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STRUCTURE-ACTIVITY RELATIONSHIP OF ISOLATED AVENANTHRAMIDE ALKALOIDS AND SYNTHESIZED RELATED COMPOUNDS AS OVIPOSITION DETERRENTS FOR *PIERIS BRASSICAE*

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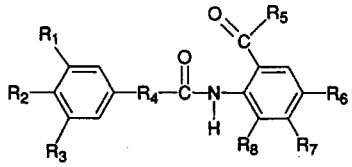
ABSTRACT.—The structure-activity relationship was investigated of compounds isolated from the eggs of *Pieris brassicae*, the large white cabbage butterfly, and eight synthesized related compounds as oviposition deterrents for this insect. The activity of all compounds was tested in a dual-choice bioassay. The two most active oviposition deterrents for *P. brassicae* were *trans*-2-[3-(4-hydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid [**8**] and *trans*-2-[3-(3,4-dihydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid [**2**]. Among members of this compound class, alteration of the substituents of the cinnamic acid part of the molecule affected the oviposition deterrent activity more profoundly than other structural changes. Modification of the anthranilic acid part of the molecule resulted in lower activity.

The white cabbage butterflies, *Pieris brassicae* L. and *P. rapae* L. (Lepidoptera), herbivorous pests of crucifers, produce egg-associated chemicals that can inhibit their oviposition (1). These chemicals can be collected by washing the eggs with H_2O or MeOH. Oviposition by *P. brassicae* and *P. rapae* is inhibited when a potential host plant is sprayed with such an egg wash (2,3). Inhibition of oviposition is especially pronounced when females have a choice between treated plants and control plants, or when dispersal activity can be manifested (4). Recently, the compounds responsible for the oviposition deterrent effect of a crude egg wash were isolated and identified as *trans*-2-[3-(3,4,5-trihydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid [**1**], *trans*-2-[3-(3,4-dihydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid [**2**], and *trans*-2-[3-(3,4-dihydroxy-5-β-D-glucopyranosyloxy-phenylpropenoyl)amino]-3,5-dihydroxybenzoic acid [**3**] (5), which are three previously unknown avenanthramide alkaloids (amides of cinnamic and anthranilic acids) (6–8). Substances that modify the oviposition behavior of *Pieris* butterflies might have practical value in preventing colonization of cabbage by these specialized insects (4). A limited SAR study has been undertaken in order to determine whether there are more active or simpler structures than the natural deterrents identified so far. Eight structurally related compounds, with changes in either both ring systems or in the way they are coupled, were synthesized and their oviposition deterrent activity was measured quantitatively. In this paper, we describe the results of these studies.

RESULTS AND DISCUSSION

The tested compounds were *trans*-2-[3-(3,4,5-trihydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid [**1**] (miriamide), *trans*-2-[3-(3,4-dihydroxyphenyl-propenoyl)amino]-3,5-dihydroxybenzoic acid [**2**], *trans*-2-[3-(3,4-dihydroxy-5-β-D-glucopyranosyloxy-phenylpropenoyl)amino]-3,5-dihydroxybenzoic acid [**3**], *trans*-2-[3-(3,4,5-trimethoxyphenyl-propenoyl)amino-3,5-dimethoxybenzoic acid methyl ester [**4**], *trans*-2-[3-(3,4,5-trihydroxyphenylpropenoyl)amino]-3-hydroxy-5-



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	OH	OH	OH	CH=CH	OH	OH	H	OH
2	OH	OH	H	CH=CH	OH	OH	H	OH
3	OH	OH	Glucose	CH=CH	OH	OH	H	OH
4	OCH_3	OCH_3	OCH_3	CH=CH	OCH_3	OCH_3	H	OCH_3
5	OH	OH	OH	CH=CH	OH	OCH_3	H	OH
6	OH	OH	OH	CH=CH	OCH_3	OH	H	OH
7	OH	OH	OH	CH=CH	OH	OH	OH	H
8	H	OH	H	CH=CH	OH	OH	H	OH
9	H	OH	H	CH=CH	OH	OH	OH	H
10	OH	OH	OH	CH_2-CH_2	OH	OH	H	OH
11	OH	OH	OH	—	OH	OH	H	OH

methoxybenzoic acid [**5**], *trans*-2-[3-(3,4,5-trihydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid methyl ester [**6**], *trans*-2-[3-(3,4,5-trihydroxyphenylpropenoyl)amino]-4,5-dihydroxybenzoic acid [**7**], *trans*-2-[3-(4-hydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid [**8**], *trans*-2-[3-(4-hydroxyphenylpropenoyl)amino]-4,5-dihydroxybenzoic acid [**9**], 2-[3-(3,4,5-trihydroxyphenylpropionyl)amino]-3,5-dihydroxybenzoic acid [**10**], and 2-[(3,4,5-trihydroxybenzoyl)amino]-3,5-dihydroxybenzoic acid [**11**].

Oviposition deterrency was quantified by means of an oviposition deterrent index (ODI) (see Experimental). The dose-response curves are given in Figure 1. The concentration at which ODI equals 50% (ED_{50}) was calculated for each compound by the method of Spearman and Kärber (9,10). The ED_{50} values with their 95% confidence intervals are given in Figure 2. We considered compounds to exert a significantly different deterrent effect when there was no overlap between the confidence intervals of their ED_{50} values. When comparing the ED_{50} value of compound **1** with the ED_{50} values of the other compounds, three groups with different ED_{50}s could be distinguished (Figure 2). The ED_{50} of compound **4** could not be calculated because the oviposition deterrent activity was only 28% at the highest dose tested (10 μg/leaf).

Removal of one or two hydroxy groups (from position 3 or from positions 3 and 5) of the cinnamic acid part of the parent molecule **1** (structures **2** and **8**) increased deterrency (Figure 2). Neither reduction nor removal of the double bond (**10** and **11**) affected activity when compared to **1**. When two hydroxy groups were removed from positions 3 and 5 of the cinnamic part of the molecule and one hydroxy group was shifted from position 3 to 4 in the anthranilic acid portion of the molecule (compound **9**), deterrency remained equal to that of compound **1**. Methylation of one of the hydroxy groups of the anthranilic part of the parent molecule **1**, as in compounds **5** or **6**, or a change in the position of one hydroxy group from position 3 to 4 of the anthranilic acid part of the molecule (structure **7**), reduced effectiveness compared to **1**. When glucose is linked to the cinnamic part of the molecule [**3**] deterrency was drastically reduced relative to that of **1**.

In conclusion, modification of the groups linked to the anthranilic part of the molecule lowers effectiveness as compared to **1**. Methylation of **1** and glucosylation of the 5-OH of the cinnamic acid moiety likewise reduces deterrency, while changes in the way both ring systems were linked has no influence on effectiveness. In contrast, mono- and dihydroxy

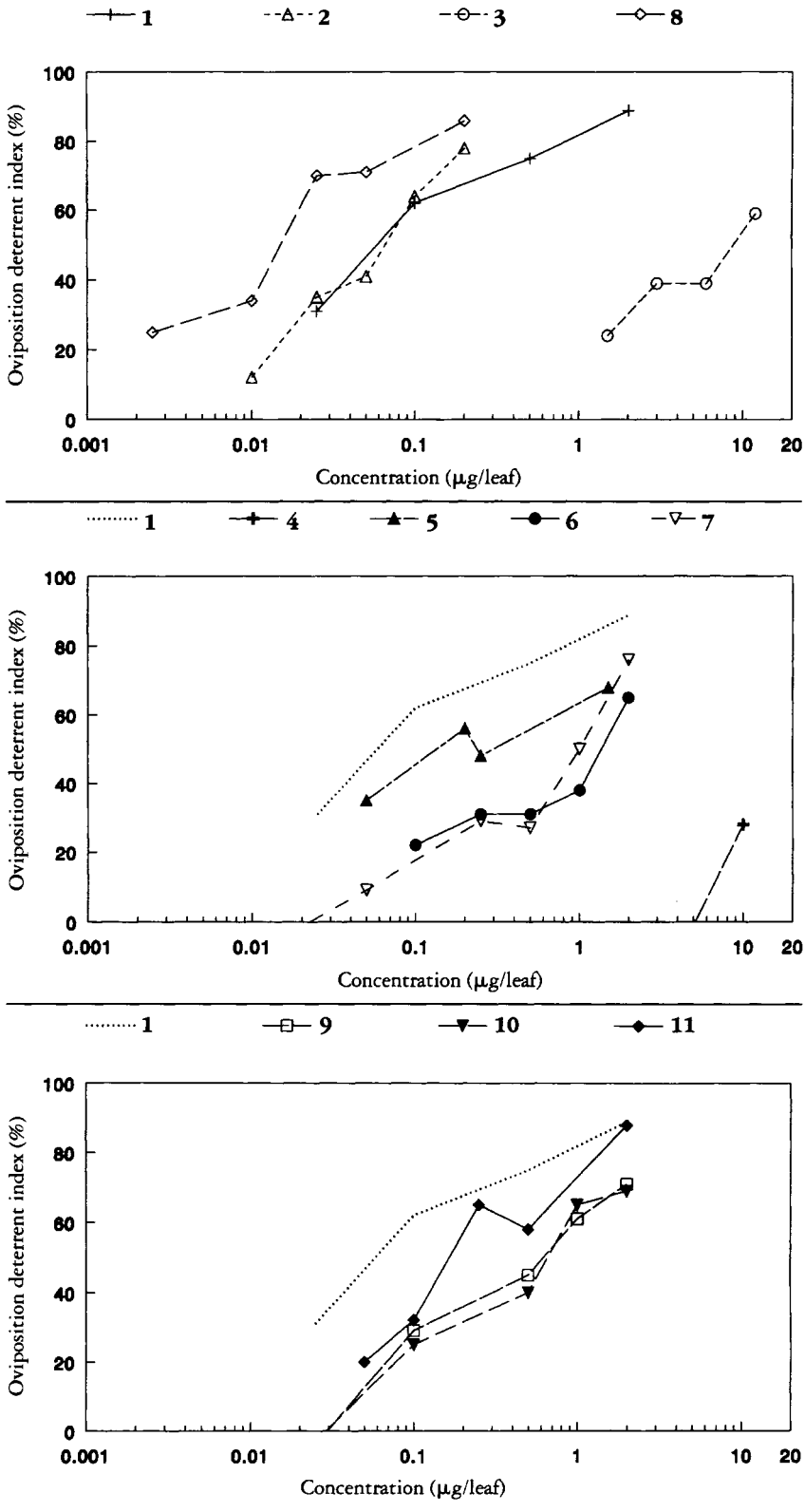


FIGURE 1. Dose-response curves of tested compounds.

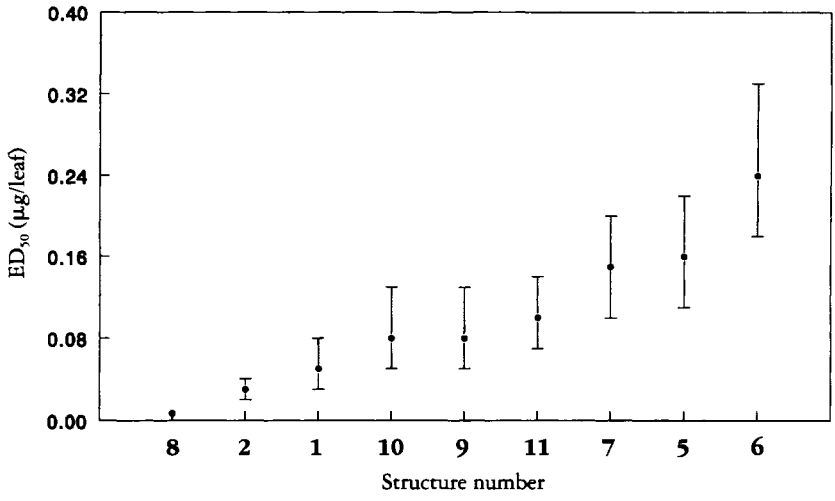


FIGURE 2. Calculated ED_{50} with 95% confidence intervals for nine of the eleven tested compounds. [The confidence interval for compound **3** runs from 1.37 to 2.16 µg/leaf. The ED_{50} of compound **4** could not be calculated (see text).]

substituted cinnamic acid groups in the molecule increase deterrent activity.

We tested derivatives of the cinnamic acid and anthranilic acid components of miriamide [**1**], caffeic acid, 3-hydroxyanthranilic acid, 3,5-dihydroxyanthranilic acid methyl ester and mixtures of these at 5 and 20 µg/leaf and found them to be inactive.

In field experiments carried out at the same time as the SAR study, with cabbage plants sprayed with pure miriamide **1**, no oviposition deterrent or dispersal activity could be measured (J.J.A. van Loon, personal communications). Additional laboratory experiments demonstrated that compound **1** was unstable when exposed to direct sunlight, due to the strongly conjugated structure of **1**.

In order to find simpler compounds that can be used effectively to prevent colonization of cabbage by cabbage butterflies, the synthesis of less conjugated derivatives such as compound **10**, or less substituted compounds or esters of cinnamic and benzoic acids, will be the subject of further research. Photostability of the other compounds described in the present study should also be investigated.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—All ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AC-E 200 spectrometer. Microanalyses were carried out on a Carlo Erba elemental analyzer model 1106. Uv spectra were recorded on a Beckman DU-7 spectrophotometer.

PLANT MATERIAL.—*Brassica oleracea* L. var. *gemmifera* cv. Titurel plants were reared in a greenhouse (20–30°, 50–80% RH, 16L:8D) in standard potting soil. Illumination consisted of natural daylight supplemented by high-pressure sodium vapor lamps hanging 0.75 m above pot level. A voucher specimen (van Setten 1073) has been deposited at the Herbarium Vadense (WAG), Wageningen, The Netherlands.

INSECTS.—*Pieris brassicae* L. (Lepidoptera: Pieridae) adults were obtained from a laboratory colony maintained on *Brassica oleracea*. This culture was established in 1981, and since then 18 generations have been produced each year. Field-collected adults have been introduced several times during this period. Rearing conditions were similar to those described by David and Gardiner (11). Voucher specimen 378,421 has been deposited at the insect collection of the Department of Entomology, Wageningen Agricultural University.

BIOASSAYS.—Bioassays were the same as those described by Blaakmeer *et al.* (5) except that there were 2 males and 4 females present in each cage and, on any one day, 12–16 replicates were run. The oviposition deterrent index was calculated in the following way: ODI=(C-T)×100/(C+T), where C and T represent

the number of egg batches laid on control and treated leaves. All doses of each compound were tested on one generation of butterflies.

The isolation of **1**, **2**, and **3** and the synthesis of **1** and **2** have been described previously (5). Compound **4** (*trans*-2-[3-(3,4,5-trimethoxyphenylpropenoyl)amino-3,5-dimethoxybenzoic acid methyl ester) was an intermediate in the synthesis of **1** [for spectral data of **1**, **2**, **3**, and **4**, see Blaakmeer *et al.* (5)]; mp of **4**: 173–174°.

trans-2-[3-(3,4,5,-Trihydroxyphenylpropenoyl)amino]-3-hydroxy-5-methoxybenzoic acid [**5**].—Compound **5** was a byproduct in the synthesis of **1** (5). After treatment of *trans*-2-[3-(3,4,5-trimethoxyphenylpropenoyl)amino]-3,5-dimethoxybenzoic acid with BBr_3 90% of **1** and 10% of **5** were isolated. ^{1}H-nmr spectrum (CD_3OD, 200 MHz) of **5**; benzoic acid part: δ 3.73 (OCH_3), 6.67 (d, J=2.7 Hz, H-4), 7.08 (d, J=2.7 Hz, H-6); cinnamic acid part: δ 6.48 (d, J=15.5 Hz, H-8), 6.60 (s, H-2/H-6), 7.42 (d, J=15.5 Hz, H-7). ^{13}C-nmr spectrum (CD_3OD, 50 mHz) of **5**; benzoic acid part: δ 56.0 (OCH_3), 109.3 (C-4/C-6), 122.0 (C-2), 124.9 (C-1), 153.3 (C-3), 159.3 (C-5), 170.8 (C-7); cinnamic acid part: δ 108.6 (C-2/C-6), 117.6 (C-8), 126.9 (C-1), 137.4 (C-4), 145.0 (C-7), 147.2 (C-3/C-5), 168.1 (C-9). *Anal.* found C 51.5, H 4.5, N 3.6; calcd for $C_{17}H_{15}NO_8 \times 2(H_2O)$, C 51.4, H 4.8, N 3.5. Uv λ max (MeOH) 355 nm.

trans-2-[3-(3,4,5-Trihydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid methyl ester [**6**].—A solution of 1M BBr_3 (8 ml, 8 mmol) in CH_2Cl_2 was added dropwise to a stirred suspension of *trans*-2-[3-(3,4,5-trimethoxyphenylpropenoyl)amino]-3,5-dimethoxybenzoic acid methyl ester [**4**] (216 mg, 0.5 mmol) in dry CH_2Cl_2 (15 ml) at −78°. After stirring for 2 h at 0°, the reaction was quenched with 1 M HCl (15 ml). The mixture was centrifuged and the residue was washed with 0.02 M HCl (2×5 ml) and H_2O (3×5 ml). After drying, *trans*-2-[3-(3,4,5-trihydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid methyl ester [**6**] (179 mg, 99%) was obtained. ^{1}H-nmr spectrum (CD_3OD, 200 MHz) of **6**; benzoic acid part: δ 3.83 (OCH_3), 6.59 (d, J=2.6 Hz, H-4), 6.90 (d, J=2.8 Hz, H-6); cinnamic acid part: δ 6.56 (d, J=15.9 Hz, H-8), 6.64 (s, H-2/H-6), 7.44 (d, J=15.5 Hz, H-7). ^{13}C-nmr spectrum (CD_3OD, 50 MHz) of **6**; benzoic acid part: δ 52.9 (OCH_3), 110.1 (C-4/C-6), 119.7 (C-2), 126.1 (C-1), 153.7 (C-3), 157.2 (C-5), 169.3 (C-7); cinnamic acid part: δ 108.7 (C-2/C-6), 117.8 (C-8), 127.1 (C-1), 137.3 (C-4), 144.6 (C-7), 147.1 (C-3/C-5), 168.3 (C-9). *Anal.* found C 51.8, H 4.5, N 3.4; calcd for $C_{17}H_{15}NO_8 \times 1.5(H_2O)$, C 52.8, H 4.7, N 3.6. Uv λ max (MeOH) 343 nm.

COMPOUNDS **7**–**9**.—These compounds were prepared in the same way as described for **1** (5). For **7**, the starting material was 3,4-dimethoxybenzoic acid methyl ester and *trans*-(3,4,5-trimethoxy)cinnamic acid. Nitration of 3,4-dimethoxybenzoic acid methyl ester gave 2-nitro-4,5-dimethoxybenzoic acid methyl ester (yield 92%) and reduction of the nitro group resulted in 2-amino-4,5-dimethoxybenzoic acid methyl ester (yield 94%). For **8**, the starting material was *trans*-4-methoxycinnamic acid and 3,5-dimethoxybenzoic acid methyl ester. *trans*-4-Methoxycinnamic acid was converted into its acid chloride (yield 98%) with thionyl chloride. *trans*-4-Methoxycinnamic acid and 3,4-dimethoxybenzoic acid methyl ester were the starting materials for the synthesis of **9**.

trans-2-[3-(3,4,5-Trihydroxyphenylpropenoyl)amino]-4,5-dihydroxybenzoic acid [**7**].—Yields of the coupling of *trans*-(3,4,5-trimethoxy)cinnamoyl chloride with 2-nitro-4,5-dimethoxybenzoic acid methyl ester and of the demethylation product were, respectively, 25% and 40%. ^{1}H-nmr spectrum of **7** (CD_3OD, 200 MHz); benzoic acid part: δ 7.50 (s, H-3), 8.22 (s, H-6); cinnamic acid part: δ 6.38 (d, J=15.8 Hz, H-8), 6.63 (s, H-2/H-6), 7.42 (d, J=15.6 Hz, H-7). ^{13}C-nmr spectrum of **7** (CD_3OD, 50 MHz); benzoic acid part: δ 108.4 (C-3), 119.5 (C-6), 131.0 (C-2), 141.9 (C-5), 152.3 (C-4), 171.5 (C-7), (C-1 not observed); cinnamic acid part: δ 108.5 (C-2/C-6), 118.4 (C-8), 127.0 (C-1), 137.2 (C-4), 143.7 (C-7), 147.1 (C-3/C-5), 166.7 (C-9). *Anal.* found C 50.5, H 4.4, N 3.6; calcd for $C_{16}H_{13}NO_8 \times 1.9(H_2O)$, C 50.4, H 4.4, N 3.7. Uv λ max (MeOH) 349 nm.

trans-2-[3-(4-Hydroxyphenylpropenoyl)amino]-3,5-dihydroxybenzoic acid [**8**].—Yields of the coupling of *trans*-4-methoxycinnamoyl chloride with 2-nitro-3,5-dimethoxybenzoic acid methyl ester (mp 154–156°) and of the demethylation product were, respectively, 71% and 93%. ^{1}H-nmr spectrum of **8** (CD_3OD, 200 MHz); benzoic acid part: δ 6.60 (d, J=2.9 Hz, H-4), 7.04 (d, J=2.8 Hz, H-6); cinnamic acid part: δ 6.63 (d, J=15.6 Hz, H-8), 6.80 (d, J=8.6 Hz, H-3/H-5), 7.47 (d, J=8.6 Hz, H-2/H-6), 7.60 (d, J=15.6 Hz, H-7). ^{13}C-nmr spectrum of **8** (CD_3OD, 50 MHz); benzoic acid part: δ 110.8 (C-4/C-6), 121.0 (C-2), 124.6 (C-1), 153.1 (C-3), 157.0 (C-5), 170.7 (C-7), cinnamic acid part: δ 116.8 (C-3/C-5), 117.4 (C-8), 127.4 (C-1), 131.1 (C-2/C-6), 144.1 (C-7), 161.0 (C-4), 168.1 (C-9). *Anal.* found C 56.8, H 4.4, N 4.1; calcd for $C_{16}H_{13}NO_6 \times 1.3(H_2O)$, C 56.7, H 4.6, N 4.1. Uv λ max (MeOH) 319 nm.

trans-2-[3-(4-Hydroxyphenylpropenoyl)amino]-4,5-dihydroxybenzoic acid [**9**].—Yields of the coupling of *trans*-4-methoxycinnamoyl chloride with 2-nitro-4,5-dimethoxybenzoic acid methyl ester (mp 160–162°) and of the demethylation product were 78% and 80%. ^{1}H-nmr spectrum of **9** (CD_3OD, 200 MHz); benzoic acid part: δ 7.51 (s, H-3), 8.22 (s, H-6); cinnamic acid part: δ 6.48 (d, J=15.6 Hz, H-8), 6.81 (d, J=8.3

Hz, H-3/H-5), 7.50 (d, J=9.8 Hz, H-2/H-6), 7.55 (d, J=15.6 Hz, H-7). ^{13}C-nmr spectrum of **9** (CD_3OD, 50 MHz); benzoic acid part: δ 115.4 (C-1), 108.5 (C-3), 119.5 (C-6), 137.2 (C-2), 141.9 (C-5), 152.3 (C-4), 171.5 (C-7); cinnamic acid part: δ 116.8 (C-3/C-5), 118.4 (C-8), 127.5 (C-1), 130.9 (C-2/C-6), 143.0 (C-7), 160.9 (C-4), 166.7 (C-9). *Anal.* found C 61.3, H 4.2, N 4.2; calcd for $C_{16}H_{13}NO_6$, C 61.0, H 4.2, N 4.4. Uv λ max (MeOH) 336, 315 nm.

Synthesis of 2-[3-(3,4,5-trihydroxyphenylpropionyl)amino]-3,5-dihydroxybenzoic acid **[10]**.—*2-[3-(3,4,5-Trimethoxyphenylpropionyl)amino]-3,5-dimethoxybenzoic acid methyl ester.* DCC (1.44 g, 7 mmol) was added to a stirred solution of 3,4,5-trimethoxyphenylpropionic acid in DMF (25 ml) and the mixture was stirred at room temperature under N_2. After 1 h, 2-amino-3,5-dimethoxybenzoic acid methyl ester (1.12 g, 5.3 mmol) in DMF (10 ml) was added to the solution. After 48 h, the mixture was poured into 1 M HCl (50 ml). The aqueous layer was extracted three times with EtOAc (75 ml). After drying and evaporation, 2-[3-(3,4,5-trimethoxyphenylpropionyl)amino]-3,5-dimethoxybenzoic acid methyl ester (290 mg, 13%) was isolated.

2-[3-(3,4,5-Trimethoxyphenylpropionyl)amino]-3,5-dimethoxybenzoic acid. A solution of 2-[3-(3,4,5-trimethoxyphenylpropionyl)amino]-3,5-dimethoxybenzoic acid methyl ester (270 mg, 0.62 mmol) in H_2O (25 ml) and MeOH (25 ml) was stirred with KOH (140 mg, 2.5 mmol) for 24 h at room temperature and then quenched with 1 M HCl (3 ml). The precipitate was filtered off and was washed with H_2O (5 ml). After drying 2-[3-(3,4,5-trimethoxyphenylpropionyl)amino]-3,5-dimethoxybenzoic acid (190 mg, 73%) was collected.

2-[3-(3,4,5-Trihydroxyphenylpropionyl)amino]-3,5-dihydroxybenzoic acid **[10]**. A solution of 1M BBr_3 (6.5 ml) in CH_2Cl_2 was added dropwise to a stirred suspension of 2-[3-(3,4,5-trimethoxyphenylpropionyl)amino]-3,5-dimethoxybenzoic acid (180 mg, 0.43 mmol) in dry CH_2Cl_2 (15 ml) at −78°. After stirring for 2 h at 0°, the reaction was quenched with 1 M HCl (10 ml). The mixture was centrifuged and the residue was washed with 0.02 M HCl (2×5 ml) and H_2O (3×5 ml). After drying 2-[3-(3,4,5-trihydroxyphenylpropionyl)amino]-3,5-dihydroxybenzoic acid **[10]** was isolated (40 mg, 27%). ^{1}H-nmr spectrum (CD_3OD, 200 MHz); benzoic acid part: δ 6.56 (d, J=2.8 Hz, H-4), 6.96 (d, J=2.7 Hz, H-6); cinnamic acid part: δ 2.66 (2H), 2.77 (2H), 6.24 (s, H-2/H-6). *Anal.* found C 46.4, H 4.8, N 3.1; calcd for $C_{16}H_{15}NO_8 \times 3(H_2O)$, C 47.6, H 5.3, N. 3.5. Uv λ max (MeOH) 321 nm.

Synthesis of 2-[(3,4,5-trihydroxybenzoyl)amino]-3,5-dihydroxybenzoic acid **[11]**.—*3,4,5-Trimethoxybenzoyl chloride.* $SOCl_2$ (15 ml) was added to a solution of 3,4,5-trimethoxybenzoic acid (5.1, 21.1 mmol) in C_6H_6 (25 ml). The mixture was refluxed for 1.5 h. Removal of the excess $SOCl_2$ by azeotropic distillation followed by bulb-to-bulb distillation of the residue gave 3,4,5-trimethoxybenzoyl chloride (4.6 g, 83%).

2-[(3,4,5-Trimethoxybenzoyl)amino]-3,5-dimethoxybenzoic acid methyl ester. A solution of 2-amino-3,5-dimethoxybenzoic acid methyl ester (1.27 g, 6 mmol) in CH_2Cl_2 (10 ml) was added dropwise at 0° to a stirred solution of 3,4,5-trimethoxybenzoyl chloride (1.27 g, 5.5 mmol) in CH_2Cl_2 (15 ml) and Et_3N (758 mg, 7.5 mmol). After stirring for 48 h at room temperature, the mixture was washed with 1 M HCl (50 ml), saturated $NaHCO_3$ solution (50 ml), and saturated NaCl solution (50 ml). After drying and evaporation, 2-[(3,4,5-trimethoxybenzoyl)amino]-3,5-dimethoxybenzoic acid methyl ester (1.6 g, 79%) (mp 156–158°) was crystallized from EtOAc.

2-[(3,4,5-Trimethoxybenzoyl)amino]-3,5-dimethoxybenzoic acid. A solution of 2-[(3,4,5-trimethoxybenzoyl)amino]-3,5-dimethoxybenzoic acid methyl ester (1.22 g, 3.0 mmol) in H_2O (25 ml) and MeOH (25 ml) was stirred with KOH (674 mg, 12 mmol) for 4 h at 40° and then poured into 1 M HCl (12 ml). The precipitate was filtered off and was washed successively with H_2O (15 ml) and $CHCl_3$ (25 ml). After drying 2-[(3,4,5-trimethoxybenzoyl)amino]-3,5-dimethoxybenzoic acid (1.1 g, 95%) was collected.

2-[(3,4,5-Trihydroxybenzoyl)amino]-3,5-dihydroxybenzoic acid **[11]**. A solution of 1 M BBr_3 (8 ml, 8 mmol) in CH_2Cl_2 was added dropwise to a stirred suspension of 2-[(3,4,5-trimethoxybenzoyl)amino]-3,5-dimethoxybenzoic acid (810 mg, 2.07 mmol) in dry CH_2Cl_2 (15 ml) at −78°. After stirring for 2 h at 0°, the reaction was quenched with 4 M HCl (7.5 ml). The mixture was centrifuged and the residue was washed with 0.02 M HCl (2×5 ml) and H_2O (3×5 ml). After drying 2-[(3,4,5-trihydroxybenzoyl)amino]-3,5-dihydroxybenzoic acid **[11]** was isolated (630 mg, 95%). ^{1}H-nmr spectrum (CD_3OD, 200 MHz); benzoyl part: δ 7.07 (H-2/H-6); benzoic acid part: δ 6.63 (d, J=3.0 Hz, H-4), 7.10 (d, J=3.0 Hz, H-6). ^{13}C-nmr spectrum (CD_3OD, 50 MHz); benzoyl part: δ 108.9 (C-2/C-6), 125.2 (C-1), 139.6 (C-4), 147.4 (C-3/C-5), 169.3 (C-7); benzoic acid part: δ 111.6 (C-6), 112.1 (C-4), 122.4 (C-2), 124.0 (C-1), 153.2 (C-3), 157.3 (C-5), 171.8 (C-7). *Anal.* found C 47.8, H 3.7, N 4.1; calcd for $C_{14}H_{11}NO_8 \times 1.7(H_2O)$, C 47.8, H 4.1, N 4.0. Uv λ max (MeOH) 340, 297, 264 nm.

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