Biosynthesis of Antidesmone in Cell Cultures of *Antidesma membranaceum* (Euphorbiaceae): An Unprecedented Class of Glycine-Derived Alkaloids

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Abstract: Antidesmone (**3**), known from several species of *Antidesma* and *Hyeronima* (Euphorbiaceae), is a quinoline alkaloid with an unusual substitution pattern. It carries a linear aliphatic side chain that indicates an at least partially acetogenic origin of the carbon skeleton. Feeding experiments with ^{13}C and ^{15}N labeled precursors administered to cell cultures of *Antidesma membranaceum* yielded antidesmone, which was investigated by NMR spectroscopy. Accordingly, antidesmone is built up from a linear C_{16}-polyketide chain and a C_2 unit derived directly from glycine. It is thus the first representative of a biosynthetically novel class of alkaloids.

Introduction

The higher plant family Euphorbiaceae is notable for a rich diversity not only of growth forms but also of natural products contained therein.[1,2] Several different classes of alkaloids are known from the family, among the most unique ones being apparently polyketide-derived polycyclic secondary metabolites.[1,2] The unusual structure (**1**, Figure 1) originally proposed for antidesmone from *Antidesma membranaceum*[3] in particular attracted our attention with respect to its biosynthesis. An aliphatic C_8 side chain suggested an acetogenic origin, at least for this part of the molecule. The constitution and the substitution pattern originally proposed did, however, not fit into a simple polyketide folding scheme without branching, and especially the heterocyclic ring was biosynthetically unclear. In this paper, we report on the elucidation of the biosynthesis of antidesmone from acetate and glycine, which also initiated a major structural revision of this alkaloid to have structure **3**.[4,5]

The initial structural attribution of antidesmone and of the obviously identical[4] metabolite hyeronine A from *Hyeronima oblonga* (Euphorbiaceae), which, based on identical spectral data, had again been assigned a different structure **2**,[6] suffered substantially from inconclusive NMR data and ambiguous derivatization reactions. Therefore we employed a feeding strategy with stable isotope labeled metabolites both to elucidate the biogenesis of this natural product and, by the biosynthetic introduction of NMR-active isotopes, to facilitate its structural reinvestigation. The revised structure **3** of antidesmone was initially suggested and ultimately corroborated by the elucidation of its biosynthesis described here.[4]

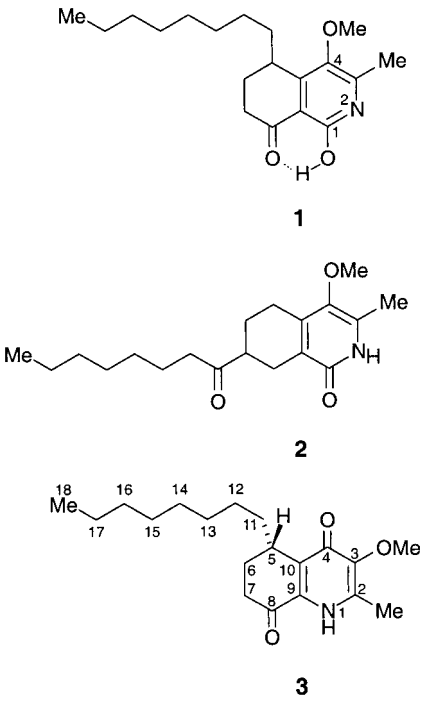

Figure 1. Structures **1** and **2** previously suggested for antidesmone and hyeronine A, respectively, and revised structure **3**.

Results

Establishing Cell Cultures. For viable seeds of *A. membranaceum*, as available from different East-African habitats, a germination rate of almost 100% was reached by an optimized pretreatment of the seeds and an appropriate sterilization method. From the sterile plants thus obtained, callus formation was induced on cut leaf margins by using two different phytohor-

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(1) Seigler, D. A. *Ann. Mo. Bot. Gard*. **1994**, *81*, 380.
(2) Hegnauer, R. *Chemotaxonomie der Pflanzen*; Birkhäuser: Basel and Stuttgart, 1966; Vol. 4, pp 102−140.
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(5) Presented in part at the SFB 369 Symposium (Secondary Metabolites: Chemistry and Biology), München, Germany, October 1999.
(6) Alves, T. M. A.; Zani, C. L. *Tetrahedron Lett.* **1999**, *40*, 205.

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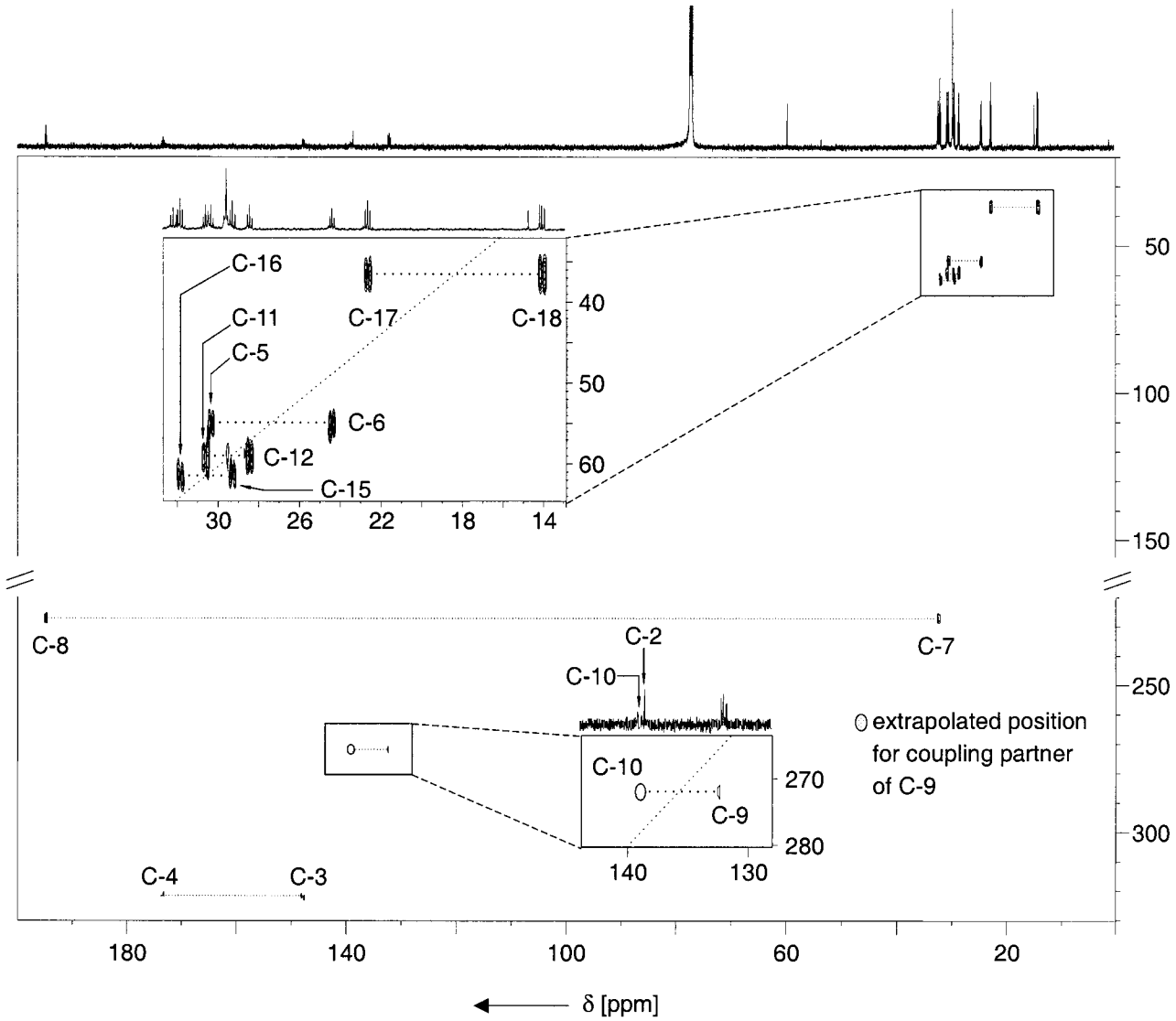

Figure 2. 2D-INADEQUATE of antidesmone isolated after feeding of [U-$^{13}C_2$]acetate. The assigment of C-10 as the coupling partner of C-9 was achieved by subtraction of the chemical shift of C-9 from the double quantum frequency of the respective trace and by 1D analysis of the extracted trace.[7,8]

mone formulations. Subcultured calli grew faster on a medium containing 2,4-dichlorophenoxyacetic acid (medium A) but were softer than the friable callus formed with higher concentrations of benzylaminopurine, lower concentrations of naphthylacetic acid, and without 2,4-dichlorophenoxyacetic acid (medium B). The alkaloid content, by contrast, was not significantly influenced by the choice of the medium.

[U-$^{13}C_2$]Acetate Feeding. Antidesmone obtained from the [U-$^{13}C_2$]acetate feeding experiment exhibited a C−C-coupling pattern in the 2D-INADEQUATE[7] spectrum (Figure 2) with the C−C correlations C-11/C-12, C-13/C-14, C-15/C-16, and C-17/C-18, which confirmed the hypothesis of an acetogenic origin of the aliphatic side chain. Additional signals also established the acetogenic nature of the whole isocyclic ring (C-5/C-6 and C-7/C-8), including both of the bridgehead atoms, C-9/C-10. The two signals at δ_c 147.6 and 173.4 ppm, corresponding to oxygen-substituted atoms in the heterocycle, could not be brought into line with the structure **1** (Figure 1) originally proposed for antidesmone, because the ^1J coupling constant (65 Hz) of these two carbon atoms necessitated their immediate proximity, which excluded their claimed 1,4-arrangement in the (erroneous) original structure **1**. One carbon atom of the heterocyclic ring (C-2, δ_c 137.9 ppm) and an exocyclic one attached to it (2-CH_3, δ_c 14.67 ppm) were not labeled in this experiment, and are thus not derived from a joint intact acetate unit.

[U-$^{13}C_6$]Glucose Feeding. The labeling pattern of the aliphatic side chain and the isocyclic ring of antidesmone after application of uniformly ^{13}C labeled D-glucose corresponded to that obtained after feeding acetate. In contrast to the above experiment with acetate, however, the ^{13}C NMR signal of the methyl carbon atom attached to the heterocycle (2-CH_3) did show weak ^{13}C satellites (Figure 3B), indicating this and, hence, also the neighboring ring carbon to be jointly derived from a single, glucose-derived precursor. The signal intensities of the satellites were, however, much lower than those of the satellites at acetate-derived positions, such as C-18 (Figure 3A and Table 1).

^{15}N Labeling. This experiment played a crucial role in the structural elucidation of antidesmone (**3**) and led to the revision of structure (**1**) mentioned above, which was then confirmed by further spectroscopic results.[4] The replacement of NO_3^- and

(7) Bax, A.; Freeman, R.; Frenkiel, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 2102.

Table 1. NMR Data of Antidesmone (**3**) after Application of Different Labeled Precursors

position	δ_c [ppm]	$^1J(^{13}C,^{13}C)$ [Hz][a]	I_s [%][b] [U-$^{13}C_2$]-acetate	(D)-[U-$^{13}C_6$]-glucose	(L)-[U-$^{13}C_3$,^{15}N]-alanine	(L)-[U-$^{13}C_4$]-aspartic acid	[U-$^{13}C_2$,^{15}N]-glycine
2	137.9	46.4[g]		n.d.[d]		n.d.[d]	n.d.[f]
3	147.6	64.9	177	n.d.[d]	97	n.d.[d]	n.d.[d]
4	173.4	65.8	111	n.d.[d]	98	n.d.[d]	n.d.[d]
5	30.32	33.6	141	50	91	29	60
6	24.47	33.4	163	58	91	23	72
7	32.24	42.9	132	56	91	21	68
8	194.9	42.7	131	58	91	n.d.[d]	n.d.[d]
9	131.9	64.2[e]	n.d.[d]	n.d.[d]	98	n.d.[d]	n.d.[d]
10	139.1	64.8[e]	n.d.[d]	n.d.[d]	106	n.d.[d]	n.d.[d]
11	30.59	34.3	162	50	102	32	65
12	28.49	34.6	151	38	107	30	60
13[c]	29.64	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]
14[c]	29.69	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]	n.d.[f]
15	29.29	34.6	126	40	100	16	55
16	31.86	34.6	129	41	91	25	66
17	22.66	34.6	162	58	101	19	63
18	14.09	34.6	198	52	111	23	78
2-CH_3	14.67	46.5[g]		20		90	454
3-OCH_3	59.38						18[h]

[a] Values from the [$^{13}C_2$]acetate feeding experiment, unless otherwise noted. [b] Intensitiy of ^{13}C satellites in relation to the intensity of the central peak in ^{13}C NMR unless otherwise noted. [c] Overlapping signals, attributions possibly exchangeable. [d] Not determined due to weak signal intensity. [e] Obtained from the alanine feeding experiment. [f] Not determined due to overlapping signals. [g] Value obtained from the glycine feeding experiment. [h] 3-OCH_3 showed a labeling degree of 18% ^{13}C in ^1H NMR.

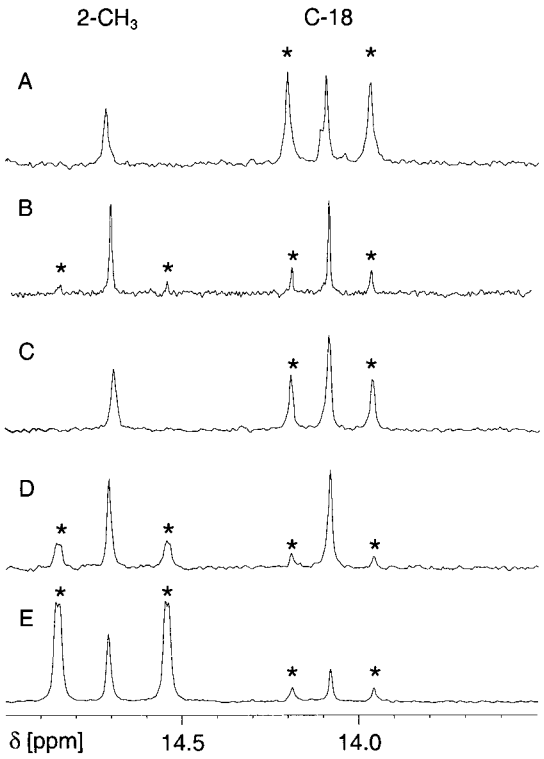

Figure 3. Comparison of ^{13}C NMR signals of 2-CH_3 and, as an "acetogenic standard", C-18 and their ^{13}C satellites (indicated by an asterisk) after feeding (A) [U-$^{13}C_2$]acetate, (B) (D)-[U-$^{13}C_6$]glucose, (C) (L)-[U-$^{13}C_3$,^{15}N]alanine, (D) (L)-[U-$^{13}C_4$]aspartic acid, or (E) [U-$^{13}C_2$,^{15}N]-glycine.

NH_4^+ by their ^{15}N labeled analogues in the medium led to antidesmone enriched up to ca. 70% with ^{15}N; the labeling ratio was determined by ^1H NMR, based on the 2-CH_3 signals, which exhibited ^{15}N-satellites with a ^3J coupling constant of 2.9 Hz. This ^{15}N labeled material showed three relevant $^1H-^{15}N$ HMBC correlations (Figure 4, top): a very strong (^3J) one from a singlet corresponding to the three methyl protons of the very close aromatic methyl group at C-2, a much weaker one (^5J) from

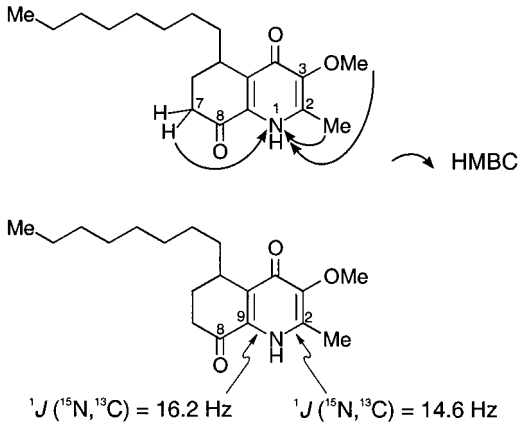

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Figure 4. HMBC correlations and $^1J(^{13}C,^{15}N)$ coupling constants (from ^{13}C NMR) obtained after ^{15}N labeling of antidesmone (**3**).

the more remote *O*-methyl group at C-3, and, diagnostically most valuable for the structure elucidation, a weak but clear interaction from H-7_{eq} (δ_H 2.59 ppm), i.e., one of the two diastereotopic protons at C-7, next to the carbonyl function at C-8. The latter finding suggested the nitrogen to be closer to H-7 than assumed before, and therefore to be located in position 1 and not in position 2. Furthermore, the chemical shift of nitrogen (δ_N −252 ppm)[9] showed the heterocyclic ring to be a pyridone, not a hydroxypyridine, tautomer. Final evidence of the structural revision was provided by ^{13}C NMR spectroscopy, as the signals of two-quarternary carbon atoms showed nitrogen satellites: one carbon atom (C-9) next to the carbonyl function at C-8 and the other one (C-2) carrying a methyl group (Figure 4, bottom). The sizes of the coupling constants of 16.2 and 14.6 Hz are typical of one-bond couplings.[10,11] Therefore, these two

(8) This assignment was confirmed later, within the alanine feeding experiment described below.

(9) Berger, S.; Braun, S.; Kalinowski, H.-O. *NMR Spectroscopy of the Non-Metallic Elements*; John Wiley & Sons: Chichester, 1996; pp 111−317.

carbon atoms attached to the nitrogen were identified to be C-9 and C-2, and antidesmone is a 4-pyridone, not a 2-pyridone. These feeding experiments establish the revised constitution **3** of antidesmone, with a tetrahydroquinolone,[12] not a tetrahydroisoquinoline structure as assumed previously.[3]

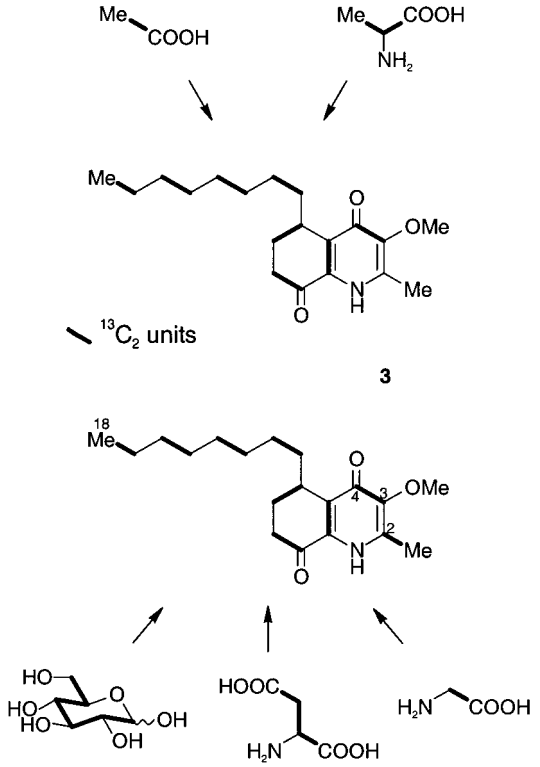
[U-$^{13}C_3$,^{15}N]Alanine Feeding. Since the two-carbon unit in antidesmone that remains unlabeled in the [U-$^{13}C_2$]acetate feeding experiment is adjacent to the nitrogen atom, it seemed possible that this C_2N domain of the molecule might be derived as an intact "building block" from the glucogenic amino acid alanine with loss of a carbon atom by decarboxylation. (L)-[U-$^{13}C_3$,^{15}N]Alanine was fed to the cell cultures and the ^{13}C NMR spectrum of the antidesmone formed was investigated as described above. Unexpectedly, the signal of 2-CH_3, which had been only weakly labeled in the glucose feeding experiment, did not show satellites of an appreciable intensity in the alanine experiment, thus excluding a direct origin of this and the attached carbon atom from alanine or from a biogenetically related C_3 precursor. At the acetate-derived positions (see above), however, the same clear incorporation pattern of C_2 units as in the acetate feeding experiment was obtained. In addition, enrichment of ^{15}N in antidesmone was observed by $^1H-^{15}N$ HMBC, but to a weaker extent than in the ^{15}N labeling experiment.

[U-$^{13}C_4$]Aspartic Acid Feeding. Initially considered an additionally carboxylated alanine analogue (but, in light of the results obtained below, rather acting as a precursor to glycine), [U-$^{13}C_4$]aspartic acid was fed to the cell cultures. In the ^{13}C spectrum of antidesmone (**3**) obtained thereafter, the most prominent ^{13}C satellites were seen at the 2-CH_3 signal indicating ^1J coupling with C-2. Less pronounced satellite signals were present at the acetate-derived positions. This proved aspartic acid to be a specific precursor of the two carbon atoms of the antidesmone backbone that are not directly derived from acetate.

[U-$^{13}C_2$,^{15}N]Glycine Feeding. The aspartate feeding experiment did not yield definitive evidence of the positions of the carbon atoms incorporated into antidesmone, nor was the origin of the nitrogen atom proved without doubt, so that [U-$^{13}C_2$,^{15}N]-glycine was fed to address these remaining questions. The incorporation pattern obtained in this experiment showed glycine to be incorporated at a high rate and intact, as a C_2N fragment, into antidesmone, accounting for the two carbon atoms also most prominently labeled in the aspartate feeding experiment. The ^{13}C NMR signal of the 2-CH_3 carbon atom displayed two couplings, a large one ($^1J_{C-C} = 46.5$ Hz) and a smaller one ($^2J = 1.5$ Hz), the latter arising from a two-bond carbon−nitrogen coupling. The C-2 signal arose as a doublet of doublets as well, once again with the $^1J_{C-C}$ coupling constant of 46.4 Hz as above, but now with a larger $^1J_{C-N}$ value of 14.9 Hz. The attribution of the carbon−nitrogen couplings was confirmed by ^1H,^{15}N-decoupled ^{13}C NMR, in which all doublets arising from nitrogen coupling were reduced to singlets. In contrast to all the other feeding experiments described above, the feeding of glycine yielded incorporations so high that even ^{13}C and ^{15}N satellites of the 2-CH_3 signal were visible in the ^1H NMR spectrum. Calculated from integration of ^1H NMR peaks, the degree of ^{13}C labeling of 2-CH_3 was ca. 25%.

In addition, glycine also served as a carbon source for the *O*-methyl group and for the acetate units. In the ^1H NMR spectrum ^{13}C satellites of the 3-OCH_3 signal were observed ($^1J_{C-H} = 144.5$ Hz), with an intensity equivalent to a ^{13}C labeling degree of ca. 18%; and the remaining (polyketide) positions showed the same incorporation pattern as in the acetate feeding experiment, but with weaker satellite intensities.

Scheme 1. ^{13}C Labeling Pattern of Antidesmone (**3**) Obtained after Feeding [U-$^{13}C_2$]Acetate or (L)-[U-$^{13}C_3$,^{15}N]Alanine (above) or (D)-[U-$^{13}C_6$]Glucose, (L)-[U-$^{13}C_4$]Aspartic Acid, or [U-$^{13}C_2$,^{15}N]Glycine (below)



Discussion

The results prove the polyketide nature of the major part of antidesmone (Scheme 1, top) and suggest a similar origin of related compounds from several species of *Antidesma* and *Hyeronima*.[6,12] The remaining two carbon atoms of the heterocyclic ring and the nitrogen are derived from glycine (Scheme 1, bottom). Alanine is not specifically incorporated into this position, while aspartic acid is. This can be explained by conversion of alanine into acetyl-CoA (without detectable glycine formation) in the cell cultures, while aspartic acid is efficiently converted into both glycine and acetyl-CoA. All enzymes required for this pathway (aminotransferase(s), malate dehydrogenase, malate synthase) are known from plants, but aspartate is usually not considered a glycine precursor. The incorporation of intact C_2 units from glycine into acetate-derived positions of antidesmone furthermore indicates substantial conversion of glycine into acetyl-CoA, which seems to be a novel pathway. Incorporation of carbon derived from glycine (presumably from the C-2 position, involving glycine synthase) into the 3-OCH_3 group probably occurs via *S*-adenosyl methionine.

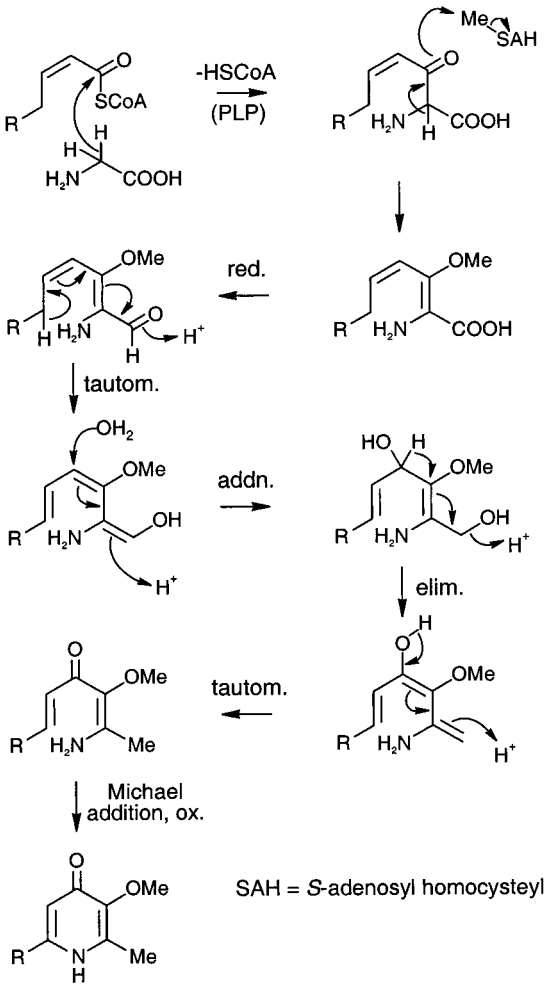
A key step in the hypothetical biosynthesis of antidesmone (Scheme 2) is the condensation of a polyketide-derived acid (probably in the form of its CoA thioester) with C-2 of glycine (presumably as a pyridoxal phosphate derivative). This (with glycine replaced by serine) would be a reaction sequence analogous to the initial step in the biosynthesis of sphingolipids,

(10) Kainosho, M. *J. Am. Chem. Soc.* **1979**, *101*, 1031.

(11) Nadzan, A. M.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 4647.

(12) A similar, more reduced tetrahydroquinolone alkaloid named hyeronimone had previously been isolated from *Hyeronima alchorneoides*: Tinto, W. F.; Blyden, G.; Reynolds, W. F.; McLean, S. *J. Nat. Prod.* **1991**, *54*, 1309.

Scheme 2. Hypothetical Biosynthesis of the Heterocyclic Ring of Antidesmone



which are well-known from various plants including Euphorbiaceae.[13] *O*-Methylation at the eventual C-3 position and oxygenation at the ultimate C-4 position of antidesmone and also the, otherwise rare,[14] hypothetic conversion of a carboxy group into a methyl group at the ultimate 2-CH_3 position (both transformations possibly being coupled to each other by a series of addition, elimination, and tautomerization reactions) seem to be prerequisites for the closure of the heterocyclic ring by Michael addition. The time and the mechanism of the formation of the carbocyclic ring of antidesmone are less obvious.[15]

Glycine-derived pyridones such as antidesmone thus constitute a novel biosynthetic class of alkaloids. From the incorporation pattern found in antidesmone derived from [U-$^{13}C_2$,^{15}N]-glycine it would appear that, in cell cultures of *A. membranaceum*, glycine is convertible into acetylCoA, that one of the C atoms of glycine can yield an *O*-methyl group, and that the carboxyl group of glycine can be reduced to the methyl oxidation level. Furthermore, it would appear from the incorporation pattern found in antidesmone derived from [U-$^{13}C_4$]aspartic acid that aspartic acid can be converted into glycine and into acetylCoA. Although these conversions have so far not been described as individual pathways, the required enzymes and the corresponding hypothetical intermediates or analogues are known from plants.

(13) Falsone, G.; Cateni, F.; Katusian, F.; Wagner, H.; Seligmann, O.; Pellizer, G.; Asaro, F. *Z. Naturforsch.* **1993**, *48b*, 1121.

(14) Another example is the formation of anethol from tyrosine, cf.: Reichling, J.; Martin, R.; Kemmerer, B. *Plant Cell Tissue, Organ Cult.* **1995**, *43*, 131.

(15) The carbocyclic ring is probably formed *after* the closure of the heterocycle, as suggested by the existence of the possible "precursor" analogue melochininone from *Melochia pyramidata* (Sterculiaceae)[16] and of the similarly substituted pyridone innovanamine from *Evodiopanax innovans* (Araliaceae).[17] Melochinone from *M. tomentosa*,[18] which differs in its tetracyclic, less saturated structure, suggesting cinnamoyl-CoA rather than acetyl-CoA as the starter of the polyketide chain, appears to coincide with antidesmone in its biosynthesis of the major part of the molecule.

Experimental Section

Materials. [U-$^{13}C_2$]Sodium acetate, (D)-[U-$^{13}C_6$]glucose, [^{15}N]ammonium [^{15}N]nitrate, potassium [^{15}N]nitrate, (L)-[U-$^{13}C_3$,^{15}N]alanine, (L)-[U-$^{13}C_3$]aspartic acid, and [U-$^{13}C_2$,^{15}N]glycine were obtained from Promochem (Wesel, Germany). The ingredients of the media came from Sigma, except for Gelrite, which was purchased from Roth GmbH & Co., Germany. Solvents were of HPLC grade.

Plant Material. Fruits of *A. membranaceum* were collected in different habitats near Malindi and Mombasa, Kenya (voucher specimens at Herb. Bringmann no. 26).

Cell Cultures. For metabolic investigations, cell cultures of *A. membranaceum* were established from axenically germinated seeds, which had previously been scarified mechanically and surface sterilized by rinsing in aqueous hypochlorite solution (1%) for 15 min. Callus tissue formed spontaneously on cut margins of leaves cultivated on solid 1/5 Linsmaier and Skoog medium[19] with full strength organics and supplemented either with 0.5 mg/L respectively of 6-benzylaminopurine, 2,4-dichlorophenoxyacetic acid, and 1-naphthalene acetic acid (medium A) or with 2 mg/L 6-benzylaminopurine and 0.01 mg/L 1-naphthalene acetic acid (medium B). The media were supplemented with 3% sucrose and 0.2% Gelrite, and the pH was adjusted to 5.8 prior to autoclaving.

Feeding Experiments. An aqueous sodium [U-$^{13}C_2$]acetate solution (4 mg/mL, pH 5.8 adjusted with HCl and filtersterilized) was fed to the callus (0.5 mL/Petri dish every second day). The calli were incubated at 25 °C for two weeks on medium A in the dark. The same method was applied for [U-$^{13}C_6$]glucose (8.6 mg/mL solution), [U-$^{13}C_3$,^{15}N]-alanine (5 mg/mL solution), [U-$^{13}C_4$]aspartic acid (5 mg/mL solution), and [U-$^{13}C_2$,^{15}N]glycine (3.5 mg/mL solution). Liquid medium (medium B without gelling agent) with all nitrogen sources (NH_4NO_3 and KNO_3) replaced by the corresponding salts in [U-^{15}N] labeled form was used for nitrogen incorporation experiments. Callus of 54.44 g fresh wt was cultivated on these media (shaking cultures) for 63 d, the medium (150 mL) was replaced and extracted every 9 d, and antidesmone was isolated from the collected media (total: 1.05 L).

NMR Spectroscopy. ^{1}H, ^{13}C, and ^{15}N NMR spectra were acquired in $CDCl_3$ at 293 K on a Bruker DMX600 spectrometer. ^{1}H NMR spectra were internally referenced to the residual $CHCl_3$ peak (7.26 ppm) and ^{13}C NMR spectra to the $CDCl_3$ signal at 77.01 ppm. The ^{15}N chemical shifts were referenced against an external standard of formamide (90% v/v) in d_6-DMSO (−267.6 ppm). For standard 1D and 2D (COSY, TOCSY, ROESY, NOESY) experiments, for the observation of ^{1}H−^{13}C correlations (^{1}H−^{13}C HMQC, ^{1}H−^{13}C HMBC), and for the investigation of ^{1}H−^{15}N correlations (^{1}H−^{15}N HMBC), a ^{1}H, ^{13}C, ^{15}N 5 mm triple probe with a *z*-gradient was used. For the determination of ^{13}C−^{13}C and ^{13}C−^{15}N coupling constants by 1D ^{13}C NMR and for 2D INADEQUATE measurements, a ^{13}C sensitive 5 mm dual probe was used.

1D ^{13}C NMR spectra were typically acquired with a sweep width of 37593 Hz in 64 K data points zero filled to 128 K. A line broadening of 0.5 Hz was applied. The relaxation delay varied between 1 and 5 s. 2D INADEQUATE spectra were typically acquired with 320 scans. The sweep width was set to 32746 Hz, the preacquisition delay to 5 s, and the evolution delay to $^1J_{C-C}$ to 5 ms. The 160 × 2024 raw data matrix was zero filled to 4 K words in F1 and F2 and processed with a 10 Hz Gaussian in F2 and with a shifted squared sine bell in F2. For

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(17) Yasue, M.; Kawamura, N. *Chem. Pharm. Bull.* **1966**, *14*, 1443.

(18) Kapadia, G. J.; Paul, B. D.; Silverton, J. V.; Fales, H. M.; Sokoloski, E. A. *J. Am. Chem. Soc.* **1975**, *97*, 6814.

(19) Linsmaier, E. M.; Skoog, F. *Physiol. Plant.* **1965**, *18*, 100.

Table 2.

precursor(s)	feeding time	amount applied	callus (fresh wt.)	callus (dry wt.)	yield of **3**
[U-$^{13}C_2$] sodium acetate	13 d	64 mg	129.68 g	9.68 g	1.62 mg
(D)-[U-$^{13}C_6$] glucose	38 d	1.161 g	134.23 g	35.80 g	1.31 mg
[^{15}N]KNO_3 + [U-$^{15}N_2$]NH_4NO_3	63 d, changed every 9 d	2.185 g $K^{15}NO_3$ + 1.898 g $^{15}NH_4$15NO_3[a]	1.05 L media (54.44 g)	− (2.83 g)	2.06 mg
(L)-[U-$^{13}C_3$,^{15}N] alanine	21 d	200 mg	112.67 g	6.03 g	1.89 mg
(L)-[U-$^{13}C_4$] aspartic acid	25 d	100 mg	83.35 g	5.36 g	1.75 mg
[U-$^{13}C_2$,2-^{15}N] glycine	23 d	70 mg	75.00 g	4.05 g	1.11 mg

[a] Media did not contain $K^{14}NO_3$ or $^{14}NH_4{}^{14}NO_3$ instead.

^{1}H−^{15}N chemical shift correlations spectra were typically acquired in a data matrix of 64 × 8192 data points with 384 scans per increment. The preacquisition delay was set to 4 s and the evolution delay for long-range ^{13}C−^{15}N correlation to 200 ms. Zero filling to 1024 words in F1 was applied. As a window function a shifted sine bell was applied in F2 and a shifted squared sine bell in F1.

Antidesmone (3). Callus tissue was lyophilized, ground, and extracted with MeOH. The solvent was removed by rotary evaporation and the extract was purified by HPLC on a semipreparative Waters Symmetry Prep C18 column (7 μm, 7.8 × 300 mm) with an H_2O/acetonitrile gradient (6:4 to 1:9 in 10 min) as the mobile phase at a flow rate of 4 mL/min. The medium was extracted with CH_2Cl_2, the solvent was removed by rotary evaporation, and the extract was purified by HPLC as above. Upon lyophilization of sampled fractions from HPLC, **3** was obtained as a yellow oil. Yields from individual feeding experiments are given in Table 2. MS, ^{1}H, and ^{13}C NMR data were in agreement with values published previously.[4]

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Supporting Information Available: 600 MHz ^{1}H and 150 MHz ^{13}C{^{1}H} NMR spectra of **3** obtained after feeding [U-$^{13}C_2$]-acetate, (D)-[U-$^{13}C_6$]glucose, (L)-[U-$^{13}C_3$,^{15}N]alanine, (L)-[U-$^{13}C_4$]aspartic acid, and [U-$^{13}C_2$,^{15}N]glycine, ^{1}H−^{15}N HMBC spectra of **3** obtained after ^{15}N labeling, and 150 MHz ^{13}C-{^{1}H,^{15}N} NMR spectra of **3** obtained after feeding [U-$^{13}C_2$,^{15}N]-glycine, respectively (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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