Pyridine Monoterpene Alkaloid Formation from Iridoid Glycosides. A Novel PMTA Dimer from Geniposide

Signe M. Frederiksen and Frank R. Stermitz*

Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523-1872

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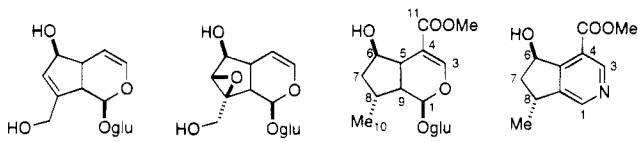
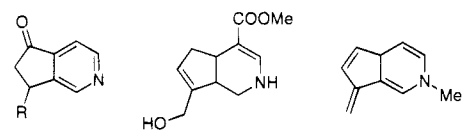
New pyridine monoterpene alkaloids (PMTAs) have been synthesized from the iridoid glycosides 8-*epi*-loganin, cornin, and antirrinoside by treatment with β-glucosidase and aqueous NH_4OAc. The PMTA from antirrinoside contained an 8-α-OAc group from the opened 7,8-epoxide moiety. Treatment of genipin, the aglycone of geniposide, with HCl(g) and NH_3(g) yielded the PMTA racemigerine, a known plant isolate, in which the C=C−CH_2OH side chain was converted to C=C−CH_3. Reaction of geniposide with β-glucosidase and aqueous NH_4OAc led to oligomeric alkaloids, but at high dilution a dimer was obtained whose structure was formally that of a Diels−Alder adduct between racemigerine and a dihydropyridine. These biomimetic semisyntheses were analyzed in terms of reaction mechanisms and the relative paucity of known plant PMTAs in comparison with the multitudinous occurrence of their presumed iridoid glycoside precursors.

Pyridine monoterpene alkaloids, PMTAs, occur naturally in a number of different plant families and genera.[1,2] They have often been prepared by semisynthesis from iridoid or secoiridoid glucosides in what are presumed to be biomimetic reactions. These reactions, of which there are many variants, generally involve treatment of the iridoid glucoside with some source of NH_3 and acid and/or a similar reaction on the aglycone formed after treatment with β-glucosidase. PMTAs have also been recognized as artifacts that occur during alkaloid isolations from iridoid-containing plants when aq NH_3 is used as a basifying agent in the separation scheme. This is usually demonstrated by the absence of PMTAs when hydroxide is used for pH adjustment. The number of known natural PMTAs is, however, strikingly small considering the number of investigated iridoid-containing plants, which have yielded more than 600 different iridoid or secoiridoid glycosides. The situation with plant occurrences can be exemplified by *Castilleja rhexifolia*, where the content of aucubin (**1**), catalpol (**2**), and penstmonoside (**3**) were about equal, but where the only PMTA found (rhexifoline, **4**) was derived from penstemonoside.[3] (PMTA structure numbering, as in **4**, corresponds to that usually used for the precursor iridoid glycosides.) Among the most common and widespread iridoid glycosides in the plant kingdom are **1**, **2**, antirrinoside (**5**), and geniposide (**6**), but the PMTAs corresponding to these iridoids have never been reported either as isolates or synthetics. We were unable to find reports on attempts to form PMTAs from **2** or **5**. PMTAs **7** and **8** were produced from **1** in minute quantities by human intestinal bacteria, and **7** was synthesized in about 4% yield by treating **2** with β-glucosidase and NH_4Cl.[4] The 1,2-dihydroPMTA **9**, named genipinin, was produced from **6** by bacteria and synthesized in about 3% yield from **6**.[5] A secoiridoid glucoside undergoes similar bacterial reactions.[6] These studies on intestinal flora transformations were undertaken because of the common occurrence of iridoid glycosides in folk medicinal plants of China and Japan.[4−6] Dark blue and other colors sometimes appear in the solution during PMTA preparations. Colors formed during the treatment of geniposide with methylamine have been shown to be due to pyrindine (**10**) and oligomeric pyrindine pigments.[7,8] Amazonian natives use *Genipa americana* fruits to produce a dark-blue decorative coloration on the skin, presumably formed from reactions of **6** with skin amino acids.[9]

The surprising absence in plant isolates of PMTAs derived from commonly occurring, high-concentration iridoids could be related to the chemical reactivity/instability of the iridoid or the PMTA, or that of the reaction intermediates. It is also possible that plant cell compartmentalization, where synthesis location and/or storage with proper enzymes for PMTA formation, might differ for different iridoids. There are clearly differences in plant part occurrence or concentration of different iridoid glycosides,[10−12] but little or nothing is known of this for PMTAs. A several-fold higher concentration of **4** was, however, found in seeds of *C. rhexifolia* compared to leaves and stems, and **4** was also isolated from *Platyptilia pica* adult moths whose larvae are mainly seed feeders.[13] In order to understand what factors might govern PMTA presence or absence in plants, we have begun some exploratory syntheses of the alkaloids from structurally different iridoid glycosides using a single, definable procedure. This involved dissolving an iridoid glucoside in 10% aqueous NH_4OAc, adding β-glucosidase, stirring the mixture at 37 °C for 24 h, and then extracting with CH_2Cl_2. This is referred to below as "the standard procedure." The total extract residue was analyzed by ^{1}H-NMR to determine a crude yield, and then PMTAs, if any, were purified and characterized.



1 **2** **3** **4**



7: R = CH_2OH
8: R = CH_3 **9** **10**

* To whom correspondence should be addressed. Phone: (970) 491-5158. Fax: (970) 491-5610. E-mail: frslab@lamar.colostate.edu.

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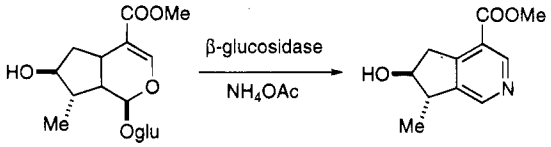
Results

Conversion of 8-*epi*-Loganin (11) to 8-*epi*-Cantleyine (12) (Eq 1). One of the more common PMTAs

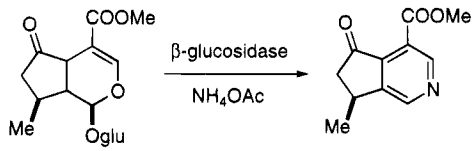


(1)

11 12

isolated from plants, or produced as an artifact, has been cantleyine,[1,2] which is derived from the iridoid loganin. On the other hand, 8-*epi*-cantleyine (**12**) has never been reported. Treatment of 8-*epi*-loganin (**11**) with the standard procedure indeed yielded **12**. The yield of **12** was estimated at 20%, with 7% subsequently isolated pure. Structure identification was accomplished by MS and by comparison of NMR spectra (see Experimental) with those for cantleyine and the starting iridoid **11**. Iridoid **11**, or its carboxylic acid derivative, occurs widely in plants of several families, such as the Scrophulariaceae, although its concentration is usually relatively low.

Conversion of Cornin (13) to Corninine (14) (Eq 2). Cornin (**13**) is not particularly widespread in oc-

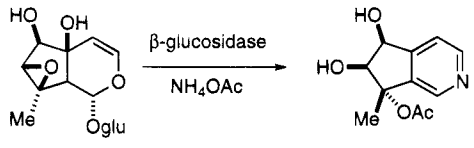


(2)

13 14

curence in plants, but where it is found (e.g., in *Cornus* spp.) it is often in relatively high concentration. Treatment of **13** under the standard conditions yielded about 80% of a mixture made up (by ^{1}H-NMR analysis) of about equal amounts of aglycone and **14**, along with smaller amounts of other pyridine-type compounds. Purification of **14** was, however, difficult, and only about 1 mg, still not quite pure, was finally isolated. Structure determination was by MS and NMR spectra, particularly in comparison with data for aucubinin A (**7**) and coelosperminone.[14] The latter is a natural isolate with the same gross structure as **7**. The absolute configuration at C-8 is unknown for **7** or for coelosperminone, and neither have been compared as to optical rotation.

Conversion of Antirrinoside (5) to PMTA 15 (Eq 3). Treatment of **5** under the standard conditions



(3)

5 15

yielded about 10% almost pure **15**, eventually crystallized in total 4% yield. The MS showed mol wt 223, rather than the 179 expected for the PMTA directly corresponding to **5**. This represented addition of C_2H_4O to yield a $C_{13}H_{13}NO_4$ molecular formula. A ^{1}H-NMR peak at δ 2.1 and ^{13}C-NMR peaks at δ 172.6 and 21.9 were consistent with presence of an acetyl group, and the molecular formula required opening of the epoxide

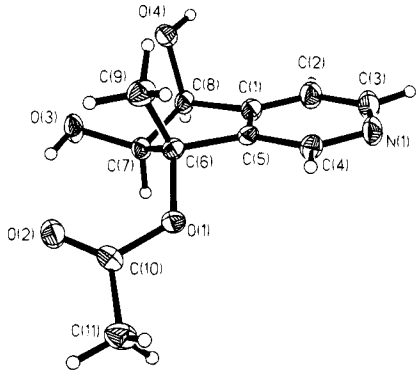
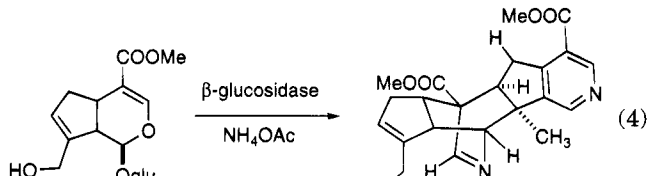


Figure 1. ORTEP projection of **15** (crystallographic numbering).

ring. Analysis of the NMR spectra (see Experimental) was in accord with this, and it remained to determine the position of acetylation. The ^{1}H-NMR spectrum showed doublets (J = 5.6 Hz) at δ 4.31 and 5.11, and the latter was assigned to H-6 based upon comparison with resonances observed in other 6-hydroxy PMTAs: δ 5.10[14] and 5.09,[15] for example. This was confirmed by observing an NOE enhancement between H-4 and the δ 5.11 resonance as well as an HMBC correlation between the two. The doublet at δ 4.31 is consistent with resonances for similar PMTAs, which bear a β-OH at C-7: δ 4.23, **12**, and δ 4.68.[16] The most likely position for the acetoxy group was therefore at C-8, and this was confirmed by a single crystal X-ray study, which also showed the stereochemistry indicated in **15** (Figure 1).

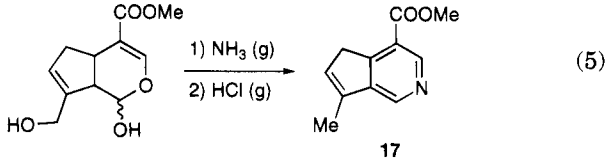
Antirrinoside was treated with aq NH_4OAc in the same manner, but without the β-glucosidase. Only starting material was recovered, and no epoxide ring-opened material was obtained. Ring opening to form **15** must occur at the PMTA stage or on one of the intermediates. The aglycone of antirrinoside was prepared in 31% yield, was treated with aq NH_4OAc, and a 23% yield of **15** was recovered. Protonation of the epoxide by NH_4^+ and subsequent ring opening by $^-$OAc would lead to **15**. Protonated epoxides are known to give products from nucleophilic attack at the most substituted carbon by either S_N1 or S_N2 mechanisms.[17]

Conversion of Geniposide (6) to Dimer 16 (Eq 4) and Genipin to Racemigerine (17) (Eq 5). Treat-



(4)

6 16



(5)

17

ment of **6** under the standard conditions yielded alkaloidal material showing a single spot on TLC and complex NMR spectra, but which was recovered relatively unchanged or unpurified after CC. Positive ion electrospray MS showed molecular ions consistent with

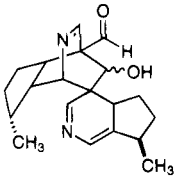
Table 1. NMR Data for Dimer **16**[a]

position	^{13}C NMR δ	^{1}H NMR δ (integral, mult., J Hz)	HMBC correlations[a]
1	148.8	8.52 (1H, s)	C-5, C-9
3	150.2	8.98 (1H, s)	C-4, C-5
4	122.9		
5	154.1		
6	32.8	3.36 (1H, dd, 21.0, 10.5)	C-5, C-7, C-9, C-4′
		3.60 (1H, dd, 21.0, 4.5)	C-5, C-7, C-9, C-4′
7	49.2	2.20 (1H, dd, 10.5, 4.5)	C-5, C-6, C-8, C-9, C-4′
8	52.0		
9	144.9		
10	28.9	1.28 (3H, s)	C-7, C-8, C-9, C-1′
11	165.9		
12	52.4	3.86 (3H, s)	C-11
1′	63.9	4.68 (1H, d, 3.0)	C-3′, C-5′, C-9′
3′	169.6	8.54 (1H, d, 3.0)	
4′	55.0		
5′	33.3	2.64 (1H, ddd, 2.6, 11.7, 6.5)	C-3′, C-4′, C-9′
6′	37.8	1.78 (1H, bd, 18.0)	
		2.48 (1H, m)	C-4′, C-5′, C-7′, C-8′
7′	127.0	5.34 (1H, bs)	C-5′, C-6′, C-8′, C-10′
8′	142.8		
9′	49.4	2.48 (1H, m)	C-1′, C-4′, C-5′, C-7′, C-8′
10′	60.4	4.06 (2H, bs)	C-7′, C-8′, C-9′
11′	173.4		
12′	52.8	3.92 (3H, s)	C-11′

[a] Optimized for J_{CH} = 5.5 Hz.

the presence of two compounds of mol wt 396 and 792. These approximate, but are not exact for, what would be a dimer and tetramer of the expected PMTA. Repetition of the glucosidase/NH_4OAc standard conditions on **7**, but at a tenfold diluted concentration of **7**, yielded a crude extract containing approximately 30–40% **16**, along with oligomers. Treatment of **7** aglycone under alternate PMTA formation conditions (eq 5) yielded racemigerine (**17**), a natural isolate from *Scaevola racemigera*.[18]

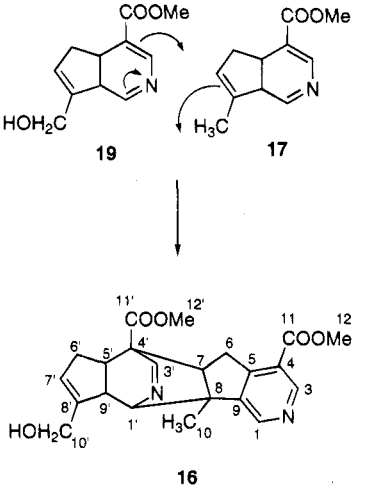
Pure **16** (9% overall yield) was obtained, and its structure proven as follows. FABMS established the molecular formula of $C_{22}H_{24}N_2O_5$ (found 397.1766, calcd 397.1763). The ^{13}C-NMR spectrum (Table 1) showed 22 carbons, including those for two carbomethoxy groups (one at an sp_2 carbon and one at an sp_3 carbon), a methyl at a quaternary carbon, a substituted pyridine, and the presence of one allylic primary alcohol moiety similar to that of the starting material. The ^{1}H-NMR spectrum (Table 1) contained the proper resonances for each of these groupings. In addition to the quaternary carbonyl resonances for the carbomethoxy groups (δ 173.4 and 165.9), there was a resonance at δ 168.2, which, by the DEPT ^{13}C NMR spectrum, represented a methine carbon. The COSY spectrum correlated this with a ^{1}H singlet resonance at δ 8.55. These resonances are very similar to those of the −CH=N− grouping in lindeniamine (**18**), an iridoid-derived reduced PMTA dimer



18

isolated from *Lindenia austro-caledonica*.[19] A Diels–Alder-type reaction between **17** and a likely intermediate (**19**) in PMTA formation from geniposide would yield

Scheme 1. Formation of Dimer **16**



an adduct consistent with all the data described so far (Scheme 1). That this was indeed the case was confirmed by NOE and HMBC experiments, which defined the structure as **16** and allowed the assignments of Table 1. Irradiation of the quaternary methyl resonance at δ 1.28 resulted in enhancements of H-1 in the pyridine ring, in H-7 of the fused cylcopentano portion (proving their *cis* relationship) and in H-1′. This last enhancement proved that the dimerization between **17** and **19** had proceeded in the regiochemical sense shown, which placed the quaternary methyl group away from, rather than on the same side as, the quaternary carbomethoxy. An NOE enhancement was also seen between the C-3′ methine proton (δ 8.54 ppm) and H-7, which showed that the azomethine bridge was on the same side as H-7 and consequently on the same side as the C-10 methyl.

Other Attempted Reactions and Isolations. Treatment of catalpol (**2**) under the standard conditions gave no PMTAs as evidenced by the absence of any pyridine ^{1}H-NMR peaks in the crude extract spectrum after reaction. Aucubin (**1**) was treated under the standard conditions and a very low yield of **7** (aucubinin A) resulted, in conformity with the literature,[4] where NH_4Cl was used instead of NH_4OAc. A CD spectrum of **7** was taken and showed no absorptions, thus proving the racemic nature of **7**. No PMTAs could be isolated from seeds of *Penstemon virgatus* and *Penstemon strictus* or from leaves of *Cornus florida* when the usual alkaloid isolation procedure with hydroxide basification[3] was used.

Discussion

The conversions of **11** to **12** (eq 1), **13** to **14** (eq 2), and **5** to **15** (eq 3) can be viewed as "normal" PMTA formation reactions. (That **5** gave **15** rather than the PMTA with an intact epoxide ring is a function of the use of NH_4OAc and does not detract from this as a "normal" reaction.) The final yields of pure PMTAs in these reactions are low, but the crude reaction extract in each case appeared to be mostly PMTA, with some unreacted aglycone. In view of the complex mechanism for the conversions (see below), low final yields are perhaps not unexpected. There are clearly problems with product stability during purification of the crude extracts, as especially evidenced for corninin (**14**). Decomposition of the PMTA venoterpine during puri-

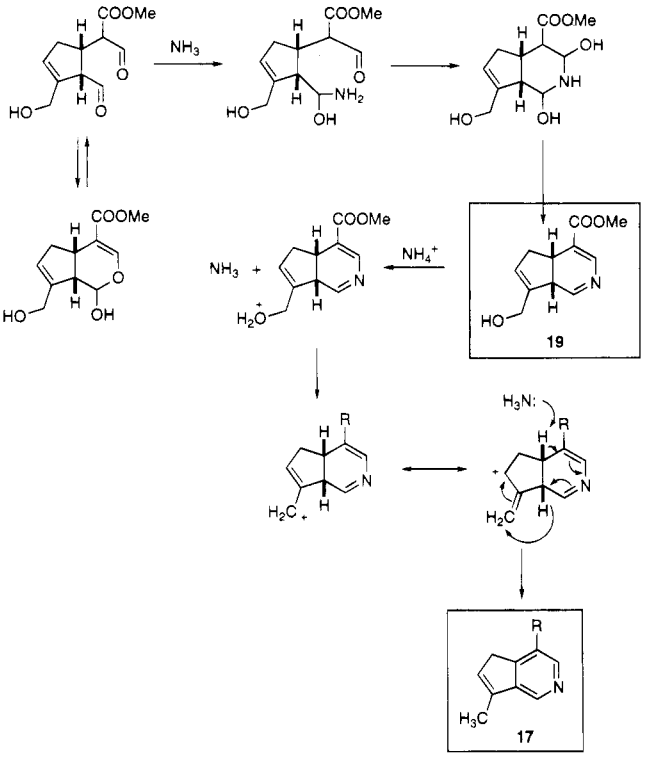
fication, for example, has also been described.[16] It is perhaps notable that the starting iridoid glycosides **11**, **13**, and **5** all contain a C-10 methyl, as do **3** and loganin, other iridoids that are precursors to the most commonly isolated PMTAs. Other than **5**, all of these glycosides are also very early precursors in iridoid biosynthesis pathways. In terms of the plant biosynthesis of PMTAs, it might only be the biosynthetically early iridoids that are present at the right place and time to be converted to the alkaloids. The presence of functionality, such as an OH, on C-10 in later biosynthetic products may also change the chemical nature of the transformation. The failure of **2** to form an isolable PMTA, the near complete failure from **1**, and the formation of alternate products from **6** (eqs 4 and 5) suggests that a C-10 OH, combined with C-7, C-8 unsaturation, may be critical factors that mitigate against straightforward PMTA formation. The presence of a hydrogen at C-5 in **11** and **13**, as opposed to the OH in **5**, did not appear to be critical for the final aromatization step. Most of the isolated PMTAs have carbomethoxy or carboxaldehyde groups at C-4, but the formation of **15** from **5** shows that this is not an absolute requirement. Firm conclusions relative to the role played in PMTA formation by the various functionalities will not be possible until a much larger group of iridoids are examined than we have yet explored.

We did not examine critically comparisons among the iridoids relative to the reaction with β-glucosidase nor the comparative stabilities of the aglycones. In our hands **6** gave a nearly quantitative yield of the aglycone upon reaction with β-glucosidase, although the yield was less with **5**. The antirrinoside (**5**) aglycone gave **15** in 23% yield, which is higher than the overall yield under standard conditions from **5** itself, but this was countered by the lower yield of aglycone from **5**. A high-yield laboratory preparation of **1** aglycone, a poor PMTA precursor, has been achieved with β-glucosidase in cases where these aglycones are used as chiral synthons,[26] so failure of the enzymatic step is probably not the reason for the low yield of PMTAs from **1**.

The subsequent reaction steps that incorporate the nitrogen can take a variety of forms, but none has as yet been proven in any study. Nearly complete mechanisms have been suggested for the conversion of **6** to **10** and subsequent pigment polymers[7,8] and for formation of **9**.[4] Scheme 2 gives a possible accounting for the formation of **19** and **17**, which presumably react to form **16**. Many variants, perhaps equally plausible, can be suggested for the reaction with ammonia. It could initiate the process by attack at C-3 of the starting aglycone or at C-1 on a preformed oxonium ion. The NH_3 could react with the top aldehyde group rather than the lower one as depicted in Scheme 2. Either amino alcohol could first eliminate water to form an imine, which subsequently attacks the second carbonyl to yield an imino alcohol instead of the aminodiol depicted. The process that converts the -CH_2OH group to the methyl is an interesting one that also comes into play in the pigment formation from reaction of **6** with methylamine.[8] As a hypothesis to be investigated in more detail, we suggest a deprotonation at C-5 and a 1,3-hydride shift from C-9 to C-10 (Scheme 2).

More detailed work, both in vitro and in vivo, is clearly needed to aid our understanding of PMTA formation mechanisms and plant biosynthesis.

Scheme 2. Possible Mode of Formation of **17** and **19**, Precursors to Dimer **16**



Experimental Section

General Experimental Procedures. Aucubin and catalpol were purified from *Castilleja integra* and *Besseya plantaginea*,[20] antirrinoside was from *Maurandya antirrhiniflora*,[21] 8-*epi*-loganin was from *Penstemon barrettiae*,[22] and cornin was from *Cornus florida*.[23] Geniposide was a gift from Glico Foods Corporation, Osaka, Japan. Organic extracts were dried over Na_2SO_4 prior to evaporation. The β-glucosidase (EC 3.2.1.21) was from Sigma Chemical Co. NMR spectra were performed on a Bruker AC300 spectrometer in $CDCl_3$ and internally referenced to residual $CHCl_3$ (δ 7.24 for ^1H and δ 77.00 for ^{13}C). MS were obtained from Fisons Instruments Quatro-SQ and VG AutoSpec spectrometers and from a Hewlett Packard 5970 MSD with Series 5980 gas chromatograph. Alkaloid isolations were attempted on leaves of *C. florida*[23] and seeds of *P. virgatus*[24] and *P. strictus* (Dean Swift Co., Jaroso, Colorado).

Enzymatic Hydrolysis of Geniposide (6) to Its Aglycone Genipin. Geniposide (1.015 g) was dissolved in H_2O (19 mL) and treated with β-glucosidase (305 mg). The reaction mixture was transferred to an extractor and continuously extracted with CH_2Cl_2 (55 mL) for 24 h, while the H_2O fraction was kept at 37 °C with heating tape. Concentration of the organic layer *in vacuo* yielded genipin (559 mg, 95% yield) as a white powder, identified by ^1H-NMR spectrum.[25]

Conversion of 8-*epi*-Loganin (11) to 8-*epi*-Cantleyine (12). 8-*epi*-Loganin (539 mg) was added to 73 mg of β-glucosidase in 50 mL 10% aq NH_4OAc and allowed to stand under an atmosphere of Ar for 24 h at 36 °C. The reaction mixture was then extracted with CH_2Cl_2 (5 × 50 mL) and the CH_2Cl_2 evaporated to yield 194 mg of a crude alkaloid mixture. CC (silica gel; 98:2 $CHCl_3$–MeOH) yielded 20 mg pure 8-*epi*-cantleyine.

GCMS *m/z* (rel. int.): M^+ 207(51), 179(38), 175(75), 147-(100), 132(26), 118(38), 91(37), 77(27), 51(32), 39(27). ^{1}H-NMR: δ 8.95 and 8.47 (2H, s, H-1 and H-3), 4.23 (1H, ddd, J = 6.4, 4.6, 2.9 Hz, H-7), 3.60 (1H, dd, J = 18.5, 6.4 Hz, H-6a), 3.89 (3H, s, H-12), 3.15 (1H, m, H-8), 1.28 (3H, d, J = 7.2 Hz, H-10), 3.19 (1H, dd, J = 18.5, 4.6 Hz, H-6b). ^{13}C-NMR: 149.7 and 148.5 (C-1 and C-3), 123.1 (C-4), 152.3 (C-5), 41.5 (C-6), 46.4 (C-8), 79.7 (C-7), 143.1 (C-9), 17.8 (C-10), 52.3 (C-12), 166.2 (C-11).

Conversion of Cornin (13) to Corninine (14) [7(*R*)-Methyl-5-oxocyclopenteno[*e*]pyridine]. Cornin (153 mg) was added to 23 mg of β-glucosidase in 13 mL 10% aqueous NH_4OAc and allowed to stand under an atmosphere of Ar for 24 h at 36 °C. The reaction mixture was then extracted with CH_2Cl_2 (5 × 15 mL) and the CH_2Cl_2 evaporated to yield 65 mg of a crude alkaloid mixture. CC (silica gel; $CHCl_3$–MeOH 8:2) yielded 1 mg of **14**. GCMS *m/z* (rel. int.): M^+ 205(63), 190(41), 174(100), 162(25), 146(53), 132(55), 118(54), 117(54), 104(27), 91(46), 77(89), 65(37), 51(61), 39(46). ^{1}H-NMR: δ 1.5 (3H, d, J = 7.5 Hz, H-10), 2.38 (1H, bd, J = 18 Hz, H-7a), 3.00 (1H, dd, J = 7.5, 18 Hz, H-7b), 3.35 (1H, m, H-8), 4.00 (3H, s, H-12), 8.85 and 9.05 (2H, s, H-1 and H-3). ^{13}C-NMR: δ 151.7 and 148.0 (C-1 and C-3), 152.6 (C-5), 202.8 (C6), 45.7 (C-7), 31.3 (C-8), 150.0 (C-9), 21.3 (C-10), 53.1 (C-12).

Conversion of Antirrinoside (5) to PMTA (15) [7-(*S*)-Acetoxy-5,6-(*R,S*)-dihydroxy-7(*R*)-methylcyclopenteno[*e*]pyridine. Antirrinoside (1.28 g) was added to 176 mg β-glucosidase in 120 mL 10% aq NH_4OAc and allowed to stand under an atmosphere of Ar for 24 h at 36 °C. The reaction mixture was then extracted with CH_2Cl_2 (5 × 120 mL) and the CH_2Cl_2 evaporated to yield 82 mg of a crude alkaloid mixture. CC (silica gel; $CHCl_3$–MeOH 9:1) yielded 31 mg pure **15**. FabH$^+$MS *m/z* 224.1 $C_{11}H_{14}NO_4$; mp (EtOAc): 170 °C. ^{1}H-NMR: δ 8.71 (1H, s, H-1), 8.59 (1H, d, J = 5.0 Hz, H-3), 7.41 (1H, d, J = 5.0 Hz, H-4), 5.11 (1H, d, J = 5.6 Hz, H-6), 4.31 (1H, d, J = 5.6 Hz, H-7), 1.70 (3H, s, H-10), 2.10 (3H, s, CH_3COO). ^{13}C -NMR: δ 150.1 (C-1), 147.0 (C-3), 120.9 (C-4), 148.4 (C-5), 78.7 and 72.8 (C-6 and C-7), 90.2 (C-8), 139.9 (C-9), 21.8 and 21.9 (C-10 and *C*H_3COO), 172.5 (C=O). A single crystal subjected to an X-ray diffraction study (see below) confirmed the relative and absolute stereochemistry.

Preparation of Antirrinoside (5) Aglycone. Antirrinoside (776 mg) was dissolved in 19 mL of H_2O containing β-glucosidase (233 mg). The yellow solution was then transferred into a liquid–liquid extractor and extracted with CH_2Cl_2 (55 mL) for 24 h, while the H_2O-fraction was kept at 36 °C with heating tape. The CH_2Cl_2 solution was dried and the solvent evaporated *in vacuo* to yield a yellow oil (131 mg, 31%). ^{1}H-NMR (CD_3OD): δ 6.20 (H-3, d), 5.40 (H-1, d), 4.90 (H-4, d), 4.25 (H-6, s), 3.35 (H-7, s), 2.40 (H-9, d), 1.45 (H-10, s).

Conversion of Antirrinoside Aglycone to 15. Aglycon (131 mg) was dissolved in 10% NH_4OAc (in 95% EtOH) and stirred for 24 h while the reaction mixture was kept at 37 °C under Ar. The solvent was evaporated, and the residue dissolved in an emulsion of 25 mL H_2O and 25 mL $CHCl_3$. The layers were separated and the water fraction was extracted with $CHCl_3$ (4 × 25 mL). Evaporation of the organic solvent yielded 34 mg (23%) of **15**, pure by ^{1}H-NMR spectrum.

Conversion of Genipin to Racemigerine (17). Genipin (700 mg) was dissolved in anhydrous MeOH (35 mL) and NH_3(g) was bubbled through for 4 h. The solution changed from colorless to yellow to brownish red. The MeOH was evaporated, and the red residue was redissolved in MeOH (35 mL). HCl (g) was bubbled through the solution for 2 h, during which time the solution turned dark purple. The MeOH was evaporated to yield a crude alkaloid mixture (817 mg). CC (silica gel, $CHCl_3$–MeOH 85:15) yielded a yellow oil containing impure racemigerine (73 mg). The compound was unstable and prone to turn purple on standing in solution and on exposure to air and was not further purified. The following data were obtained on the impure material, with the ^{1}H-NMR data corresponding closely to the literature[18] and the ^{13}C-NMR spectrum, not previously reported, in full accord with the structure. ^{1}H-NMR: δ 8.98 and 8.65 (2H, s, H-1 and H-3), 6.3 (1H, bs, H-7), 3.95 (3H, s, H-12), 3.65 (2H, m, H-6), 2.15 (3H, bs, H-10). ^{13}C-NMR: δ 146.7 and 143.1 (C-1 and C-3), 121.7 (C-4), 154.8 (C-5), 39.3 (C-6), 131.1 (C-7), 137.0 (C-8), 141.1 (C-9), 12.7 (C-10), 166.0 (C-11), 51.9 (C-12).

Conversion of Geniposide (6) to Dimer 16. Geniposide was reacted as above with β-glucosidase and aq NH_4OAc, resulting in alkaloidal material that gave one spot on TLC. The ^{1}H and ^{13}C-NMR spectra were complex, but the GC-MS (*m/z* 189, 188, 174, 158, 157, 144, 130, 103, 102) was essentially identical with that reported[18] for **17**. The positive ion ESMS, however, showed two major peaks (*m/z* 794, 396) suggestive of an oligomer or oligomeric mixture. To avoid formation of oligomers, a reaction more dilute in **6** was carried out. Geniposide (520 mg) was added to 76 mg of β-glucosidase in 400 mL 10% aq NH_4OAc and allowed to stand under an atmosphere of Ar for 24 h at 36 °C. The reaction mixture was then extracted with CH_2Cl_2 (4 × 500 mL) and the CH_2Cl_2 evaporated to yield 323 mg of a crude alkaloid mixture. CC (silica gel; $CHCl_3$–MeOH 9:1) yielded 24 mg of dimer **16**. FabH$^+$MS *m/z* 397.1766; NMR spectra: Table 1.

X-ray Diffraction Structure Determination for 15.[27] **Crystal data.** $C_{11}H_{13}NO_4$; color, habit: clear light yellowish rods; crystal size (mm): 0.40 × 0.36 × 0.42; crystal system: hexagonal; space group: $P6_1$; unit cell dimensions: a = 16.429(2) Å, c = 7.592(2) Å; volume: 1774.3(6) Å^3; Z = 6; formula weight: 223.2; density (calcd): 1.253 mg/m^3; absorption coefficient: 0.096 mm^{-1}; F(000): 708.

Data Collection. Diffractometer used: Siemens P4/Unix; radiation: MoKα (λ = 0.71073 Å); temperature (K): 173; monochromator: highly oriented graphite crystal; 2θ range: 2.0 to 60.0 °; scan type: 2θ–θ; scan speed: variable, 3.00 to 60.00 °/min. in ω; scan range (ω): 1.00 ° plus Kα–separation; background measurement: stationary crystal and stationary counter at beginning and end of scan, each for 50.0% of total scan time; standard reflections: three measured every 97 reflections; index ranges: $-1 \le h \le 20$, $-23 \le k \le 1$, $-1 \le l \le 10$; reflections collected: 4581; independent reflections: 2087 (R_{int} = 5.70%); observed reflections: 1560 [$F > 4.0\sigma(F)$]; absorption correction: N/A.

Solution Refinement. System used: Siemens SHELXTL PLUS (UNIX); solution: direct methods; refinement method: full-matrix least-squares; quantity

Table 2. Atomic Coordinates ($\times 10^5$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^4$)

	x	y	z	*U*(eq)
N(1)	32803(30)	28635(31)	33030	297(17)
O(1)	28287(20)	48006(21)	4373(72)	213(11)
O(2)	27575(25)	55867(24)	−19538(75)	304(14)
O(3)	30592(24)	43241(22)	−38011(73)	278(13)
O(4)	37290(23)	31257(24)	−31058(69)	258(13)
C(1)	29889(30)	28045(29)	−3183(78)	217(15)
C(2)	28892(37)	20146(36)	5399(85)	300(19)
C(3)	30508(40)	20868(38)	23581(91)	330(21)
C(4)	33647(31)	36140(34)	24577(81)	235(15)
C(5)	32392(27)	36184(29)	6361(79)	190(14)
C(6)	33228(29)	43967(28)	−5266(80)	195(14)
C(7)	27747(28)	38380(29)	−21860(83)	199(14)
C(8)	28970(30)	29610(30)	−22506(78)	211(15)
C(9)	43358(29)	51524(32)	−8547(92)	279(16)
C(10)	25996(30)	53784(29)	−4088(90)	236(15)
C(11)	21163(36)	57158(35)	8084(93)	307(18)

[a] Equivalent isotropic *U* defined as one third of the trace of the orthogonalized $\mathbf{U}_{ij}$ tensor.

minimized: $\Sigma w(F_o - F_c)^2$; absolute structure: N/A; extinction correction: N/A; hydrogen atoms: riding model, fixed isotropic U; weighting scheme: $w^{-1} = \sigma^2(F) + 0.0287F^2$; number of parameters refined: 144; final *R* indices (obs. data): $R = 6.83\%$, $wR = 10.91\%$; *R* indices (all data): $R = 8.14\%$, $wR = 23.05\%$; goodness-of-fit: 0.61; largest and mean Δ/σ: 0.004, 0.001; data-to-parameter ratio: 10.8:1; largest difference peak: 1.33 $eÅ^{-3}$; largest difference hole: -0.51 $eÅ^{-3}$.

Table 2 lists atomic coordinates.

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(27) Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained, on request, from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

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