Phytochemistry, Vol. 27, No. 3, pp. 839–843, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

HYDROXYCINNAMIC ACID–SPERMIDINE AMIDES FROM POLLEN OF *ALNUS GLUTINOSA*, *BETULA VERRUCOSA* AND *PTEROCARYA FRAXINIFOLIA**

BARBARA MEURER, VICTOR WRAY,† ROLF WIERMANN‡ and DIETER STRACK§

Botanisches Institut der Universität zu Köln, Gyrhofstrasse 15, 5000 Köln 41, F.R.G.; †GBF, Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-3300 Braunschweig, F.R.G.; ‡Botanisches Institut der Universität Münster, Schlossgarten 3, D-4400 Münster, F.R.G.

(*Received* 20 *July* 1987)

Key Word Index—*Alnus glutinosa*; *Betula verrucosa*; *Pterocarya fraxinifolia*; Betulaceae; Juglandaceae; pollen; hydroxycinnamic acid amide; *p*-coumaric acid; ferulic acid; polyamine; spermidine.

Abstract—Hydroxycinnamic acid–spermidine amides have been isolated from pollen and identified from their ^{1}H NMR and mass spectral data: N^1,N^5-di-(*E*)-*p*-coumaroylspermidine from *Pterocarya fraxinifolia* (Lam.) Spach., N^5,N^{10}-di-(*E*)-feruloylspermidine from *Betula verrucosa* Ehrh. and a mixture of two di-(*E*)-*p*-coumaroylspermidines from *Alnus glutinosa* (L.) Gaertn.

INTRODUCTION

We have previously reported the isolation and identification of hydroxycinnamic acid (HCA)-spermidine amides, namely caffeoylferuloyl- and diferuloylspermidines, from pollen of *Corylus avellana* L. [1] and localized these compounds in the structure of the exine [2]. In a further study on their occurrence in pollen of related genera [3] we found that similar compounds may frequently accumulate in pollen of the Hamamelididae. They may form patterns which seem to be characteristic of the generic, subfamily and family levels.

Such conjugates of HCAs with the aliphatic amines putrescine, spermidine, and spermine have been reported from a large number of families throughout the plant kingdom [4, 5]. Their accumulation was shown to be linked with cell multiplication [6] or fertility of reproductive organs [7, 8]. They may also protect plants from viral, bacterial or fungal infestation [9, 10] and may be produced as phytoalexins [11].

In the present study we report the isolation and identification of new types of N^5-acylated spermidines in pollen. These are N^1,N^5-di-(*E*)-*p*-coumaroyl-spermidine (**P**) from *Pterocarya fraxinifolia* (Lam.) Spach. and N^5,N^{10}-di-(*E*)-feruloylspermidine (**B**) from *Betula verrucosa* Ehrh. In addition a mixture of two di-(*E*)-*p*-coumaroylspermidines (**AI, AII**) was isolated from *Alnus glutinosa* (L.) Gaertn.

RESULTS AND DISCUSSION

Pollen material was first treated with water to remove flavonoids followed by 80% aqueous methanol to extract the HCA-spermidine amides [1]. Repeated extractions (× 12) yielded 1.3, 2.0, and 1.9% HCA-spermidine amides dry weight from pollen of *Pterocarya fraxinifolia*, *Alnus glutinosa*, and *Betula verrucosa*, respectively. Total extraction could not be achieved, but continued methanolic extraction of the same material gave a further 40–50 nmol of the amides per hr from 100 mg pollen material. These results are in good agreement with those obtained earlier [1, 2].

Purification of the crude extract by ion exchange chromatography gave N^1,N^5-di-(*E*)-coumaroyl-spermidine (**P**) from *P. fraxinifolia*, N^5,N^{10}-di-(*E*)-feruloylspermidine from *B. verrucosa* (**B**), and a mixture of two di-(*E*)-*p*-coumaroylspermidines (**AI, AII**) from *A. glutinosa*. Chromatographic and spectral data are given in Tables 1–3. The data of $N^1;N^{10}$-(*E*)-caffeoyl-(*E*)-feruloylspermidine from pollen of *Corylus avellana* L. [1] and chemically synthesized N^1,N^5,N^{10}-tribenzoyl-spermidine are listed for comparison.

All compounds usually underwent configurational isomerization (*E*/*Z*) and rotational isomerization (hindered rotation about the N^5-amidic bond) during the purification procedure which caused highly complex ^{1}H NMR spectra with up to eight signals per proton (only the main sets of signals are listed in Table 3). It is assumed that the configuration of the naturally occurring compounds is the *E* configuration [1]. The occurrence of isomers of compounds in the preparations examined was confirmed by their negative-ion FAB mass spectra, where only a single peak occurred at *m/z* 436 $[M-H]^-$ for **P** and for the preparation from *A. glutinosa* pollen. A single peak at *m/z* 496 $[M-H]^-$ supported the structure of a diferuloyl-spermidine as the major compound from *Betula* pollen. Furthermore the ratio of HCA and spermidine moieties was always 2:1 in the ^{1}H NMR spectra. Strong alkaline hydrolyses gave only *p*-coumaric acid from the compounds isolated from *P. fraxinifolia* and *A. glutinosa* and ferulic acid from the compounds isolated from *B. verrucosa*, while strong acid hydrolyses yielded only spermidine in all cases (Table 2).

*Dedicated to the memory of Dr. Lutz Grotjahn, a good colleague and friend, who died on 17 June 1987.

§Author to whom correspondence should be addressed. Present address: Institut für Pharmazeutische Biologie der Technischen Universität Braunschweig, Mendelssohnstrasse 1, D-3300 Braunschweig, F.R.G.

Table 1. Chromatographic, UV spectroscopic (longer wavelength region) and mass spectrometric (MS) data of HCA–spermidine amides (**P**, **AI**, **AII**, **B**) from pollen

	TLC*		HPLC	UV	MS
Compound	R_f	Appearance	R_t, min	λ_{max}, nm	$[M-H]^-$
P	0.62	abs/d-blue	13.9	309, 298 sh	436
AI	0.62	abs/d-blue	13.9	309, 294	436
AII	0.88	abs/d-blue	14.4	309, 294	436
B	0.83	blue/gr-blue	15.8	318, 298 sh	496

*On microcrystalline cellulose with $CHCl_3$–HOAc (3:2, satd with H_2O) as solvent. Fluorescence under UV (350 nm) without and with NH_3 vapour (abs, absorbing; d, dark; gr, greenish).
sh, shoulder.

Table 2. Chromatographic (TLC, HPLC) and UV spectroscopic (longer wavelength region) data of alkaline and acid hydrolysis products (prod) of the HCA-spermidines amides (**P**, **AI**, **AII**, **B**) from pollen in comparison with reference material

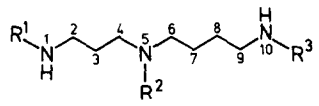
	TLC*		UV	HPLC	
Compound	R_f	Appearance	λ_{max}, nm	R_t, min	
P-prod	0.37	abs/d-blue	309, 298 sh	10.3	6.7
AI/**AII**-prod	0.37	abs/d-blue	309, 298 sh	10.3	6.7
B-prod	0.58	blue/gr-blue	319, 295 sh	11.5	6.7
p-Coumarate	0.37	abs/d-blue	309, 297 sh	10.3	—
Ferulate	0.57	blue/gr-blue	321, 293 sh	11.5	—
Spermidine	—	—	—	—	6.7

AI and **AII** were analysed as a mixture. Alkaline-liberated HCAs were analysed by TLC, HPLC, and UV spectroscopy and the acid-liberated benzoylated spermidines (see Experimental section) only by HPLC
*On microcrystalline cellulose with toluene–HOAc (2:1, satd with H_2O) as solvent. Appearance: fluorescence under UV (350 nm) without and with NH_3 vapour (abs, absorbing; d, dark; gr, greenish).
sh, shoulder.

Although the presence of isomers gave rise to complex ^{1}H NMR spectra the acylation pattern of the spermidine residue could be deduced from the 1D and 2D ^{1}H NMR spectra. Spermidine can be acylated at any of the three nitrogen (N^1, N^5 or N^{10}) and the following criteria—evident from previous work [1], from data for the synthetic tribenzoylspermidine (Table 3), and for internal consistency of the present data—were used for interpretation of the spectra: Proton signals of $-CH_2-$ next to a non-acylated $-NH_2$ or $-NH-$ occur at δ2.7–3.1 ppm; the signal of the central $-CH_2-$ of the $-(CH_2)_3-$ moiety occurs at δ2.0 ppm and signals of the central $-CH_2-CH_2-$ of the $-(CH_2)_4-$ moiety occur at δ1.8–1.5 ppm. Acylation of the terminal amino groups (N^1, N^{10}) causes a downfield shift of the adjacent $-CH_2-$ signal of 0.4 ppm, while $-CH_2-$ signals next to an acylated central nitrogen (N^5) shift by up to 0.6 ppm downfield. Acylation of the central nitrogen also gives rise to a hindered rotation of the phenylpropanoid substituent and results in a doubling of all the signals in the spectrum as both configurations will be approximately equivalent in energy as spermidine is almost symmetrical. This was confirmed by the 2D ^{1}H NMR spectrum of synthetic N^1,N^5,N^{10}-tribenzoylspermidine. The H-7′ and H-8′ signals of the phenylpropanoid residue in the N^5-position are typically shifted downfield to about δ6.9 and 7.6 ppm (see the N^1,N^{10}-disubstituted type of compound for comparison, Table 3). The interpretation of the spectra was additionally complicated by the occurrence of *E*/*Z* isomerism of the phenylpropanoid residue.

All signals of the ^{1}H NMR spectrum of **P** from pollen of *P. fraxinifolia* were doubled due to hindered rotation of the *p*-coumaroyl residue in the N^5-position. The substitution pattern of the spermidine moiety, however, was evident from the 2D ^{1}H NMR shift correlated spectrum which showed couplings for two sets of signals for the sequences $-(CH_2)_3-$ and $-(CH_2)_4-$ at δ3.42–2.0–3.65 and 3.38–1.9–3.58 ppm, and δ3.65–1.78–1.78–3.00 and 3.58–1.75–1.75–3.00 ppm, respectively. Application of the above criteria gave evidence of acylation at the N^1 and N^5

Table 3. ^{1}H NMR spectral data of natural HCA- and synthetic benzoylspermidine amides in CD_3OD (for comparison with N^1,N^{10} substitution the spectral data of N^1,N^{10}-(*E*)-caffeoyl-(*E*)-feruloylspermidine [1] were included)



P ($R^1 = R^2 = p$-coumaroyl, $R^3 = H$)

H-2	3.42 *m*	3.38 *m*	H-7′ 7.59 *d* 7.56 *d* 7.52 *d* 7.49 *d*
H-3	2.0 *m*	1.9 *m*	H-8′ 6.93 *d* 6.88 *d* 6.59 *d* 6.52 *d*
H-4	3.65 *m*	3.58 *m*	H-2′, H-6′ 7.49 *d** 7.41 *d** 7.41 *d** 7.54 *d**
H-6	3.65 *m*	3.58 *m*	H-3′, H-5′ 6.77 *d** 6.84 *d** 6.85 *d** 6.85 *d**
H-7, H-8	1.78 *m*	1.75 *m*	$J(7'-8') = 15$ Hz
H-9	3.0 *m*	3.0 *m*	$J(2'-3') + J(5'-6') = 8.7$ Hz

B ($R^1 = H$, $R^2 = R^3 =$ feruloyl)

H-2	2.95 *m*	2.90 *m*	H-7′ 7.46 *d* 7.47 *d* 7.59 *d* 7.57 *d*
H-3	1.95 *m*	2.00 *m*	H-8′ 6.45 *d* 6.46 *d* 6.47 *d* 6.49 *d* 6.59 *d* 6.96 *d*
H-4	3.6 *m*	3.65 *m*	$J(7'-8') = 15$ Hz
H-6	3.65 *m*	3.65 *m*	H-7′ 6.65 *d* 6.64 *d*
H-7	1.8 *m*	1.76 *m*	H-8′ 6.06 *d* 6.03 *d* $J(7'-8') = 12$Hz
H-8	1.67 *m*	1.64 *m*	H-2′ 7.08 *d* 7.04 *d* 7.12 *d* 7.18 *d* 7.27 *d*
H-9	3.4 *m*	3.4 *m*	H-6′ 6.89 *dd* 7.025 *dd* 7.18 *dd* 7.14 *dd*
			H-5′ 6.78 *d* 6.80 *d* 6.82 *d* 6.84 *d* 6.845 *d*
			-OMe† 3.84 *s* 3.85 *s* 3.91 *s* 3.92 *s* 3.94 *s*
			$J(2'-6') = 1.9$ Hz; $J(5'-6') = 8.1$ Hz

AI/AII (*N*,*N*-di-*p*-coumaroylspermidines)

Main fragments	Minor fragments		
3.55 *m*	3.69 *m*‡		H-7′ 7.62 *d* 7.60 *d* 7.48 *d* 7.46 *d*
$-(CH_2)_3-$ 2.04 *m*	2.09 *m*		H-8′ 6.92 *d* 6.94 *d* 6.54 *d* 6.47 *d*
2.97 *m*	3.06 *m*		$J(7'-8') = 15.7$ Hz
			H-7′ 6.66 *d* 6.68 *d*
			H-8′ 6.02 *d* 6.05 *d*
3.55 *m*	3.53 *m*	3.39 *m*	$J(7'-8') = 12$ Hz
$-(CH_2)_4-$ 1.78 *m*	1.82 *m*	1.58 *m*	H-2′, H-6′ complex at 7.25 to 7.60
1.72 *m*	1.82 *m*	1.54 *m*	
3.28 *m*	3.02 *m*	3.26 *m*	H-3′, H-5′ complex at 6.75 to 6.87
			$J(2'-3') + (5'-6') = 8.7$ Hz

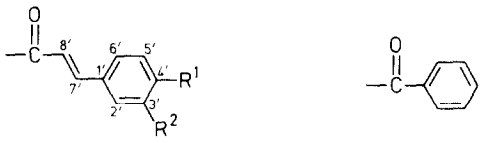
N^1,N^{10}-(*E*)-caffeoyl-(*E*)-feruloylspermidine

		Caffeoyl	Feruloyl
H-2	3.46 *t*		
H-3	2.01 *m*	H-7′ 7.46 *d*	7.49 *d*
H-4	3.10 *t*	H-8′ 6.46 *d*	6.54 *d* $J(7'-8') = 15.7$ Hz
H-6	3.11 *t*	H-2′ 7.08 *d*	7.18 *d*
H-7	1.84 *m*	H-6′ 6.95 *dd*	7.07 *dd* $J(2'-6') = 1.9$ Hz
H-8	1.74 *m*	H-5′ 6.81 *d*	6.84 *d* $J(5'-6') = 8.1$ Hz
H-9	3.41 *t*	-OMe —	3.93†

N^1,N^5,N^{10}-Tribenzoylspermidine

H-2	3.68 *t*	3.39 *t*	benzoyl signals 7.92–7.25
H-3	2.04 *p*§	1.92 *p*	
H-4	3.53 *t*	3.27 *t*	
H-6	3.64 *t*	3.27 *t*	
H-7	1.83 *p*	1.67 *p*	
H-8	1.76 *p*	1.47 *p*	
H-9	3.50 *t*	3.27 *t*	

Table 3. Continued



p-Coumaroyl	$R^1 = OH$; $R^2 = H$	Benzoyl
Caffeoyl	$R^1 = OH$; $R^2 = OH$	
Feruloyl	$R^1 = OH$; $R^2 = OMe$	

*Intensity of two protons.
†Intensity of three protons.
‡Several other cross peaks.
§Pentet.

positions. Substitution at N^5 is additionally confirmed by the downfield chemical shifts of H-7′ and H-8′ to 6.93–7.59 ppm and 6.88–7.56 ppm, respectively. The structure of the *p*-coumaroyl residues, indicated in the spectrum by four sets of signals, could be deduced from the signals for H-7′ and H-8′ with $J(7'-8') = 15$ Hz indicating the *E* configuration of the double bonds. The signals for the aromatic moiety show characteristic AA′BB′-patterns with $J(2'-3') + J(2'-5') = 8.7$ Hz. Thus the 1D and 2D ^{1}H NMR spectra show two sets of signals each corresponding to the structure of N^1, N^5-di-(*E*)-*p*-coumaroylspermidine (**P**). Comparative HPLC analysis of a freshly prepared methanolic extract with the NMR sample gave only one peak at $R_t = 13.5$ min for the crude extract and two peaks at $R_t = 12.9$ and 13.5 min for the NMR sample. This indicated that isomerization must be due to the purification procedure and that only one configuration of **P** occurs naturally in pollen of *P. fraxinifolia*.

From pollen of *B. verrucosa* N^5, N^{10}-di-(*E*)-feruloylspermidine (**B**) was identified as the major HCA amide. Again, the acylation pattern of the spermidine residue was deduced from cross peaks in the 2D ^{1}H NMR shift correlated spectrum. Doubling of the spermidine signals supported the occurrence of isomers resulting from the hindered rotation of the feruloyl residue at the N^5-position. The couplings and chemical shifts of the proton signals of the $-(CH_2)_3-$ moieties at δ2.95–1.95–3.60 and 2.90–2.00–3.65 ppm and the $-(CH_2)_4-$ moieties at 3.65–1.80–1.67–3.40 and 3.65–1.76–1.64–3.40 ppm clearly indicated a free terminal amino group in the N^1 position. The N^5 and N^{10} positions were acylated with feruloyl residues, but interpretation of the downfield signals was additionally complicated by the occurrence of *Z* isomers. The 2D ^{1}H NMR shift correlated spectra, however, allowed the identification of several signals for H-7′ and H-8′: doublets at δ7.46 and 7.47 ppm showed cross peaks with the complex signal for H-8′ at δ6.45–6.49 ppm, and a $J(7'-8') = 15$ Hz indicated the *E*-configuration of the double bonds of these feruloyl residues. The occurrence of two doublets each for H-8′ and H-7′ at δ6.95 and 6.96 ppm and 7.56 and 7.59 ppm with $J = 15$ Hz supported the N^5-position of one of the phenylpropanoid moieties. There were two sets of signals visible for the *Z*-configuration of the double bond at δ6.06 and 6.65 ppm and 6.03 and 6.64 ppm with $J(7'-8') = 12$ Hz. The substitution pattern of the aromatic part was deduced from the characteristic coupling patterns for H-2′, H-5′, and H-6′ signals with $J(2'\text{-}6') = 1.9$ Hz and $J(5'\text{-}6') = 8.1$ Hz and several singlets at δ3.8–4.0 ppm indicating the occurrence of –OMe– groups. Only major signals are listed in Table 3. Comparative HPLC analyses of freshly prepared methanolic extracts of pollen from several *Betula* species [3] as well as the FAB mass spectrum ($m/z = 496$ $[M-H]^-$) again confirmed the occurrence of only one compound.

Although the negative-ion FAB mass spectrum of the preparation from *A. glutinosa* pollen showed only one peak corresponding to a M_r of 437, and hence di-*p*-coumaroylspermidine, the ^{1}H NMR spectra were more complex than those from the other preparations. The 2D COSY ^{1}H NMR spectra indicated a *E*/*Z* mixture of isomers, while the complexity of the signals in the region 3.6 to 1.5 ppm suggested more than one set of *N*,*N*-disubstituted compounds were present. The most prominent cross peaks in this region of the spectrum were indicative of a $H_2N(CH_2)_3N(COR)-$ or $RCONH(CH_2)_3NH-$ fragment corresponding to either $N^5, N^{10}-$ or $N^1, N^{10}-$ dicoumaroylspermidine. The presence of N^5 substitution was supported by the occurrence of two major sets of signals for H-7′ and H-8′ (*p*-coumaroyl residue): doublets at δ7.62 and 7.60 (H-7′) were coupled with doublets at δ6.92 and 6.94 ppm [H-8′, $J(7'-8') = 15$ Hz]. These downfield shifts seem to be characteristic N^5-substituted spermidines. Spermidine signals of the major fragments, however, were not identical with those obtained for **P** from *P. fraxinifolia*. We assume that N^5, N^{10}-di-(*E*)-*p*-coumaroylspermidine occurs in pollen of *A. glutinosa*. The acylation pattern of the second compound could not be deduced from the 2D ^{1}H NMR spectral data. The occurrence of at least two compounds in this preparation was supported by HPLC analysis which showed two distinct peaks at $R_t = 13.9$ and 14.4 min for the freshly prepared crude extract.

In summary, conjugates of HCAs with spermidine have been described from several plant species [1, 12–14], but this is the first report of N^5-acylated spermidine in the plant kingdom. However, such compounds have been identified from bacteria. They are excreted as iron-binding ligands (siderophores) into iron deficient media and their structures were identified as 2-hydroxybenzoyl- and 2,3-dihydroxybenzoyl-*N*-L-threonyl-N^5-[N^1, N^{10}-di-(2,3-dihydroxybenzoyl)]spermidines [15, 16].

EXPERIMENTAL

Plant material. Pollen of *Alnus glutinosa* (L.) Gaertn. and *Betula verrucosa* Ehrh. were collected from the area around

Münster, F.R.G, those of *Pterocarya fraxinifolia* (Lam.) Spach. from the Botanic Garden in Münster. The pollen material was freeze-dried immediately after harvest.

Extraction and isolation. Pollen was first treated × 3 with H_2O (4 g dry wt/20 ml, 2 hr with stirring at room temp.) to remove flavonoids followed by 80% aq. MeOH (× 6) to extract the HCA–spermidine amides. Each extract was centrifuged (3500 *g* for 15 min) and the supernatants were combined. The methanolic extracts were evapd to dryness *in vacuo* and the residues suspended in 10 ml of 0.1% aq. NaCl (30 min stirring at room temp.) The compounds were purified by chromatography on a CM-Sephadex C-25 column (28 × 2 cm i.d.; Pharmacia, Uppsala, Sweden) using increasing concns of aq. NaCl soln (100 ml each of 0.1, 0.3, 0.5, 0.7% aq. NaCl, followed by 200 ml of 1.0 and 1.2% aq. NaCl soln). **P** from *P. fraxinifolia* and **AI** and **AII** from *A. glutinosa* were eluted with 1% aq. NaCl and **B** from *B. verrucosa* with 1.2% aq. NaCl. Elution was monitored by UV at 254 nm (Uvicord; LKB). The fractions were evapd to dryness *in vacuo*, the residues redissolved in MeOH, and excess salt removed by centrifugation (3500 *g*, 15 min). Chromatographic (TLC, HPLC) and UV spectroscopic data are listed in Table 1.

Quantification. The total amounts of HCAs were estimated as follows: 100 mg pollen was first extracted × 4 with 2 ml H_2O (1 hr with stirring at room temp.), followed by × 12 80% aq. MeOH in the same way. The methanolic extractions were centrifuged (3500 *g*, 15 min) and the HCA content of the supernatants measured by their UV absorbance at 315 nm for **B** and 294 nm for **P**, **AI**, and **AII** (λ_{max} of the supernatants). For quantitative calculations a log ε value ($cm^{-1} \times mol^{-1}$) of 4.33 (codonocarpine) was used [13].

Hydrolysis. HCAs were liberated from the amides by alkaline hydrolysis (4 M NaOH, 4 hr at 100°) and extracted after acidification (HCl) with Et_2O. The ferulic and *p*-coumaric acids were identified by their UV spectra, co-chromatography with standard material and their appearance under UV at 350 nm with and without treatment of NH_3 vapour. The spermidine residues were analysed (HPLC) after acid hydrolysis (4 M HCl, 4 hr at 100°) and subsequent benzoylation [17] (see below).

Preparation of N^1, N^5, N^{10}-tribenzoylspermidine was carried out from benzoylchloride (Sigma) and spermidine × 3HCl (Serva). The aq. spermidine soln (5 ml of 10 mM) was diluted with 10 ml 2 M NaOH prior to the addition of 200 μl benzoylchloride. This mixture was allowed to stand for 20 min with stirring at room temp. After the addition of 20 ml NaCl soln (aq. satd) the mixture was extracted with Et_2O. The Et_2O phase was evapd to dryness, dil. in 5 ml of 1% aq. NaCl and purified by the procedure used for the HCA–spermidines. The benzoylspermidine was eluted from the CM Sephadex C-25 column (equilibrated with 1% aq. NaCl) with 200 ml of 1% aq. NaCl.

HPLC. The liquid chromatograph used was from LKB. The chromatographic column was prepacked with Nucleosil C-18 (5 μm, 250 × 4 mm i.d.; Macherey-Nagel). Injections were performed via a 20 μl loop and quantitative calculations were obtained with a Shimadzu Data Processor (Chromatopac C-R3A). Benzoylamines were eluted isocratically with 1.5% H_3PO_4 and 50% MeCN in H_2O as solvent (UV detection at 230 nm). Separations of the HCA amides were achieved with a linear gradient from 30% solvent B (1.5% H_3PO_4, 20% HOAc and 25% MeCN in H_2O) in solvent A (1.5% H_3PO_4 in H_2O) to 100% solvent B within 20 min (UV detection at 320 nm). Flow rate was 1 ml/min.

^1H NMR spectra were recorded on Bruker WM 400 and AM 300 NMR spectrometers at ambient temp. and locked to the major deuterium resonance of the solvent, CD_3OD. Shifts are recorded in ppm relative to TMS and couplings in Hz. The 2D ^1H homonuclear shift-correlated (COSY) spectra were recorded with a 90°–t_1–90° –FID (t_2) or a 90°–t_1–45° –FID (t_2) pulse sequence. The data were multiplied by a sine-bell function and one level of zero filling was used in both t_1 and t_2. All 1D and 2D spectra were taken with the standard Bruker Aspect 3000 software. ^1H NMR spectral data are listed in Table 3.

FAB mass spectra were recorded in the negative-ion mode of operation on a Kratos MS 50 spectrometer with a Kratos FAB source; glycerol was used as matrix.

Acknowledgements—This work was supported by the Deutsche Forschungsgemeinschaft (D. S., R. W.) and the Fonds der Chemischen Industrie (D.S., R.W.). Further support by a Heisenberg fellowship from the Deutsche Forschungsgemeinschaft (D.S.) and by a grant (Promotionsförderung) from the Studienstiftung des Deutschen Volkes (B.M.) is gratefully acknowledged. Dr L. Grotjahn recorded the MS spectra.

REFERENCES

1. Meurer, B., Wray, V., Grotjahn, L., Wiermann, R. and Strack, D. (1986) *Phytochemistry* **25**, 433.
2. Gubatz, S., Herminghaus, S., Meurer, B., Strack, D. and Wiermann, R. (1986) *Pollen Spores* **28**, 347.
3. Meurer, B., Wiermann, R. and Strack, D. (1987) *Phytochemistry* **27**, 823.
4. Martin-Tanguy, J., Cabanne, F., Perdrizet, E. and Martin, C. (1978) *Phytochemistry* **17**, 1927.
5. Smith, T. A., Negrel, J. and Bird, C. R. (1983) *Adv. Polyamine Res.* **4**, 347.
6. Martin, C., Kunesch, G., Martin-Tanguy, J., Negrel, J., Paynot, M. and Carré, M. (1985) *Plant Cell Rep.* **4**, 158.
7. Cabanne, F., Dalebroux, M. A., Martin-Tanguy, J. and Martin, C. (1981) *Physiol. Plantarum* **53**, 399.
8. Martin-Tanguy, J., Perdrizet, E., Prevost, J. and Martin, C. (1982) *Phytochemistry* **21**, 1939.
9. Martin, C. and Martin-Tanguy, J. (1981) *C. R. Acad. Sci. Paris Ser. III* **293**, 249.
10. Pandey, D. K., Tripathi, R. N., Tripathi, R. D. and Dixit, S. N. (1983) *Grana* **22**, 31.
11. Smith, T. A. and Best, G. R. (1978) *Phytochemistry* **17**, 1093.
12. Cabanne, F., Martin-Tanguy, J. and Martin, C. (1977) *Physiol. Vég.* **15**, 429.
13. Doskotch, R. W., Ray, A. B. and Beal, J. L. (1971) *Chem. Commun.* 300.
14. Englert, G., Klinger, K., Hamet, R., Schlitter, E. and Vetter, W. (1973) *Helv. Chim. Acta* **56**, 474.
15. Tait, G. H. (1975) *Biochem. J.* **146**, 191.
16. Leong, S. A., Peterson, T. and Neilands, J. B. (1979) *J. Biol. Chem.* **254**, 1860.
17. Flores, H. E. and Galston, A. W. (1982) *Plant Physiol.* **69**, 701.