acetate/acetone/dichloromethane/methanol/water (40:30:12:10:8 *v/v*). Fractions (25 ml) were monitored by TLC with the same solvent, using a sugar-specific (naphthoresorcinol reagent[79]) as well as a cyanide-specific (sandwich picrate assay[80]) method of visualizing the spots. Evaporation of appropriate fractions yielded 79 mg (0.24 % of dry weight) of crude tetraphyllin A (**2**) and 710 mg (2.15 % of dry weight) of crude tetraphyllin B (**3**), purified by HPLC on Lichrosorb RP-18, 10 μm, using 9 ml min^{-1} of water/methanol (85:15 *v/v*). Retention times of **2** and **3** were 16.5 and 5.4 min, respectively. Investigation of the sample of **2** by ^1H NMR (270 MHz) demonstrated that a small amount of deidaclin (**1**) was present (ratio of 20:1); the glucosides were separated by normal-phase HPLC and characterized as described elsewhere.[2] Similarly, investigation of the sample of **3** revealed the presence of **4** (ratio of 30:1); the major glucoside was purified by repeated crystallization from ethyl acetate/methanol. A sample of purified **3** was treated overnight with a 1:1 mixture of acetic anhydride and pyridine, the mixture evaporated, and the resulting acetate recrystallized from ether. The penta-*O*-trimethylsilyl derivative of tetraphyllin B was obtained by heating the glucoside for 20 min at 40 °C with a 1:1:1 mixture of trimethylchlorosilane, hexamethyldisilazane and pyridine, followed by evaporation.

Tetraphyllin B: m.p. 168–169 °C, lit.[22] m.p. 169–170 °C; $[\alpha]_D^{26}$ −75° (*c* 0.5, methanol), lit.[21,22] $[\alpha]_D^{20}$ −35.6° (water), $[\alpha]_D^{25}$ −35.6° (*c* 1, water); IR (KBr): 3400 (s), 3300 (s), 3200 (s), 2980 (m), 2950 (m), 2920 (m), 2870 (m), 2240 (w), 1620 (w) cm^{-1}.

Tetraphyllin B pentaacetate: m.p. 113–113.5 °C, lit.[22] m.p. 114–115 °C; $[\alpha]_D^{21}$ −53.5° (*c* 0.5, methanol), lit.[22] $[\alpha]_D^{25}$ −25° (*c* 1, chloroform); IR (KBr): 1750 (s), 1735 (s), 1635 (w) cm^{-1}.

Isolation of cyanohydrin glucosides from A. volkensii. Dried and pulverized tubers (58 g) were extracted with 600 ml of aqueous methanol and the extract was chromatographed in a manner similar to that described for *T. tetrandra*, but using ethyl acetate/methanol (6:1 *v/v*). The crude cyanogenic[80] fraction (0.6 g) was purified by preparative HPLC on Lichrosorb RP-18, 10 μm, using 3 ml min^{-1} of water/methanol (4:1 *v/v*), to give 208 mg (0.36 % of dry weight) of a mixture of **3** and **4** in a ratio of 1:1 (270 MHz ^1H NMR), eluted as a single peak at 13.2 min. In spite of numerous variations of the mobile phase, only poor separation was achieved on Lichrosorb RP-8 (the best result was obtained with water containing 2 % of methanol and 2 % of acetonitrile). Full separation of the glucosides was achieved by HPLC on Lichrosorb Si60, 7 μm, using 4 ml min^{-1} of ethyl acetate/methanol/water (78:20:2 *v/v*), where **3** and **4** had *k'* of 1.0 and 0.9, respectively (separation factor of 1.11; the column had 10000 theoretical plates). The sample of **3** obtained had physical (m.p.) and spectroscopic (^1H and ^{13}C NMR) properties identical with those of the material isolated from *T. tetrandra*. The derivatives of **4** were obtained as described above for **3**.

Volkenin: colourless syrup; $[\alpha]_D^{22}$ +27° (*c* 0.7, methanol), lit.[1] $[\alpha]_D^{27}$ +20° (*c* 0.5, methanol); IR (FT spectrum; a KBr pellet was obtained after

evaporation of the syrup with KBr in methanol and desiccation): 3600–3200 (s), 2980 (m), 2930–2880 (m), 2240 (w), 1620 (m) cm^{-1}.

Volkenin pentaacetate: m.p. 124–124.5 °C; $[\alpha]_D^{22}$ +41.5° (*c* 0.4, methanol); IR (KBr): 1750 (s), 1635 (w) cm^{-1}. Anal. $C_{22}H_{27}NO_{12}$: C, H, N.

Enzymatic hydrolysis of volkenin and tetraphyllin B. Tetraphyllin B (13.5 mg) in 1 ml of water and 0.5 ml of *Helix pomatia* enzymes[1,80] (crude solution, Sigma Chemical Co., cat. No. G0876, β-glucuronidase activity of 10^4 units ml^{-1}) was stirred vigorously with 2 ml of diethyl ether for 4 h (room temperature). The progress of the reaction was followed by TLC (ethyl acetate/methanol, 4:1). The starting material (**3**) gradually disappeared, with simultaneous development of a spot corresponding to glucose in the aqueous phase. The ether layer was separated, the water phase extracted repeatedly with ether, and the combined ether extracts dried ($MgSO_4$) and evaporated. The ^1H NMR spectrum (270 MHz) of the residue indicated the presence of two compounds in a ratio of 2:1. The minor compound was (1*S*,4*S*)-1,4-dihydroxy-2-cyclopentene-1-carbonitrile;[21] ^1H NMR ($CDCl_3$): δ 2.42 (H5A) and 2.64 (H5B) ($^2J_{AB}$ −14.7 Hz, $^3J_{AX}$ 3.8 Hz, $^3J_{BX}$ 6.6 Hz), 5.11 (m, H4), 6.04 and 6.25 (H2 and H3, dd, $^3J_{AB}$ 5.5 Hz). The major compound was (*S*)-4-hydroxy-2-cyclopenten-1-one;[21] ^1H NMR ($CDCl_3$): δ 2.29 (H5A) and 2.80 (H5B) ($^2J_{AB}$ −18.7 Hz, $^3J_{AX}$ 2.2 Hz, $^3J_{BX}$ 6.0 Hz), 5.07 (m, H4), 6.25 (dd, $^3J_{AB}$ 5.5 Hz, $^4J_{AX}$ −1.2 Hz) and 7.57 (dd, $^3J_{AB}$ 5.5 Hz, $^3J_{BX}$ 2.5 Hz),[1] in agreement with lit.[81,82] data. Repeated evaporation of methanolic solutions of the mixture caused disappearance of the cyanohydrin, leaving the ketone (1.2 mg, 26%): MS (EI): *m/z* 98 (M^+); UV (CH_3OH): λ_{max} 212 nm (cf. Ref. 82); $[\alpha]_D^{27}$ ca. −60° (*c* 0.1, methanol), lit.[81] $[\alpha]_D^{22}$ +96° (*c* 0.1, methanol) for the (*R*) form; the CD spectrum exhibited a positive Cotton effect at 320 nm and a negative at 220 nm[1,81,82] (in methanol; when the concentration of the solution was determined by UV spectroscopy assuming ε_{max} 11 000 at 212 nm,[82] the calculated intensity of the long-wavelength CD maximum was [θ] ca. +8000°, lit.[81] 7990°), confirming the (*S*) configuration.

Similar hydrolysis of 15 mg of volkenin yielded a 6:1 mixture of the ketone and the cyanohydrin, with ^1H NMR spectra as those reported above. The ketone (1.1 mg, 21%) had $[\alpha]_D^{27}$ ca. +60° (*c* 0.1, methanol) and a CD spectrum with a negative Cotton effect at 320 nm and a positive at 220 nm (in methanol);[1] MS (EI): *m/z* 98 (M^+); UV (CH_3OH): λ_{max} 212 nm (cf. Ref. 82).

Derivatives of volkenin and tetraphyllin B. During the fractionation of the extract of *A. volkensii* on Lichrosorb RP-18, a fraction eluted before the mixture of **3** and **4** was obtained, from which the pure amides **7a** and **8a** (ca. 6 mg of each) were isolated by repeated HPLC on Lichrosorb Si60 (7 μm, 4 ml min^{-1} of ethyl acetate/methanol/water, 78:20:2; retention times 24.2 and 25.4 min, respectively). An additional amount of **8a** (3.5 mg; 250 MHz ^1H NMR spectrum identical with that of the previous sample) was isolated in the same way from a non-crystalline sample of **4** (20 mg) which had been stored for several months at +5 °C after purification.

Amide **7a**: colourless gum; ^1H NMR (250 MHz, CD_3OD): δ 2.17 and 2.58 (H5, $^2J_{AB}$ −14.2 Hz, $^3J_{AX}$ 2.7 Hz, $^3J_{BX}$ 6.7 Hz), 3.15–3.35 (m, H2′, H3′, H4′ and H5′), 3.66 and 3.82 (H6′, $^2J_{AB}$ −11.9 Hz, $^3J_{AX}$ 5.4 Hz, $^3J_{BX}$ 2.4 Hz), 4.28 (d, H1′, $^3J_{AB}$ 7.5 Hz), 4.92 (m, H4), 5.97 and 6.18 (H2 and H3, $^3J_{AB}$ 5.5 Hz, J_{AX} 0.8 Hz, J_{BX} 2.2 Hz); ^{13}C NMR (125.7 MHz, CD_3OD): δ 45.1 (C5), 76.6 (C4), 94.2 (C1), 135.4 and 141.8 (C2 and C3), 178.7 (CO), 62.8, 71.7, 75.1, 78.3, 78.4 and 100.3 (glucose carbons); IR (FT spectrum, obtained as for **4**): 3600–3100 (s), 1670 (s), 1600 (m) cm^{-1}.

Amide **8a**: colourless gum; ^1H NMR (250 MHz, CD_3OD): δ 2.10 and 2.64 (H5, $^2J_{AB}$ −14.2 Hz, $^3J_{AX}$ 3.3 Hz, $^3J_{BX}$ 6.8 Hz), 3.15–3.35 (m, H2′, H3′, H4′ and H5′), 3.64 and 3.80 (H6′, $^2J_{AB}$ −11.9 Hz, $^3J_{AX}$ 5.4 Hz, $^3J_{BX}$ 2.5 Hz), 4.38 (d, H1′, $^3J_{AB}$ 7.5 Hz), 4.90 (m, H4), 5.82 and 6.21 (H2 and H3, $^3J_{AB}$ 5.5 Hz, J_{AX} 1.1 Hz, J_{BX} 2.1 Hz); ^{13}C NMR (125.7 MHz, CD_3OD): δ 46.3 (C5), 76.8 (C4), 94.0 (C1), 133.5 and 143.4 (C2 and C3), 178.7 (CO), 62.8, 71.7, 75.3, 78.3, 78.4 and 100.4 (glucose carbons); IR (FT spectrum, obtained as for **4**): 3600–3100 (s), 1670 (s), 1600 (m) cm^{-1}.

The amide **7a** was also formed on prolonged heating under reflux of **3** in water (monitored by TLC with ethyl acetate/acetane/chloroform/methanol/water 40:30:12:10:8). The amide **7a** was synthesized from **3** (200 mg) by hydrolysis[22] to **7b** with $Ba(OH)_2$ [880 mg of the octahydrate in 3.5 ml of water, heating at ca. 100 °C for 1 h, saturation with CO_2, filtration, ion exchange on Dowex 50 (H^+) and evaporation]; the resulting oily

acid **7b** (195 mg, 91 %) was methylated in methanol with ethereal diazomethane to give the ester **7c**,[22] purified by HPLC on Lichrosorb Si60 (7 μm, 4 ml min^{-1} of ethyl acetate/methanol/water, 78:20:2; retention time 17.5 min). The ester **7c** (20 mg) was quantitatively converted into **7a** by treating with an excess of ammonia in methanol (1 h, room temp.); spectroscopic (^1H and ^{13}C NMR) and chromatographic (HPLC and TLC) properties of the material were identical with those of the sample isolated as described above.

Acid **7b**: colourless gum; $[\alpha]_D^{21}$ −83° (*c* 0.9, methanol); ^1H NMR (250 MHz, CD_3OD): δ 2.13 and 2.68 (H5, $^2J_{AB}$ −14.4 Hz, $^3J_{AX}$ 4.5 Hz, $^3J_{BX}$ 6.7 Hz), 3.1–3.4 (m, H2′, H3′, H4′ and H5′), 3.64 and 3.81 (H6′, $^2J_{AB}$ −12.0 Hz, $^3J_{AX}$ 5.5 Hz, $^3J_{BX}$ 2.5 Hz), 4.26 (d, H1′, $^3J_{AB}$ 7.5 Hz), 5.01 (m, H4), 6.10 and 6.19 (H2 and H3, $^3J_{AB}$ 5.6 Hz, J_{AX} 1.4 Hz, J_{BX} 1.9 Hz); ^{13}C NMR (62.9 MHz, CD_3OD): δ 45.6 (C5), 76.3 (C4), 91.7 (C1), 133.4 and 142.7 (C2 and C3), 176.3 (CO), 62.8, 71.5, 74.8, 77.9, 78.1 and 100.1 (glucose carbons); IR (FT spectrum, obtained as for **4**): 3600–2600 (s), 1730 (s), 1625 (m) cm^{-1}.

Ester **7c**: colourless gum; $[\alpha]_D^{21}$ −85° (*c* 0.7, methanol), lit.[22] $[\alpha]_D^{25}$ −48.6° (*c* 1, water); ^1H NMR (250 MHz, CD_3OD): δ 2.06 and 2.67 (H5, $^2J_{AB}$ −14.4 Hz, $^3J_{AX}$ 4.5 Hz, $^3J_{BX}$ 6.7 Hz), 3.1–3.35 (m, H2′, H3′, H4′ and H5′), 3.63 and 3.80 (H6′, $^2J_{AB}$ −11.9 Hz, $^3J_{AX}$ 5.5 Hz, $^3J_{BX}$ 2.5 Hz), 4.26 (d, H1′, $^3J_{AB}$ 7.5 Hz), 5.00 (m, H4), 6.17 and 6.20 (H2 and H3, $^3J_{AB}$ 5.6 Hz, J_{AX} 1.1 Hz, J_{BX} 1.4 Hz) (cf. Ref. 22); ^{13}C NMR (62.9 MHz, CD_3OD): δ 46.2 (C5), 53.3 (OCH_3), 76.3 (C4), 91.4 (C1), 133.0 and 143.4 (C2 and C3), 175.2 (CO), 63.0, 71.7, 75.0, 78.2, 78.3 and 100.4 (glucose carbons); IR (FT spectrum, obtained as for **4**): 1730 (s), 1630 (m) cm^{-1}.

When the acid **7b** (200 mg) was left for ca. 1 week in methanol (ca. 30 ml) at room temp., the solution evaporated and the residue chromatographed (Lichrosorb Si60, 7 μm, 4 ml/min of ethyl acetate/methanol/water 70:28:2), 30 mg of **7c** (identified by comparison with authentic material by 250 MHz ^1H NMR) and 50 mg of **9a** (retention time 31 min) was isolated. The lactone **9a** (15 mg) was acetylated as described for **3**, and the acetate was recrystallized from acetone/ether.

Lactone **9a**: crystalline mass; $[\alpha]_D^{22}$ +28° (*c* 0.8, methanol); ^1H NMR (250 MHz, CD_3OD): δ 2.21 and 2.61 (H5, $^2J_{AB}$ −13.9 Hz, $^3J_{AX}$ 2.0 Hz, $^3J_{BX}$ 6.6 Hz), 3.2–3.4 (m, H2′, H3′, H4′ and H5′), 3.64 and 3.81 (H6′, $^2J_{AB}$ −12.0 Hz, $^3J_{AX}$ 5.1 Hz, $^3J_{BX}$ 2.2 Hz), 4.40 (d, H1′, $^3J_{AB}$ 7.7 Hz), 4.80 (m, H4), 5.94 and 6.05 (H2 and H3, $^3J_{AB}$ 5.6 Hz, $J_{AX} < 1.0$ Hz, J_{BX} 2.2 Hz); ^{13}C NMR (62.9 MHz, CD_3OD): δ 45.0 (C5), 77.2 (C4), 95.2 (C1), 138.2 and 138.5 (C2 and C3), 173.1 (CO), 62.8, 71.6, 75.4, 78.3, 78.8 and 101.3 (glucose carbons); IR (FT spectrum, obtained as for **4**): 3600–3200 (s), 1600 (s) cm^{-1}.

Tetraacetate **9b**: m.p. 245 °C dec.; $[\alpha]_D^{24}$ +102° (*c* 0.3, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 2.05, 2.08, 2.09 and 2.10 (CH_3CO), 2.48 and 2.80 (H5, $^2J_{AB}$ −14.5 Hz, $^3J_{AX}$ 4.1 Hz, $^3J_{BX}$ 7.0 Hz), 3.93 (o, H5′), 4.18 and 4.25 (H6′, $^2J_{AB}$ −12.5 Hz, $^3J_{AX}$ 2.4 Hz, $^3J_{BX}$ 4.8 Hz), 4.35 (q, H2′, $^3J_{1,2}$ 7.8 Hz, $^3J_{2,3}$ 10.0 Hz), 4.84 (d, H1′, $^3J_{A,B}$ 7.8 Hz), 5.10 (H4′) and 5.37 (H3′) (each q, $^3J_{2,3}$ 10.0 Hz, $^3J_{3,4}$ 9.1 Hz, $^3J_{4,5}$ 10.1 Hz), 5.85 (m, H4) 6.04 and 6.30 (H2 and H3, $^3J_{AB}$ 5.6 Hz, J_{AX} 1.4 Hz, J_{BX} 2.1 Hz) (cf. Fig. 1); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 20.5, 20.6. 20.7 and 21.0 (CH_3CO), 43.3 (C5), 73.9 (C4), 88.8 (C1), 133.4 and 138.4 (C2 and C3), 167.8, 169.5, 169.8, 170.5 and 170.9 (CO), 61.6, 68.1, 71.3, 77.2, 77.4 and 93.9 (glucose carbons); IR (KBr): 1750 (s), 1725 (s), 1710 (s), 1635 (w) cm^{-1}; MS (FAB): *m/z* 457 (M + 1), 397 (M − $C_2H_3O_2$); Anal. $C_{20}H_{24}O_{12}$: C, H.

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References

1. Jaroszewski, J. W. and Olafsdottir, E. S. *Tetrahedron Lett. 27* (1986) 5297.
2. Jaroszewski, J. W. and Jensen, B. *Acta Chem. Scand., Ser. B 39* (1985) 867.
3. Conn, E. E. In: Akazawa, T., Asahi, T. and Imaseki, H., Eds., *The New Frontiers in Plant Biochemistry,* Japan Scientific Societies Press, Tokyo 1983, p. 11.
4. Conn, E. E. In: Stumpf, P. K. and Conn, E. E., Eds., *The Biochemistry of Plants, A Comprehensive Treatise,* Vol. 7: *Secondary Plant Metabolites,* Academic Press, New York 1981, p. 479.
5. Dewick, P. M. *Nat. Prod. Rep.* (1984) 545.
6. Clapp, R. C., Ettlinger, M. G. and Long L., Jr. *J. Am. Chem. Soc. 92* (1970) 6378.
7. Tober, I. and Conn, E. E. *Phytochemistry 24* (1985) 1215.
8. Cramer, U. and Spener, F. *Biochim. Biophys. Acta 450* (1976) 261.
9. Cramer, U. and Spener, F. *Eur. J. Biochem. 74* (1977) 495.
10. Santoso, S., Kemmer, T. and Trowitzsch, W. *Justus Liebigs Ann. Chem.* (1981) 658.
11. Bartlett, P. A. and Barstow, J. F. *Tetrahedron Lett. 23* (1982) 623.
12. Bartlett, P. A. and Barstow, J. F. *J. Org. Chem. 47* (1982) 3933.
13. Dennis, R. L., Plant, W. J., Skinner, C. G., Sutherland, G. L. and Shive, W. *J. Am. Chem. Soc. 77* (1955) 2362.
14. Gipson, R. M., Skinner, C. G. and Shive, W. *Arch. Biochem. Biophys. 111* (1965) 264.
15. Wray, V., Davis, R. H. and Nahrstedt, A. *Z. Naturforsch., C 38* (1983) 583.
16. Nahrstedt, A. and Davis, R. H. *Comp. Biochem. Physiol. B 75* (1983) 65.
17. Nahrstedt, A. and Davis, R. H. *Comp.. Biochem. Physiol. B 82* (1985) 745.
18. Fischer, F. C., Fung, S. Y. and Lankhorst, P. P. *Planta Med. 45* (1982) 42.
19. Spencer, K. C. and Seigler, D. S. *Biochem. Syst. Ecol. 13* (1985) 303.
20. Spencer, K. C., Seigler, D. S. and Nahrstedt, A. *Phytochemistry 25* (1986) 645.
21. Gainsford, G. J., Russell, G. B. and Reay, P. F. *Phytochemistry 23* (1984) 2527.
22. Russell, G. B. and Reay, P. F. *Phytochemistry 10* (1971) 1373.
23. De Wilde, W. J. J. O. *Blumea 22* (1974) 37.
24. Green, P. S. *Kew Bull. 26* (1972) 539.
25. Gondwe, A. T. D., Seigler, D. S. and Dunn, J. E. *Phytochemistry 17* (1978) 271.
26. De Wilde, W. J. J. O. *A Monograph of the Genus* Adenia *Forsk. (Passifloraceae),* Veenman, H. and Zonen N. V., Wageningen 1971.
27. Spencer, K. C. and Seigler, D. S. *Planta Med. 43* (1981) 175.
28. Spencer, K. C. and Seigler, D. S. *Onderstepoort J. Vet. Res. 49* (1982) 137.
29. Spencer, K. C. and Seigler, D. S. *Phytochemistry 21* (1982) 653.
30. Spencer, K. C., Seigler, D. S. and Domingo, J. L. *Phytochemistry 22* (1983) 1815.
31. Spencer, K. C. and Seigler, D. S. *Phytochem. Bull. 16* (1984) 3.
32. Spencer, K. C. and Seigler, D. S. *Biochem. Syst. Ecol. 13* (1985) 23.
33. Spencer, K. C. and Seigler, D. S. *Biochem. Syst. Ecol. 13* (1985) 421.
34. Spencer, K. C., Seigler, D. S. and Fraley, S. W. *Biochem. Syst. Ecol. 13* (1985) 433.
35. Seigler, D. S., Spencer, K. C., Statler, W. S., Conn, E. E. and Dunn, J. E. *Phytochemistry 21* (1982) 2277.
36. Nahrstedt, A. In: Vennesland, B., Conn, E. E., Knowles, C. J., Westley, J. and Wissing, F., Eds., *Cyanide in Biology,* Academic Press, London 1981, p. 145.
37. Jaroszewski, J. W. *J. Nat. Prod. 49* (1986) 927.
38. Jaroszewski, J. W. and Ettlinger, M. G. *Magn. Reson. Chem. 25* (1987) 555.
39. Cocu, F. G., Wolczunowicz, G., Bors, L. and Posternak, Th. *Helv. Chim. Acta 53* (1970) 739.
40. Cocu, F. G., Wolczunowicz, G., Bors, L. and Posternak, Th. *Helv. Chim. Acta 53* (1970) 1511.
41. Angeli, P., Brasili, L., Giardiná, D. and Melchiorre, C. *J. Pharm. Sci. 70* (1981) 489.
42. Donaldson, R. E., Saddler, J. C., Byrn, S., McKenzie, A. T. and Fuchs, P. L. *J. Org. Chem. 48* (1983) 2167.
43. Zagorski, M. G. and Salomon, R. G. *J. Am. Chem. Soc. 106* (1984) 1750.
44. Smith, A. B., III, Toder, B. H., Richmond, R. E. and Branca, S. J. *J. Am. Chem. Soc. 106* (1984) 4001.
45. Bohlmann, F. and Fiedler, L. *Chem. Ber. 114* (1981) 227.
46. Brewster, J. H. *J. Am. Chem. Soc. 81* (1959) 5475.
47. Brewster, J. H. *J. Am. Chem. Soc. 81* (1959) 5483.
48. Brewster, J. H. *J. Am. Chem. Soc. 81* (1959) 5493.
49. Brewster, J. H. *Tetrahedron 13* (1961) 106.
50. Brewster, J. H. *Topics Stereochem. 2* (1967) 1.
51. Brewster, J. H. *Topics Curr. Chem. 47* (1974) 29.
52. Kauzmann, W., Clough, F. B. and Tobias, I. *Tetrahedron 13* (1961) 57.
53. Veibel, S. and Eriksen, F. *Bull. Soc. Chim. [5] 3* (1936) 277.

54. Veibel, S. and Lillelund, H. *Bull. Soc. Chim. [5] 5* (1938) 494.
55. Pigman, W. W. and Richtmyer, N. K. *J. Am. Chem. Soc. 64* (1942) 369.
56. Fischer, E. and Helferich, B. *Liebigs Ann. Chem. 383* (1911) 68.
57. Bourquelot, E. and Bridel, M. *Ann. Chim. Phys. [8] 29* (1913) 145.
58. Fischer, E. *Ber. Dtsch. Chem. Ges. 52* (1919) 197.
59. Poretz, R. D. and Goldstein, I. J. *Biochemistry 9* (1970) 2890.
60. Bissett, F. H., Clapp, R. C., Coburn, R. A., Ettlinger, M. G. and Long, L., Jr. *Phytochemistry 8* (1969) 2235.
61. Rees, D. A. *J. Chem. Soc. B* (1970) 877.
62. Schurig, V. and Gil-Av, E. *Israel J. Chem. 15* (1977) 96.
63. Sliwka, H.-R. and Hansen, H.-J. *Helv. Chim. Acta 67* (1984) 434.
64. Mislow, K. and Steinberg, I. V. *J. Am. Chem. Soc. 77* (1955) 3807.
65. Hill, R. K. and Edwards, A. G. *Tetrahedron Lett.* (1964) 3239.
66. Sato, T., Gotoh, Y., Wakabayashi, Y. and Fujisawa, T. *Tetrahedron Lett. 24* (1983) 4123.
67. Miura, S., Kurozumi, S., Toru, T., Tanaka, T., Kobayashi, M., Matsubara, S. and Ishimoto, S. *Tetrahedron 32* (1976) 1893.
68. Deardorff, D. R., Matthews, A. J., McMeekin, D. S. and Craney, C. L. *Tetrahedron Lett. 27* (1986) 1255.
69. Chapman, O. L., Mattes, K. C., Sheridan, R. S. and Klun, J. A. *J. Am. Chem. Soc. 100* (1978) 4878.
70. Coburn, R. A. and Long, L., Jr. *J. Org. Chem. 31* (1966) 4312.
71. Kim, H. S., Jeffrey, G. A., Panke, D., Clapp, R. C., Coburn, R. A. and Long, L., Jr. *J. Chem. Soc., Chem. Comun.* (1970) 381.
72. Hirsch, J. A. *Topics Stereochem. 1* (1967) 199.
73. Eliel, E. L. and Gilbert, E. C. *J. Am. Chem. Soc. 91* (1969) 5487.
74. Woldbæk, T., Berkessel, A., Horn, A. and Klæboe, P. *Acta Chem. Scand., Ser. A 36* (1982) 719.
75. Ettlinger, M. G. *Personal communication.*
76. Steyn, R. and Sable, H. Z. *Tetrahedron 27* (1971) 4429.
77. Lambert, J. B., Papay, J. J., Khan, S. A., Kappauf, K. A. and Magyar, E. S. *J. Am. Chem. Soc. 86* (1974) 6112.
78. Saebø, S., Cordell, F. R. and Boggs, J. E. *J. Mol. Struct. 104* (1983) 221.
79. Stahl, E. *Dünnschicht-Chromatographie; Ein Laboratoriumshandbuch,* Springer, Berlin 1967, p. 840.
80. Brimer, L., Christensen, S. B., Mølgaard, P. and Nartey, F. *J. Agric. Food Chem. 31* (1983) 789.
81. Gill, M. and Rickards, R. W. *Tetrahedron Lett.* (1979) 1539.
82. Tanaka, T., Kurozumi, S., Toru, T., Miura, S., Kobayashi, M. and Ishimoto, S. *Tetrahedron 32* (1976) 1713.

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