Notes

Tryptamine-Derived Amides and Alkaloids from the Seeds of *Annona atemoya*

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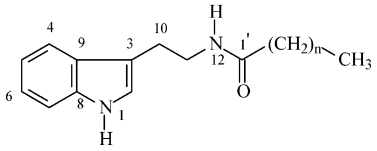
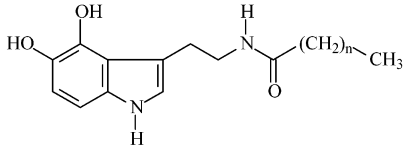
A series of *N*-fatty acyl tryptamines, including a mixture of *N*-nonadecanoyltryptamine (**1**), *N*-behenoyltryptamine (**2**), *N*-lignoceroyltryptamine (**3**), *N*-cerotoyltryptamine (**4**), and *N*-octacosanoyl tryptamine (**5**), and a mixture of *N*-tricosanoyl-4,5-dihydroxytryptamine (**6**), *N*-lignoceroyl-4,5-dihydroxytryptamine (**7**), *N*-pentacosanoyl-4,5-dihydroxytryptamine (**8**), and *N*-heptacosanoyl-4,5-dihydroxytryptamine (**9**), along with two alkaloids, atemoine (**10**) and cleistopholine, were isolated from the EtOAc extract of seeds of *Annona atemoya*. The structures of the new compounds, **1** and **5**−**10**, were determined on the basis of spectral evidence.

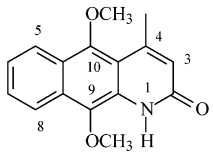
Although annonaceous acetogenins constitute a major group of the chemical constituents of Formosan Annonaceae, a large number of alkaloids have also been described.[1−3] Previously, we have isolated 17 annonaceous acetogenins, including six new compounds, 12,15-*cis*-squamostatin-D, 12,15-*cis*-squamostatin-A, artemoin-A, artemoin-B, artemoin-C, and artemoin-D, from the seeds of *A. atemoya* Hort. (*A. cherimolia* × *A. squamosa*).[4] As part of our continuing investigation on the alkaloids of this species, a series of *N*-fatty acyl tryptamines, a mixture of *N*-nonadecanoyltryptamine (**1**), *N*-behenoyltryptamine (**2**),[5,6] *N*-lignoceroyltryptamine (**3**),[5,6] *N*-cerotoyltryptamine (**4**),[5,6] and *N*-octacosanoyltryptamine (**5**), and a mixture of *N*-tricosanoyl-4,5-dihydroxytryptamine (**6**), *N*-lignoceroyl-4,5-dihydroxytryptamine (**7**), *N*-pentacosanoyl-4,5-dihydroxytryptamine (**8**), and *N*-heptacosanoyl-4,5-dihydroxytryptamine (**9**), together with two alkaloids, artemoine (**10**) and cleistopholine,[7,8] were obtained by systematic extraction and isolation from the seeds of *A. atemoya*. Besides the seven new compounds, **1** and **5**−**10**, compounds **2**−**4** and cleistopholine were isolated for the first time from this species.



Compound	n
1	17
2	20
3	22
4	24
5	26



Compound	n
6	21
7	22
8	23
9	25



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The mixture of tryptamine derivatives (**1**−**5**) was isolated as a white powder. Its UV spectrum showed the typical absorptions of an indole moiety.[9,10] The ^{1}H and ^{13}C NMR spectra of the mixture of **1**−**5** were identical to those of the mixture of tryptamine derivatives.[5,6] The structure of the tryptamine moiety was determined by the ^{1}H NMR spectrum, which contained signals at δ 7.61 (1H, dd, J = 7.8, 1.0 Hz, H-4), 7.38 (1H, dd, J = 7.8, 1.0 Hz, H-7), 7.21 (1H, ddd, J = 7.8, 7.8, 1.0 Hz, H-6), and 7.13 (1H, ddd, J = 7.8, 7.8, 1.0 Hz, H-5) from the *ortho*-substituted benzene ring moiety, δ 7.04 (1H, d, J = 2.6 Hz, H-2) ascribable to the pyrrole ring moiety, along with two methylene protons at δ 3.60 (2H, t, J = 6.5 Hz, H-11) and 2.98 (2H, t, J = 6.5 Hz, H-10). The resonances of the acid moiety included those of the amide carbonyl (δ_C 173.1), the methylenes α and β to the amide function, one terminal methyl group, and those for the remaining methylenes of the aliphatic chain (Table 1). Furthermore, an HMQC experiment permitted unequivocal assignment of all proton-bearing carbons, while an HMBC experiment allowed full NMR assignments for the molecules (Table 1). The FABMS of the mixture showed a series of five $[M + H]^+$ peaks (*m/z* 441, 483, 511, 539, and 567) and a base peak at *m/z* 143. HRFABMS of the five peaks gave molecular formulas of $C_{38}H_{66}ON_2$, $C_{36}H_{62}ON_2$, $C_{34}H_{58}ON_2$, $C_{32}H_{54}ON_2$, and $C_{29}H_{48}ON_2$, respectively. A prominent peak at *m/z* 143 (base peak) in the FABMS and EIMS further confirmed the tryptamine moiety. This peak, attributable to a vinylindole fragment, is generated from tryptamine through a McLafferty rearrangement.[9] The NMR and mass spectra clearly indicated that compounds **1**−**5** each contained a tryptamine unit coupled with a different alkyl chain. Altogether, the individual components of this mixture were identified as *N*-nonadecanoyltryptamine (**1**), *N*-behenoyltryptamine (**2**),

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Table 1. ^{1}H (400 MHz, *J* in Hz) and ^{13}C NMR (100 MHz) Spectral Data of the Mixture of Tryptamine Derivatives **1**−**5**[a] in $CDCl_3$

position	δ_H (mult., *J* Hz)	δ_C (mult.)	HMBC[b]
1	8.03 (br s)		
2	7.04 (d, 2.6)	122.3 (d)	C-3, C-9, C-8
3		113.3 (s)	
4	7.61 (dd, 7.8, 1.0)	118.8 (d)	C-8, C-6
5	7.13 (ddd, 7.8, 7.8, 1.0)	119.6 (d)	C-9
6	7.21 (ddd, 7.8, 7.8, 1.0)	122.3 (d)	C-8, C-4
7	7.38 (dd, 7.8, 1.0)	111.2 (d)	C-9, C-5
8		136.7 (s)	
9		127.4 (s)	
10	2.98 (t, 6.5)	25.4 (t)	C-2, C-3, C-9, C-11
11	3.61 (t, 6.5)	39.6 (t)	C-3, C-10, C-1′, C-2′
12	5.45 (br s)		
1′		173.1 (s)	
2′	2.09 (t, 7.6)	36.9 (t)	C-1′, C-3′
3′	1.57 (t, 7.6)	25.7 (t)	
aliphatic CH_2 of fatty acids	1.25 (br s)	29.8 (t)−29.4 (t)	
terminal CH_3 of fatty acids	0.88 (t, 6.5)	14.1 (q)	

[a] Assigned by COSY, HMQC, and HMBC spectra. [b] Important long-range ^{1}H−^{13}C correlations.

N-lignoceroyltryptamine (**3**), *N*-cerotoyltryptamine (**4**), and *N*-octacosanoyltryptamine (**5**). A mixture of tryptamine derivatives **2**−**4** was isolated from both *Annona reticulata* and *Rollinia mucosa* (Annonaceae).[5,6] Compounds **1** and **5** are new compounds.

The mixture of 4,5-dihydroxytryptamine derivatives (**6**−**9**) was isolated as a white powder. The UV spectrum also showed identical absorptions to indole derivatives. On comparison with the fragmentation of compounds **1**−**5**, a base peak appearing at *m/z* 175 in the FABMS indicated the presence of a dihydroxytryptamine moiety. The structure of the dihydroxytryptamine moiety was suggested by the ^{1}H NMR spectrum, which exhibited *ortho*-coupling signals at δ 7.13 (1H, d, J = 8.4 Hz) and 6.69 (1H, d, J = 8.4 Hz) on the benzene ring, 6.91 (1H, s, H-2), and two typical methylene protons at δ 3.43 (2H, t, J = 6.8 Hz, H-11) and 2.81 (2H, t, J = 6.8 Hz, H-10). The resonances of the acid moieties included those of the amide carbonyl (δ_C 172.1), the methylenes α and β to the amide function, one terminal methyl group, and those for the remaining methylenes of the aliphatic chain (Table 2). An HMQC experiment permitted unequivocal assignment of all proton-bearing carbons, while a HMBC experiment allowed full NMR assignments of the mixture (Table 2). Two hydroxy groups were substituted at C-4 and C-5. Finally, the FABMS of the mixture showed a series of four $[M + H]^+$ peaks (*m/z* 529, 543, 557, and 585). HRFABMS of the four peaks gave molecular formulas of $C_{37}H_{64}O_3N_2$, $C_{35}H_{60}O_3N_2$, $C_{34}H_{58}O_3N_2$, and $C_{33}H_{56}O_3N_2$, respectively. As described above, the NMR and mass spectra clearly indicated that compounds **6**−**9** each contained a 4,5-dihydroxytryptamine unit coupled with a different alkyl chain. Thus, these individual components of the mixture were determined to be *N*-tricosanoyl-4,5-dihydroxytryptamine (**6**), *N*-lignoceroyl-4,5-dihydroxytryptamine (**7**), *N*-pentacosanoyl-4,5-dihydroxytryptamine (**8**), and *N*-heptacosanoyl-4,5-dihydroxytryptamine (**9**). This is the first example of naturally occurring *N*-fatty acyl tryptamines possessing a 4,5-dihydroxytryptamine moiety.[5,6]

Artemoine (**10**) was obtained as yellow amorphous powder with mp 212−214 °C. The HREIMS gave a molecular ion at *m/z* 269.1071 (calcd 269.1052), appropriate for a molecular formula of $C_{16}H_{15}O_3N$. The UV spectrum showed absorption bands at λ 248, 255, and 320 nm, which were typical of a linear benzoquinolin-2-one system. An IR absorption band at 1685 cm^{-1} and a signal appearing at δ 161.7 in the ^{13}C NMR spectrum suggested that a carbonyl group was present. In the ^{1}H and ^{13}C NMR spectrum ($CDCl_3$), the presence of an *ortho*-disubstituted aromatic function was observed as a typical ABCD system [δ_H/δ_C 8.18 (dd, J = 7.7, 1.2 Hz, H-5)/123.5 (C-5), 8.07 (dd, J = 7.7, 1.2 Hz, H-8)/124.7 (C-8), 7.57 (ddd, J = 7.7, 7.7, 1.2 Hz, H-7)/123.0 (C-7), 7.47 (ddd, J = 7.7, 7.7, 1.2 Hz, H-6)/121.1 (C-6)] and the signals for the lactam portion [δ_H/δ_C 6.46 (s, H-3)/127.9 (C-3)] of the quinoline system. The ^{1}H NMR spectrum indicated the presence of two methoxyl groups at δ 3.99 and 3.98 and one methyl group at δ 2.79. A NOESY correlation was observed between the methyl group and H-3, as well as between the methyl group and the methoxyl group at δ 3.98. The methyl group was allocated to C-4, and the methoxyl signals at δ 3.99 and 3.98 were ascribable to OMe-9 and OMe-10, respectively. In the ^{13}C NMR spectrum, 12 aromatic carbon atoms between δ 152.3 and 121.1, two methoxy carbons at δ 64.1 and 61.9, one signal for a methyl carbon at δ 23.3, and one carbonyl carbon at δ 161.7 were consistent with the structure of **10**. The complete structural elucidation of **10** was confirmed by HMBC and HMQC experiments. On the basis of the above results, compound **10** was characterized as a new alkaloid, 9,10-dimethoxy-4-methylhydrobenzo[*g*]-quinolin-2-one, which we have named artemoine.

Table 2. ^{1}H (400 MHz, *J* in Hz) and ^{13}C NMR (100 MHz) Spectral Data of the Mixture of 4,5-Dihydroxytryptamine Derivatives **6**−**9**[a] in $CDCl_3$

position	δ_H (mult., *J* Hz)	δ_C (mult.)	HMBC[b]
2	6.91 (br s)	127.9 (d)	C-3, C-9, C-8
3		111.3 (s)	
4		150.3 (s)	C-8, C-6
5		146.3 (s)	C-9
6	6.69 (d, 8.4)	112.4 (d)	C-8, C-4
7	7.13 (d, 8.4)	111.7 (d)	C-9, C-5
8		130.9 (s)	
9		123.1 (s)	
10	2.81 (t, 6.8)	25.6 (t)	C-2, C-3, C-9, C-11
11	3.43 (t, 6.8)	39.9 (t)	C-3, C-10, C-1′
1′		172.1 (s)	
2′	2.04 (t, 7.6)	37.6 (t)	C-1′, C-3′
3′	1.48 (t, 7.6)	25.6 (t)	
aliphatic CH_2 of fatty acids	1.22 (br s)	29.1 (t)−28.7 (t)	
terminal CH_3 of fatty acids	0.80 (t, 6.5)	14.1 (q)	

[a] Assigned by COSY, HMQC, and HMBC spectra. [b] Important long-range ^{1}H−^{13}C correlations.

The identity of a known alkaloid was verified by comparison with UV, IR, ^{1}H NMR, ^{13}C NMR, and MS data with published values of cleistopholine.[7,8]

Experimental Section

General Experimental Procedures. Melting points were determined on a Laboratory Devices Mel-Temp II and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra (in EtOH) were obtained on a Hitachi 220-20 spectrophotometer. IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H NMR (400 and 200 MHz), ^{13}C NMR, NOESY, and DEPT spectra were obtained on a Varian NMR spectrometer. EIMS and FABMS were recorded on a JEOL JMS-SX/SX 102A mass spectrometer or Quattro GC-MS spectrometer having a direct inlet system. HREIMS and HRFABMS were measured on a JEOL JMS-HX 110 mass spectrometer. Si gel 60 (Merck, 230−

400 mesh) was used for column chromatography, precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC, and precoated Si gel plates (Merck, Kieselgel 60 F-254, 0.50 mm) were used for preparative TLC. Spots were detected by spraying with Dragendorff's reagent or 50% H_2SO_4 and then heating on a hot plate.

Plant Material. Seeds of *A. atemoya* were collected in Chia-Yi City, Taiwan, in June 1994. A voucher specimen is deposited in the Graduate Institute of Natural Products (voucher number: Annona 14), Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. The fresh seeds of *A. atemoya* (3.0 kg) were extracted repeatedly with EtOAc (10 L × 6) at room temperature for 24−48 h. The combined EtOAc extracts were evaporated under reduced pressure to yield brown syrup (ca. 180 g, wet weight). The syrup was partitioned between $CHCl_3$ and water. The free bases in the $CHCl_3$ solution were extracted with 3% HCl to leave the acidic portion and $CHCl_3$ solution (part A). The acidic portion was basified with NH_4-OH and extracted with $CHCl_3$. The $CHCl_3$ solution was dried and evaporated to leave a brownish viscous residue (part B, 2.0 g). Part B was placed on a silica gel column using gradient mixtures of *n*-hexane−EtOAc−MeOH to afford 20 fractions. Fraction 3 (0.3 g), eluted with *n*-hexane−Me_2CO (10:1), was further separated using silica gel column chromatography and preparative TLC (hexane−Me_2CO (14:1)) to yield the mixture of tryptamine derivatives (**1**−**5**) (12 mg). Fraction 6 (0.4 g), eluted with $CHCl_3$−MeOH (10:1), was further separated using silica gel column chromatography and prepartive TLC ($CHCl_3$−MeOH (15:1)) to obtain 4,5-dihydroxytryptamine derivatives (**6**−**9**) (9 mg). Fraction 11 (158 mg), eluted with $CHCl_3$−MeOH (9:1), was further separated using silica gel column chromatography ($CHCl_3$−MeOH (15:1)) to afford artemoine (**10**) (5 mg). Fraction 13 (55.0 mg), eluted with $CHCl_3$−MeOH (8:1), was further separated using silica gel column chromatography ($CHCl_3$−MeOH, 10:1) and gave cleistopholine (3 mg).

Mixture of tryptamine derivatives 1−5: white amorphous powder; UV (MeOH) λ_{max} (log ϵ) 205 (3.70), 221 (3.75), 280 (3.05) nm; IR (KBr) ν_{max} 3390, 1675 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; FABMS *m/z* $[M + H]^+$ 567 (8), 539 (6), 511 (6), 483 (5), 441 (5); EIMS *m/z* 341 (15), 313 (23), 215 (5), 187 (9), 144 (24), 143 (100), 130 (20); HRFABMS *m/z* $[M + H]^+$ 567.5217 ($C_{38}H_{67}ON_2$, calcd 567.5253), $[M + H]^+$ 539.4952 ($C_{36}H_{63}ON_2$, calcd 539.4940), $[M + H]^+$ 511.4615 ($C_{34}H_{59}ON_2$, calcd 511.4627), $[M + H]^+$ 483.4321 ($C_{32}H_{55}ON_2$, calcd 483.4314), $[M + H]^+$ 441.3867 ($C_{29}H_{49}ON_2$, calcd 441.3845).

Mixture of 4,5-dihydroxytryptamine derivatives 6−9: white amorphous powder; UV (MeOH) λ_{max} (log ϵ) 206 (3.65), 220 (3.78), 283 (3.10) nm; IR (KBr) ν_{max} 3400, 1675 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; FABMS *m/z* $[M + H]^+$ 585 (4), 557 (5), 543 (3), 529 (4); EIMS *m/z* 247 (4), 219 (8), 176 (21), 175 (100), 162 (23); HRFABMS *m/z* $[M + H]^+$ 585.5013 ($C_{37}H_{65}O_3N_2$, calcd 585.4995), $[M + H]^+$ 557.4677 ($C_{35}H_{61}O_3N_2$, calcd 557.4682), $[M + H]^+$ 543.4534 ($C_{34}H_{59}O_3N_2$, calcd 543.4526), $[M + H]^+$ 529.4378 ($C_{33}H_{57}O_3N_2$, calcd 529.4369).

Artemoine (10): brown amorphous powder; mp 230−233 °C; UV (EtOH) λ_{max} (log ϵ) 248 (4.34), 255 (3.76), 320 (3.84) nm; IR (KBr) ν_{max} 3500 (NH), 1685 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.08 (1H, br s, NH), 8.18 (1H, dd, J = 7.7, 1.2 Hz, H-5), 8.07 (1H, dd, J = 7.7, 1.2 Hz, H-8), 7.57 (1H, ddd, J = 7.7, 7.7, 1.2 Hz, H-7), 7.47 (1H, ddd, J = 7.7, 7.7, 1.2 Hz, H-6), 6.46 (1H, s, H-3), 3.99 (3H, s, OMe-9), 3.98 (3H, s, OMe-10), 2.79 (3H, s, Me-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7 (C-2), 152.3 (C-4), 151.7 (C-9), 148.8 (C-10), 135.8 (C-9a), 133.7 (C-8a), 127.9 (C-3), 127.5 (C-10a), 124.7 (C-8), 123.5 (C-5), 123.0 (C-7), 122.7 (C-4a), 121.1 (C-6), 64.1 (OMe-9), 61.9 (OMe-10), 23.3 (Me-4); EIMS (70 eV) *m/z* 269 ($[M]^+$, 45), 254 (100), 239 (50), 224 (13), 210 (35), 195 (5); HREIMS *m/z* $[M]^+$ 269.1071 ($C_{16}H_{15}O_3N$, calcd 269.1052).

Cleistopholine (11): yellow powder; mp 186−188 °C; UV (EtOH) λ_{max} (log ϵ) 250 (4.53), 265 (4.02), 323 (3.76) nm; MS and ^1H and ^{13}C NMR data were identical with published data.[6,7]

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References and Notes

(1) Chen, C. Y.; Chang, F. R.; Yen, H. F.; Wu, Y. C. *Phytochemistry* **1998**, *49*, 1443−1447.
(2) Chen, C. Y.; Chang, F. R.; Wu, Y. C. *J. Chin. Chem. Soc.* **1997**, *44*, 313−319.
(3) Chen, C. Y.; Chang, F. R.; Teng, C. M.; Wu, Y. C. *J. Chin. Chem. Soc.* **1999**, *46*, 77−86.
(4) Chang, F. R.; Chen, J. L.; Lin, C. Y.; Chiu, H. F.; Wu, M. J.; Wu, Y. C. *Phytochemistry* **1999**, *51*, 883−889.
(5) Maeda, U.; Hara, N.; Fujimoto, Y.; Srivastava, A.; Gupta, Y. K.; Sahai, M. *Phytochemistry* **1993**, *34*, 1633−1635.
(6) Chavez, D.; Acevedo, L. A.; Mata, R. *J. Nat. Prod.* **1999**, *62*, 1119−1122.
(7) Waterman, P. G.; Muhammad, I. *Phytochemistry* **1985**, *24*, 523−527.
(8) Tadic, D.; Cassels, B. K.; Leboeuf, M.; Cavé, A. *Phytochemistry* **1987**, *26*, 537−541.
(9) Riemer, B.; Hofer, O.; Greger, H. *Phytochemistry* **1997**, *45*, 337−341.
(10) Folstar, P.; Schols, H. A.; van der Plas, H. C.; Pilnik, W.; Landheer, C. A.; van Veldhuizen, A. *J. Agric. Food Chem.* **1980**, *28*, 872−874.

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