Plant Analysis by Butterflies: Occurrence of Cyclopentenylglycines in Passifloraceae, Flacourtiaceae, and Turneraceae and Discovery of the Novel Nonproteinogenic Amino Acid 2-(3′-Cyclopentenyl)glycine in *Rinorea*[1]

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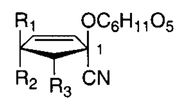
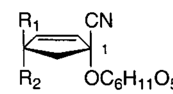
Following records about feeding habits of nymphalid butterflies, a novel nonproteinogenic L-amino acid, (*S*)-2-(3′-cyclopentenyl)glycine (**11**), was discovered in *Rinorea ilicifolia*, a species where the presence of a cyclopentanoid natural product of this kind was neither known nor anticipated from the taxonomic point of view. Another novel amino acid, (2*S*,1′*S*,2′*S*)-2-(2′-hydroxy-3′-cyclopentenyl)glycine (**12**), the stereochemistry of which was determined by single-crystal X-ray diffraction, was shown to occur in species belonging to Flacourtiaceae, Passifloraceae, and Turneraceae. These species, many of which serve as hosts for nymphalid butterflies (Acraeinae, Heliconiinae, Argynninae), also produce 2-(2′-cyclopentenyl)-glycine. Cyclopentenylglycines are proposed to be novel chemical recognition templates for plant−insect interactions. Ratios between the epimers of (2*S*)-2-(2′-cyclopentenyl)glycine, which co-occur in plants, were determined by ^{1}H NMR spectroscopy. Contrary to a previous report, the (2*S*,1′*R*) epimer always appears to predominate over the (2*S*,1′*S*) epimer. Stereochemical aspects of biosynthesis of natural cyclopentanoid cyanogenic glycosides are discussed in relation to these findings.

Species belonging to a narrowly defined, pantropical cluster of flowering plants consisting of Passifloraceae, Turneraceae, some tribes of Flacourtiaceae, and two small, closely allied families, Achariaceae and Malesherbiaceae, are known to produce, with only a few exceptions,[3] cyclopentanoid cyanohydrin glycosides such as **1**−**7** ($C_6H_{11}O_5$ = *β*-D-glucopyranosyl) and derivatives.[4−14] Some of these plants also produce cyclopentanoid fatty acids (**8**).[15,16] An established or assumed precursor of these cyclopentanoid natural products is L-2-(2′-cyclopentenyl)glycine.[17−20] Both epimers of the amino acid, the (2*S*,1′*R*) epimer **9** and the (2*S*,1′*S*) epimer **10**, have been detected in plants.[9−11,21] There are no reports about the occurrence of cyclopentanoids such as **1**−**10** outside the Passifloraceae, Flacourtiaceae, Turneraceae, Malesherbiaceae, and Achariaceae (hereafter referred to as the passifloraceous group).

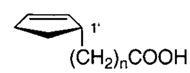
The taxa characterized by production of the cyclopentanoids **1**−**10** are known to be host plants for a group of nymphalid butterflies (family Nymphalidae).[22] Thus, many species belonging to *Acraea* (Nymphalidae, subfamily Acraeinae), a large and as a whole polyphagous genus, feed preferably or exclusively on *Adenia*, *Passiflora*, *Smeathmannia*, and *Tryphostemma* of the Passifloraceae, on *Caloncoba*, *Hydnocarpus*, *Kiggelaria*, *Oncoba*, *Rawsonia*, and *Xylotheca* of the Flacourtiaceae, or on *Wormskioldia* belonging to the Turneraceae.[23] Nearly all heliconiines (subfamily Heliconiinae) feed on Passifloraceae, usually *Passiflora*.[22−24] Members of *Cymothoe* (Limenitinae) feed, among others, on *Caloncoba*, *Kiggelaria*, and *Rawsonia*.[23] *Euptoieta* (Argynninae) use *Turnera* (Turneraceae) and *Passiflora*, whereas *Parthenos* (Limenitinae) use *Adenia* and *Passiflora* as hosts.[23] Numerous other, perhaps more sporadic, examples of the attachment of members of the Nymphalidae family to species belonging to Passifloraceae, Flacourtiaceae, and Turneraceae have been recorded.[23]





1 $R_1 = R_2 = R_3 = H$
2 $R_1 = R_3 = H, R_2 = OH$
3 $R_1 = OH, R_2 = R_3 = H$
4 $R_1 = R_3 = OH, R_2 = H$

5 $R_1 = R_2 = H$
6 $R_1 = OH, R_2 = H$
7 $R_1 = H, R_2 = OH$



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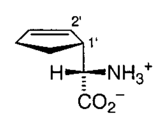

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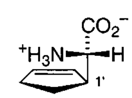

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These larval food habits suggest the presence of a common chemical basis for the selection of hosts.[25] Cyanogenesis (the ability to produce hydrogen cyanide upon damage of tissue) is one obvious chemical characteristic of the host plants involved; the presence of cyclopentanoids such as **1**−**10** is another one. The members of Heliconiinae are indeed cyanogenic.[26] Although many of them synthesize *β*-D-glucopyranosides of acetone and butanone cyanohydrin (linamarin and lotaustralin, respectively) from the respective amino acids valine and isoleucine,[27−30] the presence of cyclopentanoid cyanohydrin glycosides in heliconiines fed on *Passiflora* was recently reported.[31] Moreover, the glycoside **4** (gynocardin) was reported to be present in *Acraea horta* (Acraeinae), which uses *Kiggelaria africana* (Flacourtiaceae) as the host.[32]

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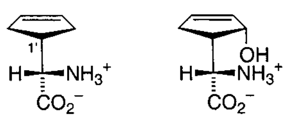
Table 1. Content of Cyclopentenylglycines in Species of Flacourtiaceae, Passifloraceae, Turneraceae, and Violaceae

species	content of **9** + **10**	ratio **9**:**10**	other amino acids
Androsiphonia adenostegia[a,b]	0.005%	high[i]	
Caloncoba echinata[c]	0.1%	100:15	**11**, 0.0015%; **12**, 0.03%
Kiggelaria africana[c]	0.01%	100:21	
Mathurina penduliflora[d,e]	0.025%	100:7	
Passiflora citrina[a]	0.004%	100:19	**12**, 0.0003%
Passiflora cuneata[a]	0.0002%	high[i]	
Passiflora indecora[a]	0.001%	high[i]	
Passiflora quadrangularis[a]	0.03%	100:14	
Passiflora suberosa[a]	0.02%	100:8	
Passiflora subpeltata[a]	0.03%	100:6	**12**, 0.03%
Rawsonia lucida[c,f]	0.0007%	high[i]	
Rinorea ilicifolia[g]	*h*	*h*	**11**, 0.07%
Turnera angustifolia[d]	0.2%	100:4	**11**, 0.24%; **12**, 0.01%

[a] Passifloraceae. [b] Data from ref 10. [c] Flacourtiaceae. [d] Turneraceae. [e] Data from ref 11. [f] Data from ref 9. [g] Violaceae. [h] Not detected. [i] Only the (2*S*,1′*R*) epimer **9** was detected.

The work reported herein extends our knowledge of the occurrence of the cyclopentanoid natural products to *Rinorea*, a noncyanogenic genus belonging to Violaceae. The cyclopentanoid found in *Rinorea ilicifolia* is **11**. This novel amino acid represents the first observation of a secondary metabolite of this kind outside the passifloraceous group. The discovery of **11** is based upon the literature reports[33,34] that *Rinorea*, in addition to the passifloraceous group, is a major host plant source for the acraeine butterflies. Also, a strong association of the Flacourtiaceae-feeding *Cymothe*[23] with *Rinorea* is known.[35,36] The fact that *Rinorea* was found to be noncyanogenic, but contains a cyclopentanoid amino acid **11** similar to **9** and **10**, provides a hint about a possible chemical basis for the association of members of *Acraea* and *Cymothe* with the passifloraceous group, now extended to include *Rinorea*. Moreover, new sources of the epimers **9** and **10** within the Passifloraceae, Flacourtiaceae, and Turneraceae are reported. The hydroxylated amino acid **12** was identified in several of the plants.



11 **12**

Results and Discussion

Extracts of *Rinorea ilicifolia* (Violaceae) and of selected plants belonging to the Flacourtiaceae, Passifloraceae, and Turneraceae (Table 1), i.e., the families that produce cyclopentanoid cyanohydrin glycosides, were fractionated by ion exchange followed by silica gel chromatography, the fractions being monitored using the ninhydrin reaction and by ^{1}H NMR. The extract of *R. ilicifolia* contained **11** as the sole cyclopentanoid amino acid. In the extract of *Passiflora subpeltata*, the hydroxylated analogue **12** was detected along with **9** and **10**. Pure amino acids **11** and **12** were obtained using preparative, reversed-phase HPLC.

The structures of the novel amino acids **11** and **12** were apparent from standard NMR experiments. The positive molar rotation, $[\Phi]_D = +17.6°$ (1 M HCl), confirms the L-configuration (*S* configuration) of **11**. The relative stereochemistry of **12**, which was ambiguous on the basis of NMR data alone, was established by single-crystal X-ray diffraction analysis. A perspective drawing of the solid state conformation is shown in Figure 1. The observed bond lengths and angles are in agreement with expected values.[37] The ammonium group is involved in two intramolecular hydrogen bonds, one to the carboxylate group and one to the hydroxy group. The absolute configuration of

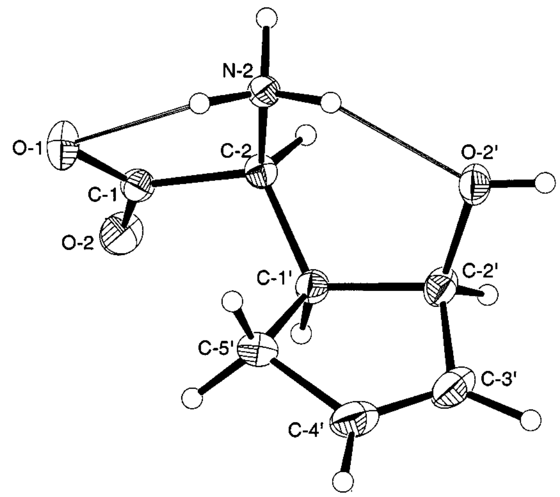


Figure 1. Molecular structure of **12** as determined by X-ray diffraction at −150 °C. Displacement ellipsoids enclose 50% probability; hydrogens are represented by spheres of arbitrary size; intramolecular hydrogen bonds are indicated by thin lines.

the amino acid moiety could be safely assumed to be *S* (L-amino acid). Accordingly, the Flack absolute structure parameter[38] for this configuration was calculated as x = 0.0(2), and as x = 0.93(19) for the other enantiomer. Final atomic coordinates and other crystallographic data for **12** are included in the Supporting Information.[39]

The content of the cyclopentanoid amino acids **9**−**12** in crude plant isolates was estimated by quantitative ^{1}H NMR spectroscopy using the standard addition method. Thus, a ^{1}H NMR spectrum of the isolate was recorded with a long relaxation delay, a known amount of synthetic 2-(2′-cyclopentenyl)glycine (mixture of all stereoisomers) was added, the quantitative ^{1}H NMR spectrum was recorded again, and the amounts of the amino acids present were calculated from the increase of integrals of the resonances of interest. Even though the crude isolates contained impurities of other amino acids (in particular isoleucine, tyrosine), no or very minor other resonances were observed in the olefinic region of the spectra of **9**−**12**. All spectra were recorded in D_2O at pD 6.2 ± 0.1 in order to obtain reproducible data. The epimers **9** and **10** could be distinguished on the basis of the recently described pH-dependence of their chemical shifts.[40] Thus, the ^{1}H NMR analysis yielded ratios between the amino acids present, including the ratios between the epimers **9** and **10**. Examples of the ^{1}H NMR analysis are shown in Figure 2. The results are collected in Table 1 together with recently reported results on *Androsiphonia adenostegia* (Passifloraceae), *Mathurina penduliflora* (Turneraceae), and *Rawsonia lucida* (Flacourtiaceae).[9−11]

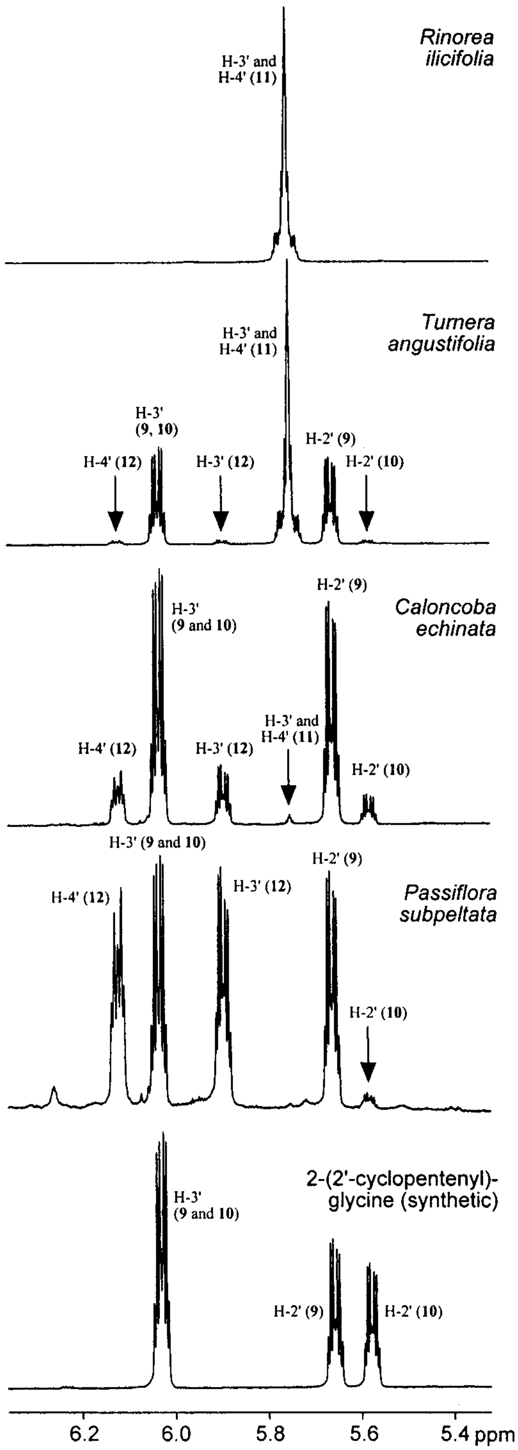


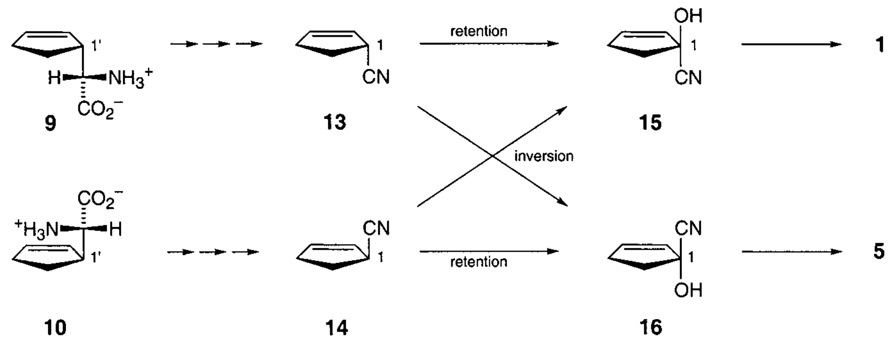
Figure 2. Determination of cyclopentenylglycines in plant extracts by 400 MHz ^{1}H NMR (25 °C). Shown are olefinic regions of spectra of a synthetic mixture of 2-(2′-cyclopentenyl)glycine stereoisomers (**9** and **10** and their enantiomers) and of amino acid fractions from representative plant species. All solutions in D_2O at pD = 6.2 ± 0.1.

All plants that were studied (Table 1) belong to genera that are hosts for nymphalid butterflies, mainly acraeines.[23] All plant species contained (2*S*,1′*R*)-2-(2′-cyclopentenyl)glycine (**9**) except for *R. ilicifolia*, which produced only **11**. In addition to **9**, most of the species contained much smaller but readily detectable amounts of the (2*S*,1′*S*) epimer **10** (Figure 2, Table 1). Hence, it appears that the epimer **9** always predominates over the epimer **10**. This finding contrasts with the first report on the isolation of **9** and **10** from plants.[21] Thus, Cramer et al. isolated mixtures of **9** and **10** from *Caloncoba echinata* leaves and *Hydnocarpus anthelmintica* seeds (Flacourtiaceae) and stated that the ^{1}H NMR spectra of the isolated and synthetic material were identical.[21] This would imply that the ratio of **9** and **10** in *C. echinata* leaves and *H. anthelmintica* seeds was 1:1.[21] However, in the present study the ratio between **9** and **10** in *C. echinata* leaves was 100:15 (Figure 2), and similar or higher ratios were observed in all other species investigated thus far (Table 1). Along with **9** and **10**, *C. echinata* and *T. angustifolia* contained **11**, and *C. echinata*, *Passiflora citrina*, *P. subpeltata*, and *T. angustifolia* contained **12** (Table 1). The interconversion of **9** and **10** in vivo may occur via a common, conjugated enol form of the epimeric α-ketoacids formed from **9** and **10** by transamination.[41]

The leaves of *R. ilicifolia* used for the isolation of **11** were not cyanogenic. To our knowledge, cyanogenesis has never been reported from *Rinorea* or from Violaceae at large. On the other hand, all Flacourtiaceae, Passifloraceae, and Turneraceae species reported in Table 1 have previously been reported to be cyanogenic and to produce cyclopentanoid cyanohydrin glycosides[2,9−11,13,17,42,43] except for *P. subpeltata*, which was reported to produce linamarin.[7] One question that emerges from the isolation of **11** and **12** is whether these amino acids can serve as precursors of cyanogenic glycosides. All currently known cyclopentanoid cyanogenic glycosides are formally derivatives of 2-cyclopentenone cyanohydrin (cf. **1**−**7**, in some derivatives[2,42] the double bond is altered by oxygenation). Thus, no cyanogenic glycosides with a 3-cyclopentenone cyanohydrin structure, which would be formed from **11** and **12** as the precursors, are known. This is especially striking in the case of *T. angustifolia*, which contains **11** as the major cyclopentanoid amino acid, but which nevertheless produces **1** and **5** as the only reported cyanohydrin glycosides.[18]

During the biosynthesis of cyclopentanoid cyanogenic glycosides, the stereogenic center C-1′ of the amino acid is converted to the cyanohydrin center C-1 (Scheme 1). The intriguing feature of the biosynthesis of these glycosides is that they are usually encountered as mixtures of glycosides with enantiomeric aglycones, i.e., **1** co-occurs with **5**, **2** with **6**, and **3** with **7** (in variable ratios).[2,4−11,18,19] This demonstrates the parallel production of the cyanohydrins **15** and **16** (Scheme 1). However, since all biosynthetic studies reported so far have been carried out with synthetic mixtures of all four stereoisomers of the precursor amino acid,[17−20] the stereochemical course of formation of the cyanohydrins **15** and **16** (Scheme 1) is unknown. One possibility is that the stereochemistry of the cyanohydrins **15** and **16** is determined by the stereochemistry of the amino acids **9** and **10**. In such a case each of the intermediate[18,19,44] nitriles, **13** and **14**, is converted to only one cyanohydrin, either **15** or **16**, in a stereospecific hydroxylation step occurring either with a retention or with an inversion of the configuration at C-1 (Scheme 1). Another possibility is that only one of the epimeric amino acids, either **9** or **10**, and hence only one of the two nitriles, either **13** or **14**, serves as a precursor for both cyanohydrins, **15** as well as **16**. If so, two enantiomeric cyanohydrins would be formed from a single stereoisomer of the precursor amino acid. This implies that the substrate specificity in the nitrile hydroxylation step (Scheme 1) is relatively broad. This is in agreement with the observation of the ability of *T. angustifolia* and *P. morifolia* to biosynthesize unnatural cyanogenic glycosides from externally supplied nitriles[19] and with the ability of the latter to inhibit biosynthesis of the natural glycosides.[18]

Scheme 1



It should also be pointed out that *A. adenostegia*,[10] *K. africana*,[42] *M. penduliflora*,[11] *P. citrina*,[2] *P. cuneata*,[2] *P. indecora*,[2] *P. quadrangularis*,[43] *P. suberosa*,[42] and *R. lucida*[9] predominantly produce cyanohydrin glycosides (**1**−**4** and derivatives), which have the same configuration at C-1 as in the major 2-(2′-cyclopentenyl)glycine epimer **9**. These glycosides (**1**−**4**) and their derivatives appear indeed to be generally more common than **5**−**7**.[2,4−14,42,43] Also, the cyclopentanoid fatty acids (**8**) have the same configuration at C-1′ as in **9**.[15,16] By contrast, *T. angustifolia* contains **5** as the major cyanogenic constituent,[18] which has the same configuration at C-1 as the minor epimer **10**. The content of the cyclopentanoid cyanohydrin glycosides in plants is thus not a simple function of the available pool of cyclopentenylglycines present, but the majority of cyclopentanoid natural products have the same stereochemistry as the major natural epimer **9**.

Adopting the hypothesis that there is a common recognition template for nymphalid butterflies in the passifloraceous group as well as in *Rinorea*, the chemicals responsible for the attraction could logically be cyclopentenylglycines. To our knowledge, this would be the first case of a nonproteinogenic amino acid serving as the chemical basis for insect attraction by plants. Whether or not some Lepidoptera can synthesize cyclopentanoid cyanohydrin glycosides from sequestered cyclopentenylglycines, which would be an evolutionary extension of their synthesis of linamarin and lotaustralin,[45] has yet to be determined.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 25 °C on a Bruker AMX 400 spectrometer (proton frequency 400.13 MHz) using tetramethylsilane (CD_3OD solutions) or sodium 4,4-dimethyl-4-silapentanesulfonate (D_2O solutions) as internal standard. Quantitative ^{1}H NMR spectra were recorded using 90° pulses with interpulse intervals of 13 s; T_1 relaxation times of cyclopentenylglycines, determined by the inversion recovery method, were ≤2.1 s. NOESY spectra were obtained with mixing times of 500−800 ms. HMBC spectra were optimized for $^nJ_{C,H} = 7$ Hz. Column chromatography was performed on Merck silica gel 60, 0.062−0.2 mm. Fractions were monitored by TLC (Merck precoated silica gel 60 F_{254} plates) using ninhydrin (260 mg of ninhydrin and 45 mL of glacial acetic acid in 100 mL of 96% EtOH) as spray reagent. Ion-exchange chromatography was performed on Dowex-50W strongly acidic cation exchanger (50−100 mesh, cross-linkage 8%) from Sigma. The resin was soaked in distilled H_2O overnight and repeatedly washed with distilled H_2O and EtOH, and then alternately with 2 M aqueous NaOH and 2 M aqueous HCl prior to use. Authentic 2-(2′-cyclopentenyl)glycine (mixture of all stereoisomers) was synthesized from 3-chlorocyclopropene and diethyl 2-acetylaminomalonate similarly as previously described.[18,40,46]

Plant Material. Aerial parts of *Passiflora citrina* MacDougal (voucher DFHJJ6), *P. cuneata* Willd. (DFHJJ7), *P. indecora* Kunth. (DFHJJ12), *P. quadrangularis* L. (*Passiflora* × *decaisneana* Planch.[47]) (DFHJJ17), *P. suberosa* L. (DFHJJ18), *P. subpeltata* Ortega (DHJJ19), *Kiggelaria africana* L. (DFHJJ20), and *Turnera angustifolia* Miller (DFHJJ21) were grown in the Botanical Garden, University of Copenhagen. *T. angustifolia* was obtained by propagation from specimens used in the previous work.[18,19] The identity of the plants was confirmed by L. B. Jørgensen, and voucher specimens were deposited in Herbarium C (Botanical Museum, University of Copenhagen, Copenhagen). Leaves of *Rinorea ilicifolia* (Welw. ex Oliv.) Kuntze (voucher GC4700) and of *Caloncoba echinata* (Oliv.) Gilg (GC47674) were collected in southern Ghana (Agriculture Research Station, Kade, and Atewa Range Forest Reserve, respectively); voucher specimens were deposited in Herbarium GC (Ghana Herbarium, Department of Botany, University of Ghana, Legon). All plants except *R. ilicifolia* and *P. subpeltata* liberated hydrogen cyanide upon disruption of the tissue with CH_2Cl_2 [development of blue color on filter paper impregnated with 4,4′-bis(dimethylamino)diphenylmethane and bis(acetylacetonato)copper(II)[48]].

General Procedure for Extraction and Identification of Cyclopentenylglycines. Dried and milled plant material (20−30 g) was extracted 1−3 times by stirring with 200−300 mL of 50% aqueous EtOH for several hours. The extract was evaporated and freeze-dried, redissolved in 150 mL of H_2O, and decolorized by heating with charcoal (10 min at 90 °C). The solution, after adjustment of the pH to 5−6, was applied to a 1 × 40 cm column of Dowex-50W (H^+ form). The column was rinsed with excess distilled H_2O, and total amino acid fraction was eluted with 150 mL of 2 M ammonia. The eluate was evaporated, freeze-dried, and investigated by TLC (silica gel) using *t*-BuOH−2-butanone−acetone−MeOH−H_2O−concentrated ammonia (40:20:20:1:14:15). When the TLC analysis showed the presence of amino acids with R_f values similar to that of 2-(2′-cyclopentenyl)glycine standard (synthetic, giving one spot with R_f of 0.45), the amino acid fraction was chromatographed on silica gel (approximately 200 times amount by weight) using the same solvent system. Appropriate fractions (TLC) were pooled and evaporated, samples were dissolved in D_2O, pD (uncorrected pH-meter reading) adjusted to 6.2 ± 0.1, internal standard was added, and the solution was investigated by ^{1}H NMR.

(*S*)-2-(3′-Cyclopentenyl)glycine (11). *R. ilicifolia* (dried and milled leaves, 200 g) was extracted two times with 50% aqueous EtOH. The extract was evaporated, the residue (32.4 g) dissolved in 1.5 L of H_2O, and the solution decolorized with charcoal, adjusted to pH 6, and applied to a 3.8 × 30 cm column of Dowex-50W (H^+-form; 300 g). The column was rinsed with 2 L of distilled H_2O, and amino acids were eluted with 700 mL of 2 M aqueous ammonia. The eluate was evaporated and freeze-dried to give 2.9 g of a residue, which was chromatographed on a 4 × 74 cm column of silica gel (400 g), collecting 25 mL fractions. Appropriate fractions (TLC) were evaporated to give 737 mg of crude product; ^{1}H NMR spectra showed the presence of **11** and no detectable amounts of **9** or **10** (Figure 2). The isolate was further fractionated on a 2.5 × 70 cm silica gel column (150 g), collecting 10 mL fractions, to give 144 mg (0.072%) of **11**. The product was finally purified by crystallization from H_2O−acetone: $[\alpha]_D^{25}$ +12.4° (*c* 0.33, 1 M HCl), $[\Phi]_D$ +17.6°; ^1H NMR (400 MHz, CD_3OD) δ 2.31−2.40 (2 H, m), 2.45−2.59 (2H, m) and 2.76−2.85 (1H, m) (H-1′, H-2′, and H-5′), 3.53 (1H, d, $J = 6.1$ Hz, H-2), 5.67−5.72 (2H, m, H-3′ and H-4′); ^{13}C NMR (100 MHz, CD_3OD) δ 35.4, 36.5, and 40.2 (C-1′, C-2′, C-5′), 60.2 (C-2), 130.5 and 130.7 (C-3′, C-4′), 183.9 (C-1); *anal.* C 59.90%, H 8.08%, N 9.62%, calcd for $C_7H_{11}NO_2$ C 59.56%, H 7.85%, N 9.92%.

(2*S*,1′*S*,2′*S*)-2-(2′-Hydroxy-3′-cyclopentenyl)glycine (12). *P. subpeltata* (dried and milled aerial parts, 110 g) was extracted four times with 50% aqueous EtOH. The extract was evaporated, the residue dissolved in 1.5 L of H_2O, and the solution decolorized with charcoal, adjusted to pH 6, and applied to a 3.8 × 30 cm column of Dowex-50W (H^+-form; 300 g). The column was rinsed with 2 L of distilled H_2O, and amino acids were eluted with 950 mL of 2 M aqueous ammonia. The eluate was evaporated and freeze-dried to give 5.6 g of a residue, which was divided into two equal portions, each being chromatographed on a 4 × 74 cm column of silica gel (400 g). Appropriate fractions (TLC) from both columns yielded a total of 485 mg of a crude mixture containing **12** together with **9** and **10** in a ratio of 100:6 (Figure 2). Repeated purification by preparative HPLC (1.6 × 20 cm column of Lichrospher-100 RP-18, 5 μm, 5 mL/min of 5% MeCN in H_2O, spectrophotometric detection at 200 nm; the major impurity to be removed was isoleucine) yielded a total of 31 mg (0.03%) of **12**. A fraction of the material was recrystallized from H_2O−MeOH: $[\alpha]_D^{20}$ +56.5° (*c* 0.23, 1 M HCl), $[\Phi]_D$ +89°; ^1H NMR (400 MHz, CD_3OD) δ 2.33−2.48 (2H, m, H-5′), 2.73−2.80 (1H, m, H-1′), 3.87 (1H, d, $J = 5.8$ Hz, H-2), 4.86 (1H, m, H-2′), 5.85 (1H, m, H-3′), 6.03 (1H, m, H-4′); ^{13}C NMR (100 MHz, CD_3OD) δ 33.2 (C-5′), 43.4 (C-1′), 56.7 (C-2), 77.6 (C-2′), 133.8 (C-3′), 135.4 (C-4′), 174.2 (C-1); *anal.* C 53.33%, H 7.35%, N 8.75%, calcd for $C_7H_{11}NO_3$ C 53.49%, H 7.05%, N 8.91%.

X-ray Crystallographic Analysis of (2*S*,1′*S*,2′*S*)-(2′-Hydroxy-3′-cyclopentenyl)glycine (12).[39] Colorless single crystals were obtained by crystallization from H_2O−MeOH. Crystal dimensions: 0.32 × 0.12 × 0.10 mm. Crystal data: $C_7H_{11}NO_3$, $M_r = 157.17$, orthorhombic, space group $P2_12_12_1$ (No. 19), $a = 7.614(2)$ Å, $b = 8.383(1)$ Å, $c = 11.777(2)$ Å, $V = 748.1(2)$ Å^3, $Z = 4$, $D_c = 1.395$ Mg m^{-3}, $F(000) = 336$, μ(Cu Kα) = 0.920 mm^{-1}, $T = 122.0(5)$ K. Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer[49] using graphite-monochromated Cu Kα radiation ($\lambda = 1.54184$ Å). Intensities were collected using the $\omega/2\theta$ scan mode. Unit cell dimensions were determined by least squares refinement of 25 reflections (θ range 39.27−40.70°). The reflections were measured in the range $0 \le h \le 9$, $-10 \le k \le 10$, $-14 \le l \le 14$ (6.50° < θ < 74.95°). Data were reduced using DREADD.[50,51] The intensities of five standard reflections were monitored every 10^4 s (decay 7.9%, corrected). Absorption correction was applied using the program ABSORB ($T_{min} = 0.844$; $T_{max} = 0.920$).[52] A total of 3230 reflections were averaged according to the point group symmetry 222, resulting in 1532 unique reflections ($R_{int} = 0.0383$ on F_o^2). The structure was solved by the direct method using the program SHELXS97[53,54] and refined using the program SHELXL97.[55] Full matrix least-squares refinement on F^2 was performed, minimizing $\sum w(F_o^2 - F_c^2)^2$, with anisotropic displacement parameters for the non-hydrogen atoms. The positions of the hydrogen atoms were located on intermediate difference electron density maps and refined with fixed isotropic displacement parameters. The refinement (134 parameters, 1532 reflections) with the molecule having the absolute configuration as in **12** converged at $R_F = 0.0270$, $wR_{F^2} = 0.0695$ for 1489 reflections with $F_o > 4\sigma(F_o)$; $w = 1/[\sigma^2(F_o^2) + (0.0266P)^2 + 0.1290P]$, where $P = (F_o^2 + 2F_c^2)/3$; $S = 1.062$. In the final difference Fourier map maximum and minimum electron densities were 0.112 and −0.226 e Å^{-3}, respectively. Refinement of the Flack absolute structure factor x in the final refinement gave $x = 0.0(2)$.[38,55]

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Supporting Information Available: Crystal and structure refinement data, final atomic coordinates, equivalent isotropic displacement parameters for non-hydrogen atoms, bond lengths and angles, anisotropic displacement parameters for non-hydrogen atoms, final atomic coordinates with fixed isotropic displacement parameters for hydrogen atoms, torsion angles, hydrogen bonds, close intermolecular interactions, and crystal packing diagram for **12**.[39] This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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