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SUBSTRATE SPECIFICITY IN THE BIOSYNTHESIS OF CYCLOPENTANOID CYANOHYDRIN GLUCOSIDES*

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Key Word Index—*Passiflora morifolia*; Passifloraceae; *Turnera angustifolia*; Turneraceae; cyanohydrin glucosides; radiolabelling; deidaclin; tetraphyllin A; linamarin; lotaustralin; epilotaustralin; 2-(2′-cyclopentenyl)glycine; 2-cyclopentenecarbonitrile; 2-methylpropanenitrile.

Abstract—The biosynthesis of deidaclin in *Turnera angustifolia* and of linamarin in *Passiflora morifolia* were investigated using intact plant tissues. Radiolabelled precursors, 2-(2′-cyclopentenyl)[2-^{14}C]glycine and L-[U-^{14}C]-valine were fed to freshly harvested shoots either alone or together with the presumed nitrile intermediates, 2-cyclopentenecarbonitrile and 2-methylpropanenitrile. The cyanohydrin glucosides were isolated and purified, and the incorporation of the radioactive labels was determined after enzymatic degradation of the glucosides to cyanide. The labels from the amino acid precursors were incorporated into the nitrile group of their corresponding cyanohydrin glucosides, and the incorporation was in each case strongly inhibited by simultaneous feeding with either of the two nitriles. *Turnera angustifolia* was able to synthesize linamarin when fed with 2-methylpropanenitrile, even though linamarin could not be demonstrated to be present in this plant naturally.

INTRODUCTION

The first step in the formation of cyanohydrin glucosides is *N*-hydroxylation of the appropriate precursor amino acid; the resulting products are converted via oximes and nitriles into cyanohydrins, which are glucosylated to give the accumulating products of the biosynthesis (Fig. 1) [2, 3]. This pathway is believed to be valid for all types of natural cyanohydrin glucosides irrespective of the starting amino acid, although details of the individual steps are still being elucidated [4–6].

Among natural cyanohydrin glucosides, those having a cyclopentene ring in the aglucone (Fig. 2) occupy a special position in several respects. Thus, the cyclopentanoids originate from the rare amino acid 2-(2′-cyclopentenyl)glycine (Fig. 3) [7, 8]. This contrasts with the classical cyanohydrin glucosides, which are synthesized from the common amino acids phenylalanine, tyrosine, valine, leucine or isoleucine.

Secondly, the cyclopentanoids have a quite restricted distribution. They are found only in a cluster of closely related plants consisting of Passifloraceae [1, 9], Turneraceae [10], Malesherbiaceae [11], Achariaceae [12], and the cyanogenic tribes of Flacourtiaceae [13–15]. The restricted occurrence of the cyclopentanoids appears to be related to the unique ability to produce 2-(2′-cyclopentenyl)glycine, and contrasts with the occurrence

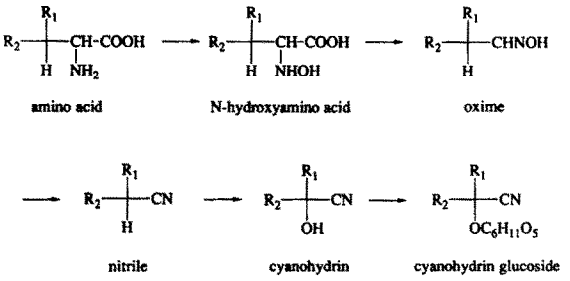

Fig. 1. Outline of the pathway for the biosynthesis of cyanohydrin glucosides.

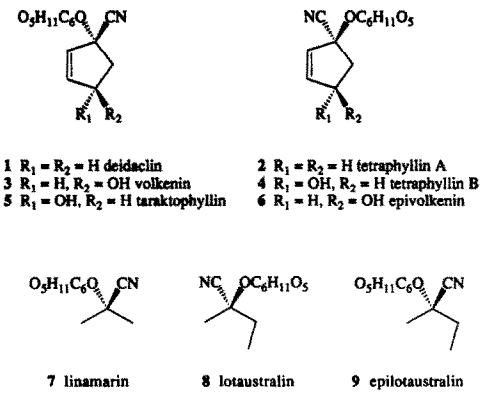

Fig. 2. Principal cyclopentanoid cyanohydrin glucosides and related cyanohydrin glucosides derived from valine or isoleucine.

*Part 14 in the series 'Natural Cyclopentanoid Cyanohydrin Glycosides.' For part 13 see ref. [1].

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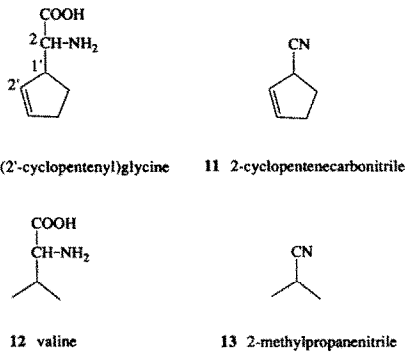

10 2-(2'-cyclopentenyl)glycine 11 2-cyclopentenecarbonitrile

12 valine 13 2-methylpropanenitrile

Fig. 3. Biosynthetic precursors and nitrile intermediates of the biosynthesis of cyclopentanoids and linamarin.

of the cyanohydrins derived from proteinogenic amino acids, which are present in all major groups of flowering plants.

Thirdly, the cyclopentanoids are special in that they usually occur as mixtures of stereoisomers with respect to the chirality of the aglucone, whereas the classical cyanohydrins are synthesized as single isomers. Thus, deidaclin and tetraphyllin A, volkenin and tetraphyllin B, and taraktophyllin and epivolkenin (Fig. 2) appear invariably to co-occur [9, 10, 16]. In addition, the plants belonging to the cyclopentanoid-producing group frequently synthesize linamarin and lotaustralin (Fig. 2); both types of cyanohydrins are sometimes found in the same plant [9, 10, 17–19]. The ability to utilize more than one amino acid in cyanohydrin biosynthesis is otherwise uncommon, being restricted to the structurally similar amino acids valine and isoleucine [16, 20].

The present investigation was carried out in order to obtain additional information about substrate specificity of cyanohydrin biosynthesis in the cyclopentanoid-producing group of plants. In particular, we were interested in the possible utilization of unnatural substrates at the amino acid step and at the nitrile step.

RESULTS AND DISCUSSION

Two plant species were selected for feeding experiments: one cyclopentanoid-producing and one linamarin-producing plant. The first was a *Turnera* species, with which the incorporation of 2-(2'-cyclopentenyl)glycine into deidaclin was originally shown [8]. The plant was grown from the same batch of seeds as used in the original work [8]. It contained deidaclin and tetraphyllin A in a ratio of 7:1, and was identified as *T. angustifolia* (see Experimental). The other plant was *Passiflora morifolia*, the cyanogenic constituents of which are reported here for the first time. The plant contained linamarin, lotaustralin and epilotaustralin in a ratio of 330:7:1. The purpose was to select a plant from the cyclopentanoid-producing taxa, but which actually synthesizes linamarin rather than a cyclopentanoid.

Radiolabelled 2-(2'-cyclopentenyl)glycine was synthesized via condensation of 3-chlorocyclopentene with diethyl 2-acetylamino[2-^{14}C]propanedioate, to give the product specifically labelled at C-2. Following the literature procedure [21], the amino acid obtained was found to be strongly contaminated with radioactive impurities, and only after purification by reverse phase HPLC could chemically and radiochemically homogeneous material be obtained. The product was a mixture of four stereoisomers with respect to the two chiral centres (C-2 and C-1') present in the molecule. Racemic 2-cyclopentenecarbonitrile was synthesized from 3-chlorocyclopentene and potassium cyanide [22].

The incorporation experiments were performed with intact plant tissue. Each plant was fed with the ^{14}C-labelled 2-(2'-cyclopentenyl)glycine or L-valine, either alone or together with 2-cyclopentenecarbonitrile or 2-methylpropanenitrile, as shown in Table 1. The plants were allowed to metabolize for 48 hours, and then their cyanohydrin glucosides were isolated and purified by HPLC. Deidaclin and tetraphyllin A mixtures isolated from *T. angustifolia* were not resolved. In order to confirm that incorporation of ^{14}C was restricted to the cyano group, the glucosides were hydrolysed with enzymes from *Helix pomatia* [23] in Conway microdiffusion cells [24], and the cyanide formed was trapped in base. Counting and determination of cyanide yielded the incorporation data shown in Table 1.

The incorporation of radioactivity from 2-(2'-cyclopentenyl)[2-^{14}C]glycine into deidaclin/tetraphyllin A and from L-[U-^{14}C]valine into linamarin was about 0.5 and 2%, respectively (Table 1, expts 1, 2, 9 and 10). Actually, these numbers correspond to considerably higher specific incorporations from the biosynthetic precursors. Thus, only L-2-(2'-cyclopentenyl)glycine is expected to serve as the biosynthetic substrate, and the L-valine used was unspecifically labelled, while the above incorporation only refers to C-2, which during the biosynthesis is converted to the cyano group. Assuming that the 2-(2'-cyclopentenyl)[2-^{14}C]glycine formed in the synthesis consisted of equal amounts of the four possible isomers, that both C-1' epimers of the L-form are used in the biosynthesis of deidaclin/tetraphyllin A [16], and that the label in L-[U-^{14}C]valine used is equally distributed between all five carbon atoms of the linamarin molecule, the obtained incorporations of 0.5 and 2% would in fact correspond to 1 and 10%, respectively. Our results confirm those of Tober and Conn [8] about the biosynthetic origin of deidaclin in *T. angustifolia*, and give direct evidence about the biosynthesis of linamarin in *Passiflora*.

No linamarin production from L-valine in *T. angustifolia* or deidaclin production from 2-(2'-cyclopentenyl)glycine in *P. morifolia* was observed (Table 1, expts 7, 8, 15 and 16). Although the glucosides isolated in these experiments were substantially labelled, this must correspond to non-specific labelling or to the presence of labelled impurities, because practically no radioactivity was recovered from the cyano group. The plants are therefore apparently unable to synthesize 'unnatural' cyanohydrin glucosides from their respective amino acids.

However, when labelled 2-(2'-cyclopentenyl)glycine or L-valine were fed into *T. angustifolia* or *P. morifolia*, respectively, together with the equimolar amounts of cold nitrile intermediates, the incorporation of label was inhibited by a factor of two or more, regardless of whether 2-cyclopentenecarbonitrile or 2-methylpropanenitrile was present (Table 1, expts 3–6 and 11–14). The inhibition of the incorporation of label from 2-(2'-cyclopentenyl)[2-^{14}C]glycine with 2-cyclopentenecarbonitrile, and from L-[U-^{14}C]valine with 2-methylpropanenitrile, is in accord

Table 1. Incorporation of radioactivity from 2-(2′-cyclopentenyl)[2-^{14}C]glycine (**10**) and L-[U-^{14}C]valine (**12**) into cyanohydrin glucosides in *T. angustifolia* and *P. morifolia*

Exp. no.	Plant	Fed with	Amino acid uptake (μCi)	Incorporation into glucoside (%)	Total amount of HCN isolated (μmol)	Incorporation into HCN (%)	Dilution
1	*T. angustifolia*	**10**	5.48	0.61	3.59	0.35	380
2	*T. angustifolia*	**10**	4.48	0.55	5.15	0.56	400
3	*T. angustifolia*	**10**+**11**	5.29	0.42	5.43	0.19	1100
4	*T. angustifolia*	**10**+**11**	4.85	0.45	6.91	0.16	1800
5	*T. angustifolia*	**10**+**13**	5.46	0.42	4.67	0.19	900
6	*T. angustifolia*	**10**+**13**	5.48	0.29	5.19	0.08	2300
7	*T. angustifolia*	**12**	4.99	0.34	3.51	0.01	—
8	*T. angustifolia*	**12**	4.99	0.40	5.59	0.00	—
9	*P. morifolia*	**12**	4.93	5.48	13.43	1.86	270
10	*P. morifolia*	**12**	4.97	8.25	10.75	2.93	140
11	*P. morifolia*	**12**+**13**	4.97	0.82	11.47	0.84	500
12	*P. morifolia*	**12**+**13**	4.99	0.56	7.95	0.22	1300
13	*P. morifolia*	**12**+**11**	4.99	3.61	12.03	0.17	2700
14	*P. morifolia*	**12**+**11**	4.95	0.81	9.43	0.12	3000
15	*P. morifolia*	**10**	5.38	3.65	14.95	0.02	—
16	*P. morifolia*	**10**	5.46	3.13	12.27	0.00	—

with their involvement in the biosynthesis of deidaclin/tetraphyllin A and linamarin, respectively. However, the 'crossed' inhibition, i.e. the inhibition of the incorporation of the label from 2-(2′-cyclopentenyl)[2-^{14}C]glycine with 2-methylpropanenitrile, and from L-[U-^{14}C]valine with 2-cyclopentenecarbonitrile, was also observed (Table 1, expts 5, 6, 13 and 14). This demonstrates a broad substrate specificity of the nitrile hydroxylating enzymes in both plants. Moreover, *T. angustifolia* actually synthesized linamarin when fed with 2-methylpropanenitrile (see Experimental), even though no linamarin could normally be detected in this plant.

It appears, therefore, that the substrate specificity of cyanohydrin glucoside biosynthesis in this group of plants is controlled at the early biosynthetic steps, possibly the *N*-hydroxylation step. The enzymes involved in the metabolism of the nitrile intermediates accept structurally related nitriles in both plants. It has been suggested that enzymes involved in cyanohydrin biosynthesis from 2-(2′-cyclopentenyl)glycine evolved through structural modifications of those that utilize valine and isoleucine [7, 16]. Our experiments support this hypothesis.

Although 2-(2′-cyclopentenyl)glycine did not serve as a substrate in *P. morifolia* (Table 1, expts 15 and 16), or, as previously shown in flax [25], it has in fact been observed to be a substrate with enzymes of cassava, which naturally synthesizes linamarin [26]. We suggest that the enzymes involved in the biosynthesis of linamarin/lotaustralin and those producing deidaclin/tetraphyllin A are closely related although not identical, and that their substrate specificity may vary to some extent from one plant species to another. These variations in specificity are responsible for varying linamarin to lotaustralin ratios, or ratios between cyclopentanoid glucosides with enantiomeric aglucones, formed from presumably similar pools of the respective amino acids in different plants. In other words, in the former case the variations are due to the varying extent to which L-isoleucine can compete with L-valine, and in the latter case to the competition between the two epimers of L-2-(2′-cyclopentenyl)glycine. The epimers co-occur naturally [27], which is probably due to a non-enzymatic equilibration of their β-ketoacid forms via a common enol form [16]. Similar considerations can be used to explain the observed co-occurrence of lotaustralin and epilotaustralin.

The central point in the biosynthesis of this group of natural cyanohydrins is thus the ability of the enzymes to accept branched aliphatic substrates with somewhat varying size of the side chain due to their relative insensitivity to the number of hydrogens on the carbon atoms next to the branch. The stereochemical relationships are visualized for the nitrile intermediates in Fig. 4. Once this hypothesis is accepted, the observed co-synthesis of 4-hydroxylated cyclopentanoid cyanohydrin

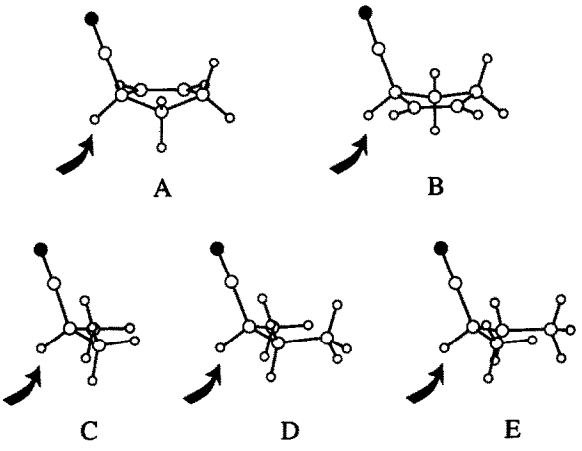

Fig. 4. Molecular models of nitriles involved in the biosynthesis of tetraphyllin A (A), deidaclin (B), linamarin (C), lotaustralin (D) and epilotaustralin (E). The models are arranged with the same orientation of the nitrile group; the arrows indicate hydroxylation to cyanohydrins with retention of configuration (from below the planes formed by the aliphatic chains or rings).

glucosides with enantiomeric aglucones (Fig. 2) can be readily explained in similar terms. Thus, the enzyme that hydroxylates a biosynthetic intermediate at C-4 may well be rigorously guided by the orientation of the substituent at C-1 (*cis* or *trans* hydroxylation), but not by the presence of the double bond, which determines the chirality of the cyclopentene ring (Fig. 5). This will result in co-synthesis of taraktophyllin and epivolkenin (cyano group and the 4-hydroxy group *trans*) or of volkenin and tetraphyllin B (cyano group and the 4-hydroxy group *cis*), but not necessarily of all four isomers at the same time, as indeed observed in the vast majority of cases. The steric tolerance at the enzymatic sites necessary to bring about this sort of substrate unspecificity does in fact not need to be very large, as seen from the evaluation of the volumes of geometry-optimized [28] molecular models of the substrates involved (Fig. 6).

EXPERIMENTAL

General methods and equipment. NMR: Bruker AM 250 or AM 500; MS: Finnigan GC-MS model 4515 B; HPLC: Waters model 590 solvent supply unit, 2.0 ml Rheodyne injector, and Waters Lambda-Max Model 481 spectrophotometric detector or Siemens SR 210 refractive index detector. Radioactivity was measured with Packard Tri-Carb model 2000CA liquid scintillation analyser, using a Packard Opti-Fluor scintillation cocktail. Autoradiograms were obtained with Kodak radiographic film. Energy minimizations and evaluations of structures of biosynthetic intermediates was carried out on a Macintosh IIci computer, using the MacMimic ver. 2.0 and the MM2(91) ver. 1.0 program packages (InStar Software).

Materials. Passiflora morifolia Mast. (Passifloraceae) and *Turnera angustifolia* Miller (Turneraceae) were grown from seeds in a greenhouse of the Botanical Garden, University of Copenhagen. The identity of *P. morifolia* is based on the description by Killip [29], supported by Vanderplank [30]. The seed material of *T. angustifolia* was the same as that used by Tober and Conn [8] under the name *T. ulmifolia*. *T. ulmifolia* L. *sensu lato* is a species complex treated by Urban [31] as comprising of 11 varieties. Modern authors tend to raise these varieties to species rank [32]. If the species rank is adopted, the name is *T. angustifolia* Miller, in agreement with the description by Robyns [33]. Voucher specimens are deposited in the Botanical Museum, University of Copenhagen.

2-(2′-Cyclopentenyl)[2-^{14}C]glycine (2.03 Ci mol^{-1}) and 2-cyclopentenecarbonitrile were synthesized as described below. L-[U-^{14}C]Valine (285 Ci mol^{-1}) and diethyl 2-acetylamino[2-^{14}C]propanedioate were purchased from Amersham. Merck silica gel 60 (0.063–0.2 mm) was used for CC as received.

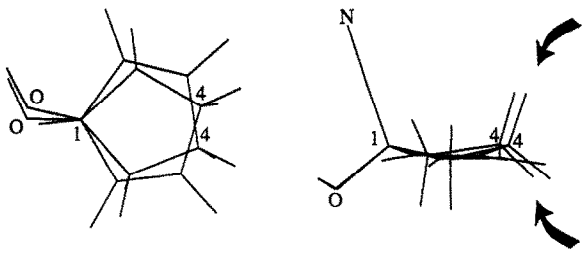

Fig. 5. Superimposition of enantiomeric cyanohydrins corresponding to deidaclin and tetraphyllin A. The fitted atoms were N, C-1, C-4, and the centre of gravity of ring carbons. Hydroxylation at C-4 can take place from below or from above the ring, to give *cis* or *trans* 1,4-dioxygenated cyclopentenes. The distance between the C-4 atoms of the enantiomers in this superimposition is 0.86 Å.

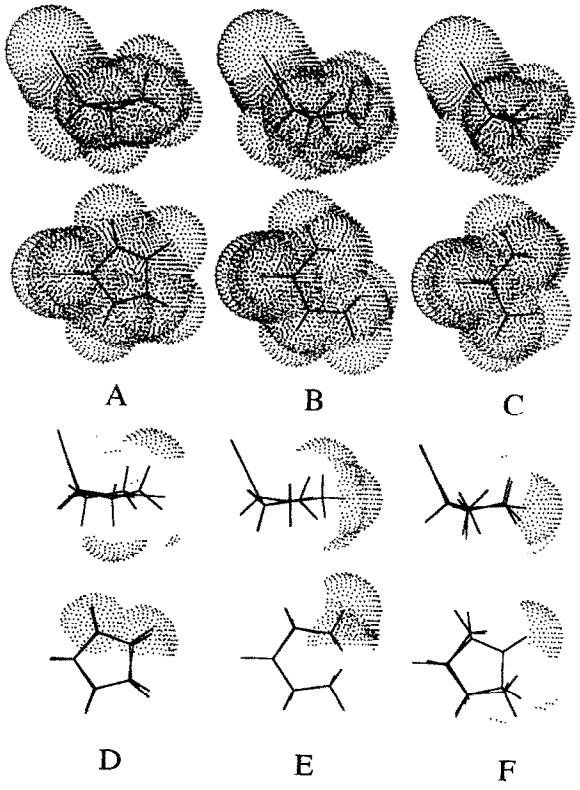

Fig. 6. Comparison of the molecular volumes of the nitrile intermediates. (A) 2-Cyclopentenecarbonitrile; (B) 2-methylbutanenitrile; (C) 2-methylpropanenitrile; (D) the difference between molecular volumes of the enantiomers of 2-cyclopentenecarbonitrile; (E) the difference between molecular volumes of the enantiomers of 2-methylbutanenitrile; (F) the difference between molecular volumes of 2-cyclopentenecarbonitrile and 2-methylbutanenitrile. The atoms fitted for the difference calculations were all corresponding carbon atoms. Each model is viewed from the side and from the top; the volumes were calculated from unscaled van der Waals radii of atoms.

Synthesis of 2-(2′-cyclopentenyl)[2-^{14}C]glycine. The labelled amino acid was obtained [21] by condensation of 400 mg diethyl 2-acetylamino[2-^{14}C]propanedioate (2.5 mCi) with 400 ml 3-chlorocyclopentene [34], followed by acid-catalysed hydrolysis. The product was initially purified on a 1 × 40 cm column of silica gel eluted with BuOH–H_2O–HOAc (4:1:1); the fractions (10 ml) were monitored by TLC (silica gel) using ninhydrin to visualize the spots. The crude product obtained still contained radioactive impurities and was purified by HPLC on a 8 × 100 mm radially compressed Waters 8C18 (5 μ) column with H_2O as the eluent, using spectrophotometric detection at 200 nm. This gave 19 mg of pure 2-(2′-cyclopentenyl)[2-^{14}C]glycine (chemical yield 7.5%) with a specific activity of 0.0144 Ci g^{-1} or 2.03 Ci mol^{-1}. Approximately the same amount of radioactive impurities was eluted before the product.

Synthesis of 2-cyclopentenecarbonitrile. Reaction [22] of 3-chlorocyclopentene (33 g) [34] with KCN (100 g), followed by vacuum distillation of the crude product, yielded a mixture of 2-cyclopentenecarbonitrile and 2-cyclopentenol (collected at

50–60° and 15 mmHg), which was further fractionated by chromatography. Thus, 2 ml of the mixture was mixed with 1 ml Et_2O and 1 ml pentane, and placed on top of a 3 × 60 cm silica gel column. The column was eluted with pentane–Et_2O (19:1), and the fractions (10 ml) monitored by TLC (silica gel, visualized with a 1:1 mixture of 1% aq. $KMnO_4$ and 5% aq. Na_2CO_3). 2-Cyclopentenol (white spot on the TLC plate) was eluted before the nitrile (brown spot on the TLC plate). Appropriate fractions were combined and evapd without heating, to give 1580 mg of the nitrile and 700 mg of the alcohol, each as a clear liquid.

2-Cyclopentenecarbonitrile. ^{1}H NMR (250 MHz, $CDCl_3$): δ3.55 (H-1, *m*), 6.00 and 5.66 (H-2 and H-3, *m*), 2.3 (H-4 and H-5, *m*, 4); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ34.1 (C-1), 135.7 and 125.3 (C-2 and C-3), 31.7 and 28.5 (C-4 and C-5), 121.4 (CN); IR (liquid sandwich); ν_{max} cm^{-1}: 3070 (m), 2950 (s), 2860 (m), 2240 (s), 910 (m); UV λ_{max}^{hexane} 197 nm, ε_{max} 970; MS (EI) *m/z* (rel. int.): 93 (30) $[M]^+$, 92 (18) $[M-1]^+$, 66 (100) $[M-HCN]^+$.

2-Cyclopentenol. ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.66 (H-1, *m*), 5.97 and 5.86 (H-2 and H-3, *m*), 2.45 (H-4A, *m*), 2.2 (H-4B and H-5B, *m*), 1.77 (H-5B, *m*); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 83.2 (C-1), 134.9 and 131.6 (C-2 and C-3), 30.9 and 30.6 (C-4 and C-5); IR (liquid sandwich): ν_{max} cm^{-1}: 3320 (broad, m), 3060 (m), 2940 (s), 2850 (s), 1635 (m), 1360 (s), 1115 (m), 1060 (s), 910 (w); UV λ_{max}^{hexane} 201 nm, ε_{max} 1660; MS (EI) *m/z* (rel. int.): 84 (33) $[M]^+$, 83 (34) $[M-1]^+$, 82 (100) $[M-2]^+$, 67 (48) $[M-17]^+$.

Isolation of cyanohydrin glucosides from P. morifolia. Fresh plant material (15 g) was extracted in boiling 80% MeOH for 3 min, homogenized, boiled again for 5 min, filtered, and the extract evapd. The residue was chromatographed on a column packed with 160 g silica gel; the fractions (25 ml) were monitored for cyanogenesis using the sandwich TLC method [23]. The cyanogenic fractions were further purified by reverse phase HPLC on a 1.6 × 25 cm column of LiChrosorb RP18 (7 μm), with 20% aq. MeOH as the mobile phase. The yield was 84 mg linamarin and 2 mg of a mixture of lotaustralin and epilotaustralin in a 7:1 ratio. The compounds were identified by ^{1}H NMR spectroscopy of the free glucosides as well as of their acetyl derivatives.

Isolation of cyanohydrin glucosides from T. angustifolia. Fresh plant material (7 g) was extracted and fractionated as described above, except that the HPLC mobile phase contained 30% MeOH. The yield was 11 mg of a mixture of deidaclin and tetraphyllin A (7:1 ratio), as shown by ^{1}H NMR spectra (free glucosides and their acetates). No tetraphyllin B was detected [10].

Feeding experiments. The radiolabelled amino acids and the nitriles were administered by immersing cut shoots (weighing 30–40 g for *T. angustifolia* and about 5 g for *P. morifolia*) in aq. solns of the compounds; 2 ml of 1.35 mM solns of the amino acids (total of 2.7 μmol), containing either 5.5 μCi 2-(2′-cyclopentenyl)[2-^{14}C]glycine or 5.0 μCi L-[U-^{14}C] valine were used. In the case of the incorporation inhibition experiments the solns also contained 2.7 μmol of either 2-cyclopentenecarbonitrile or 2-methylpropanenitrile. After the uptake of the initial solns, the amount of radioactivity left in the vials was determined, and the shoots placed in water for 48 hr; they were given 16 hr of illumination for each 24-hr period.

For each of the 16 feeding experiments (Table 1), the plant was extracted as described above, and the extract evapd. Initial investigation by TLC, followed by autoradiography, revealed the presence of radioactive glucosides. After addition of 10–30 ml MeOH to the residue the resulting suspension was centrifuged, the pellet washed with 5 ml MeOH, the combined solns evapd, redissolved in 2 ml 5% aq. MeOH, and passed through a BondElute RP18 syringe (Analytichem International; size 3 ml, containing 500 mg of the sorbent). The syringe was eluted with 8 × 1 ml 5% aq. MeOH, the combined eluates were evapd, and the glucosides purified by HPLC on a 1.6 × 25 cm column of LiChrosorb RP18 (7 μm) with 20% aq. MeOH, using refractive index detection. Portions of linamarin and of the mixtures of deidaclin and tetraphyllin A thus obtained were counted, and then dissolved in 2 ml phosphate buffer (pH 5.5) and placed in the outer chamber of Conway microdiffusion cells. The glucosides were hydrolysed for 48 hr after addition of 0.1 ml of crude *Helix pomatia* enzyme soln (Sigma). The HCN formed was trapped in 2 ml 0.1 M aq. NaOH present in the inner chamber of the cells, and its amount [35] and radioactivity were determined.

In the case of experiments (Table 1, Expts 5 and 6) in which *T. angustifolia* was fed with a solution containing 2-methylpropanenitrile, the above purification of the extracts by HPLC yielded trace amounts of non-radioactive linamarin, identified by ^{1}H NMR (free glucoside and its acetate).

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