Annosqualine: a Novel Alkaloid from the Stems of *Annona squamosa*

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Annosqualine (=(10′b*R*)-1′,5′,6′,10′b-tetrahydro-9′-hydroxy-7′,8′-dimethoxyspiro[cyclohexa-2,5-diene-1,2′-pyrrolo[2,1-*a*]isoquinoline]-3′,4-dione; **1**), a novel alkaloid with an unprecedented skeleton, and a new amide, dihydrosinapoyltyramine (=3-(4-hydroxy-3,5-dimethoxyphenyl)-*N*-[2-(4-hydroxyphenyl)ethyl]propanamide; **2**), were isolated from the stems of *Annona squamosa* L., together with six known alkaloids. The structures of all compounds were elucidated spectroscopically by means of optical rotation, ^{1}H-, ^{13}C-, and 2D-NMR, and by EI-MS, or by comparison with the spectral data of authentic samples. A possible biogenetical pathway towards annosqualine (**1**) is proposed.

Introduction. – We have isolated a number of N-containing natural products from Annonaceous plants, *e.g.*, benzylisoquinolines, acyl amides, azafluorenes, terpene alkaloids, and cyclic peptides, some of them showing interesting biological activities [1–3]. Recently, we reported the total synthesis of samoquasine A and the structure elucidation of perlolidine from the stems of *Annona squamosa* L. [4]. Our continuous phytochemical research has now led to the isolation of two new compounds, annosqualine (**1**) and dihydrosinapoyltyramine (**2**), together with six known alkaloids, dihydroferuloyltyramine (**3**) [5], demethylsonodione (**4**) [6], liriodenine (**5**) [7], annobraine (**6**) [8], thalifoline (**7**) [9], and squamolone (**8**) [10], all isolated from the stems of *Annona squamosa* L. The characterization and structure elucidation of **1** and **2** are reported herein, including a hypothetic biogenetical pathway for **1**.

Results and Discussion. – Compound **1**, obtained as a syrup, had the molecular formula $C_{19}H_{19}NO_5$ based on analysis of HR-FAB-MS data. The IR absorptions at 1677 and 1654 cm^{-1} indicated the presence of two C=O groups. In the ^{1}H-NMR spectrum of **1** (see *Fig. 1* and *Table 1*), the seven resonances at δ(H) 5.07 (*dd*, J = 9.2, 6.8 Hz, 1 H), 4.18 (*ddd*, J = 13.2, 6.2, 2.6 Hz, 1 H), 3.16 (*ddd*, J = 13.2, 11.4, 4.8 Hz, 1 H), 2.90 (*ddd*, J = 16.4, 4.8, 2.6 Hz, 1 H), 2.82 (*dd*, J = 12.8, 6.8 Hz, 1 H), 2.70 (*dddd*, J = 16.4, 11.4, 6.2, 0.8 Hz, 1 H), and 2.30 (*dd*, J = 12.8, 9.2 Hz, 1 H) were similar to those assigned to the rings *A*, *B*, and *C* of promucosine (**9**), an *N*-(methoxycarbonyl)proaporphine [11]. The four promucosine resonances at δ(H) 7.23, 6.38, 6.40, and 6.88 (4*dd*, J = 10.3 and 2.5 Hz each, 4 × 1 H) are typical for the cyclohexa-2,3,5,6-dien-1-one moiety. However, the ^{1}H-NMR chemical shifts of this moiety in **1** were significantly different, the *AA′* and *BB′* signals of **1** being shifted upfield and downfield, respectively, relative to those of **9** (see *Fig. 1*). In the ^{13}C-NMR spectra, the carbamate type *N*-methoxycarbonyl C=O resonance of **9** at δ(C) 155.4 was replaced by an amide C=O resonance at δ(C) 170.2 (C(8)) in **1**.

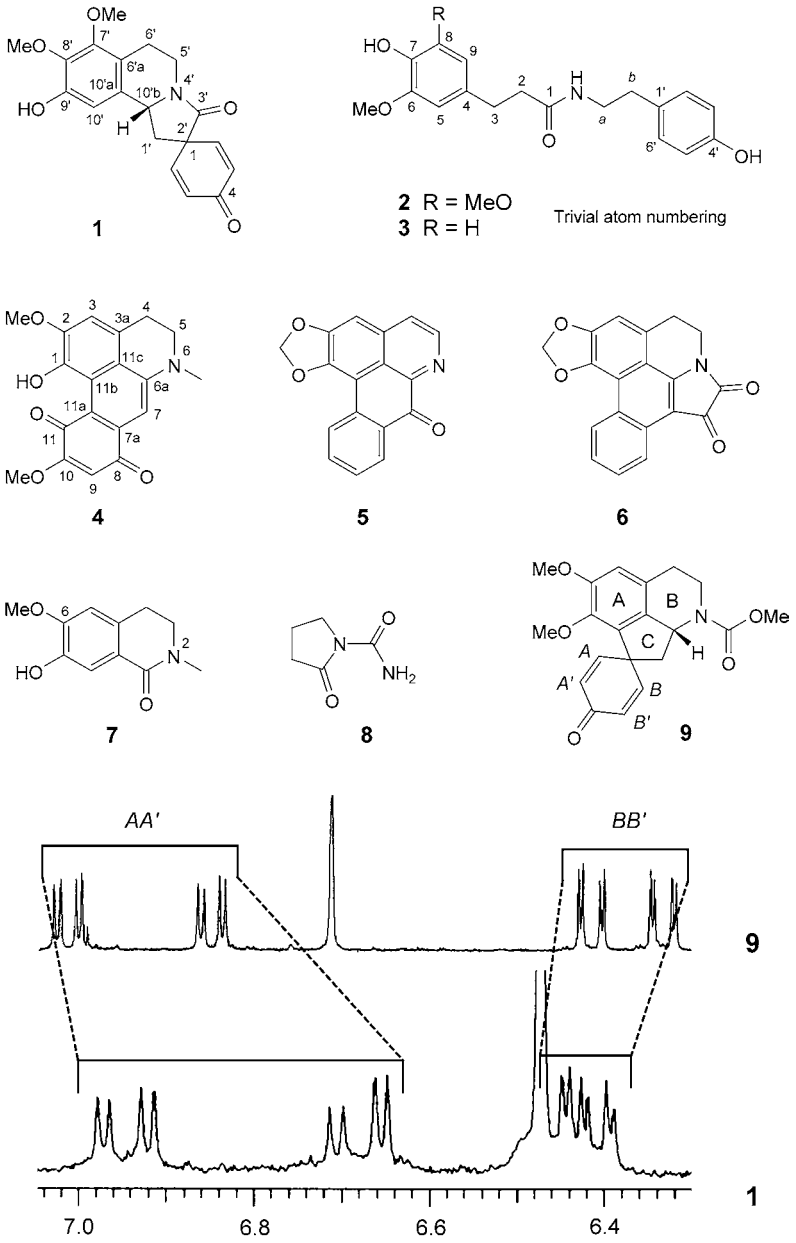


Fig. 1. *Section of the ^{1}H-NMR spectra of the cyclohexadienone moieties of annosqualine* (**1**; lower spectrum) *and promucosine* (**9**; upper spectrum). Solvent: $CDCl_3$.

The ^{1}H,^{1}H-COSY and HMBC spectra (*Fig. 2*) of **1** revealed a pyrrolidin-2-one ring. Sets of ^3J correlations between H–C(2) to C(3′) and C(1′), and between H–C(6) to C(3′) and C(1′) suggested that the cyclohexadienone moiety was spiro-fused at C(2′).

Table 1. *NMR Spectral Data of* **1**. At 400 (^{1}H) and 125 (^{13}C) MHz, in CD_3OD; δ in ppm, J in Hz.

Position	^{1}H	^{13}C
H−C(2)	7.23 (*dd*, *J* = 10.3, 2.5)	147.7
H−C(3)	6.38 (*dd*, *J* = 10.3, 2.5)	130.5
C(4)		187.7
H−C(5)	6.40 (*dd*, *J* = 10.3, 2.5)	131.9
H−C(6)	6.88 (*dd*, *J* = 10.3, 2.5)	151.2
H_α−C(1′)	2.30 (*dd*, *J* = 12.8, 9.2)	40.4
H_β−C(1′)	2.82 (*dd*, *J* = 12.8, 6.8)	
C(2′)		55.4
C(3′)		170.2
H_α−C(5′)	4.18 (*ddd*, *J* = 13.2, 6.2, 2.6)	39.3
H_β−C(5′)	3.16 (*ddd*, *J* = 13.2, 11.4, 4.8)	
H_α−C(6′)	2.70 (*dddd*, *J* = 16.4, 11.4, 6.2, 0.8)	23.2
H_β−C(6′)	2.90 (*ddd*, *J* = 16.4, 4.8, 2.6)	
C(6′a)		119.3
C(7′)		152.4
C(8′)		141.3
C(9′)		151.4
H−C(10′)	6.48 (*d*, *J* = 0.8 Hz)	108.6
C(10′a)		133.5
H−C(10′b)	5.07 (*dd*, *J* = 9.2, 6.8)	55.0
8′-MeO	3.85 (*s*)	60.9
7′-MeO	3.82 (*s*)	61.0
OH	4.61 (*s*)	

The other HMBC correlations indicated a 9′-OH group and two MeO groups at C(8′) and C(7′), respectively, as further supported by NOE correlations (*Fig. 3*) between H−C(10′) and H−C(10′b), and between H−C(10′) and H−C(1′). No correlation between the MeO groups and H−C(10′) was observed.

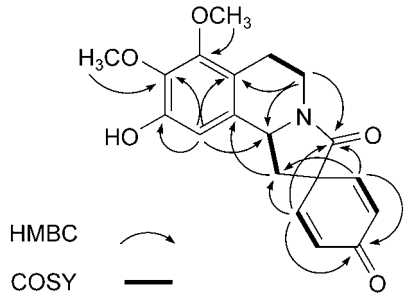


Fig. 2. *Key HMBC and ^{1}H,^{1}H-COSY correlations of* **1**

The observed EI-MS fragments further support the proposed skeleton of **1** (*Fig. 4*). From the positive optical-rotation value, the absolute configuration at C(10′b) was inferred as (*R*) [12]. This was further confirmed by NOE correlations for H−C(10′b)/H_β−C(1′)/H−C(2) and H_α−C(1′)/H−C(6) (*Fig. 3*). Thus, from the above data, the structure of annosqualine (**1**) was deduced as (10′b*R*)-1′,5′,6′,10′b-tetrahydro-9′-

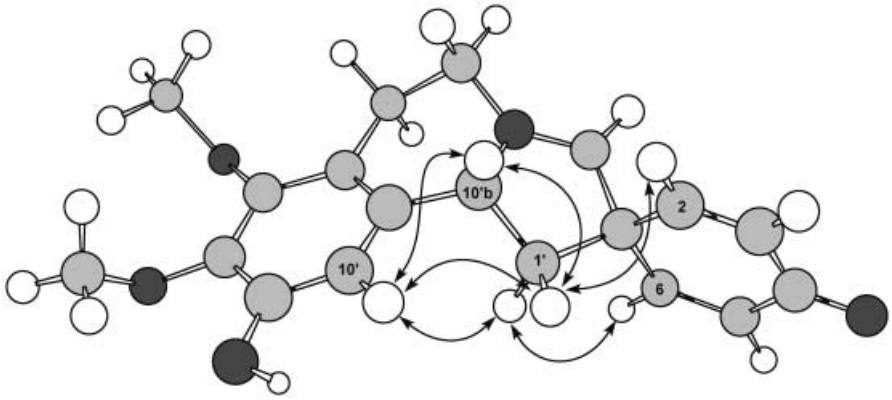


Fig. 3. *Molecular model of* **1** *with key NOESY correlations.* H-Atoms, white; C-atoms, grey; O- and N-atoms, dark.

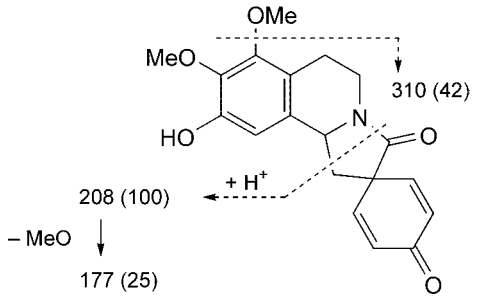


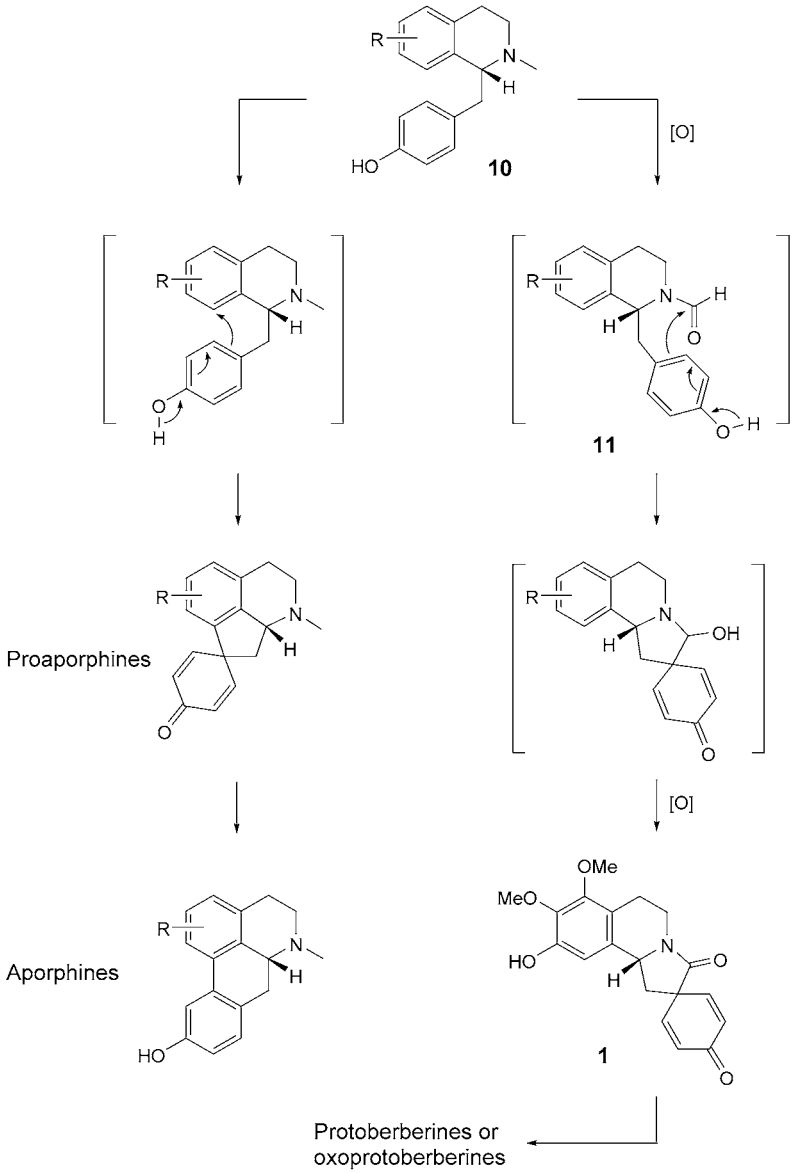
Fig. 4. *EI-MS Fragments of* **1** (*m*/*z* (rel. %))

hydroxy-7′,8′-dimethoxyspiro[cyclohexa-2,5-diene-1,2′-pyrrolo[2,1-*a*]isoquinoline]-3′,4-dione.

A possible biogenetic pathway to annosqualine (**1**) is proposed in the *Scheme* below, (1*R*)-1,2,3,4-tetrahydro-1-(4-hydroxyphenyl)methyl]-2-methylisoquinoline (**10**) acting as a potential precursor. Theoretically, **10** can be oxidized to the corresponding *N*-formyl compound **11**, followed by cyclization and oxidation to **1**. Similar to proaporphines, hypothetical intermediates in the biogenesis of aporphines, **1** was surmised to be transformed to protoberberines or oxoprotoberberines. Although a number of studies on the biogenesis of protoberberines (*e.g.*, the mechanism of berberine bridge enzyme [13]) have been proposed, **1** still gave us some imaginable space in developing isoquinoline alkaloids. The biogenesis of **1** is similar to the classic preparation of tetrahydroprotoberberines through *Mannich* condensation [14].

Two amides, dihydrosinapoyltyramine (**2**) and dihydroferuloyltyramine (**3**) were also isolated. The structure of the known compound **3** was confirmed by comparing its physical and spectroscopic data with those of an authentic sample [5]. Compound **2**, obtained as a wax, had the molecular formula $C_{19}H_{23}NO_5$ based on HR-FAB-MS. The

Scheme. *Possible Biogenetic Pathway to* **1**



^{1}H-NMR spectrum of **2** (*Table 2*) showed two pairs of ethylene moieties [δ(H) 2.40 and 2.85 (2*t*, $J = 7.2$ Hz each, 2 H each), δ(H) 2.64 and 3.42 (*t* and *q*, resp., $J = 6.8$ Hz each, 2 H each)], two MeO groups at δ(H) 3.83 (2*s*), an NH resonance (δ(H) 5.41) (*t*, $J =$ 6.8 Hz, 1 H)), a set of aromatic *AA′BB′* resonances (δ(H) 6.75 and 6.89 (*d*, $J = 8.4$ Hz each)), and an additional aromatic signal (δ(H) 6.39 (*s*, 2 H)).

Table 2. *NMR Spectral Data of* **2** and **3** . At 200 (^{1}H) and 50 (^{13}C) MHz, in $CDCl_3$; δ in ppm, J in Hz. Trivial atom numbering (see chemical formulae).

	2		**3**
	^{1}H	^{13}C	^{1}H
C(1)		172.3	
CH_2(2)	2.40 ($t, J = 7.2$)	38.9	2.39 ($t, J = 7.4$)
CH_2(3)	2.85 ($t, J = 7.2$)	31.9	2.86 ($t, J = 7.4$)
C(4)		131.9	
H–C(5)	6.39 (s)	104.9	6.70 ($d, J = 2.0$)
C(6)		146.9	
C(7)		131.8	
C(8)		146.9	6.82 ($d, J = 8.2$)
H–C(9)	6.39 (s)	104.9	6.65 ($dd, J = 8.2, 2.0$)
C_α	3.42 ($q, J = 6.8$)	40.8	3.42 ($q, J = 6.8$)
C_β	2.64 ($t, J = 6.8$)	34.6	2.65 ($t, J = 6.8$)
C(1′)		131.8	
H–C(2′)	6.89 ($d, J = 8.4$)	129.6	6.93 ($d, J = 8.8$)
H–C(3′)	6.75 ($d, J = 8.4$)	115.5	6.73 ($d, J = 8.8$)
C(4′)		154.8	
H–C(5′)	6.75 ($d, J = 8.4$)	115.5	6.73 ($d, J = 8.8$)
H–C(6′)	6.89 ($d, J = 8.4$)	129.6	6.93 ($d, J = 8.8$)
NH	5.41 ($t, J = 6.8$)		5.28 (br.)
6-MeO	3.83 (s)	56.2	
8-MeO	3.83 (s)	56.2	3.86

The ^{13}C-NMR spectrum of **2** (*Table 2*) revealed the presence of four CH_2 groups (δ(C) 31.9, 34.6, 38.9, 40.8), two isochronic MeO groups (δ(C) 56.2), two aromatic rings, and one amide C=O group (δ(C) 172.3). These data were similar to those of dihydroferuloyltyramine (**3**) [15]. According to the NOESY spectrum of **2** (*Fig. 5*), the compound consisted of 2,3-dihydrosinapic acid and tyramine moieties, as further confirmed by EI-MS (*Fig. 5*). Thus, the structure of **2** was determined as dihydrosinapoyltyramine (= 3-(4-hydroxy-3,5-dimethoxyphenyl)-*N*-[2-(4-hydroxyphenyl)-ethyl]propanamide).

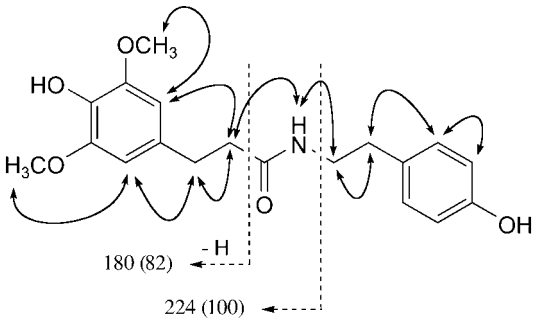


Fig. 5. *NOESY Correlations* (double arrows) *and EI-MS fragments* (dashed lines; m/z (rel. %)) of **2**

Compound **4** has been isolated from *Hernandia Sonora* [5], *H. nymphaeifolia* [16], and *Aristolochia manshuriensis* [17]. Because of the absence of detailed NMR data in

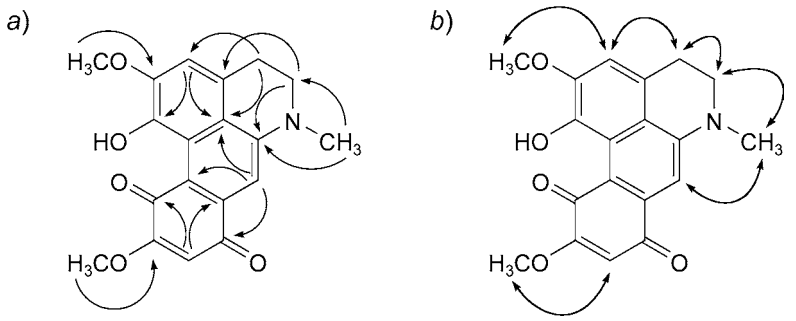


Fig. 6. a) *Key HMBC correlations and* b) *NOESY correlations of* **4**

Table 3. *NMR Spectral Data of* **4**. At 400 (^{1}H) and 100 (^{13}C) MHz, in (D_5)pyridine; δ in ppm, J in Hz.

	^{1}H	^{13}C
C(1)		143.3
C(2)		150.7
H–C(3)	7.07 (*s*)	112.9
C(3a)		125.5
C(3b)		117.6
CH_2(4)	2.97 (t, J = 6.8)	28.4
CH_2(5)	3.44 (t, J = 6.8)	50.2
C(6a)		153.2
H–C(7)	7.26 (*s*)	102.4
C(7a)		139.6
C(8)		186.1
H–C(9)	6.20 (*s*)	105.4
C(10)		164.7
C(11)		178.1
C(11a)		113.9
C(11b)		116.2
MeN	3.07 (*s*)	39.9
2-MeO	3.94 (*s*)	56.4
10-MeO	3.76 (*s*)	56.5
1-OH	11.92 (*s*)	

the literature, we reported here its ^{13}C-NMR data (*Table 3*) assigned on the basis of HMQC, HMBC, and NOESY spectra (*Fig. 6*).

The structures of compounds **5**–**8** [7–10] were confirmed by comparing their physical and spectroscopic data with those of authentic samples. Compounds **3**, **4**, **6**, and **7** were obtained for the first time from *A. squamosa*.

We would like to thank Prof. *M. Niwa*, Faculty of Pharmacy, Meijo University, Japan, for HR-FAB-MS analyses, and the *National Science Council of the Republic of China* for financial support.

Experimental Part

General. Column chromatography (CC): silica gel *60* (230–400 mesh; *Merck*). TLC spots were detected by spraying with 50% aq. H_2SO_4, followed by heating on a hot plate. HPLC: *JASCO PU-1580*, *LG-1580-04*, *DG-1580-54*, and *UV*-1575 system, with *Develosil ODS-10* and *Develosil C30-UG-5* (250 × 20 mm) prepacked

columns. Optical rotations: *JASCO P-1020* digital polarimeter. IR Spectra: *Mattson Genesis II* spectrophotometer; in cm^{-1}. ^1H-NMR, ^{13}C-NMR, $^1H,^1H$ COSY, HMBC, HMQC and NOESY Spectra: *Varian Gemini 200*, *Unity Plus 400*, and *Unity INOVA-500* spectrometers; δ in ppm, J in Hz. EI-MS: *Jeol JMS-SX/SX 102A* or *Quattro* GC/MS spectrometers; in m/z (rel. %). HR-FAB-MS: *Finnigan/Thermo Quest MAT 95XL* spectrometer.

Plant Material. Fresh stems of *A. squamosa* were collected from Pingtung, Taiwan, in May 2000, and identified by Dr. *Hsin-Fu Yen* (National Museum of Natural Science, Taichung, Taiwan). A voucher specimen (*Annona 6*) was deposited at the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. Fresh stems of *A. squamosa* (15 kg) were extracted repeatedly with MeOH at r.t. The combined MeOH extracts were evaporated under reduced pressure to yield a dark-brown syrup (550 g), which was partitioned between $CHCl_3$ and H_2O. Before being evaporated, the $CHCl_3$ layer was extracted with 3% aq. HCl soln. (acidic layer; see below) to leave a brownish viscous residue (160 g), which was subjected to CC (SiO_2; $CHCl_3$/MeOH mixtures of increasing polarity): 22 fractions (Fr.) on the basis of TLC. *Fr. 17* was further purified by CC (SiO_2; $CHCl_3/CH_3OH$ 5:1) to yield **6** (2 mg). *Fr. 20* (eluted with $CHCl_3$/MeOH 4:1) was purified by RP-HPLC (*Develosil ODS-10* and *Develosil C30-UG*-5; MeNC/H_2O 1:4 → 4:21) to give **1** (1.9 mg), **3** (5 mg), **2** (8 mg), and **7** (10 mg). The acidic layer from the above $CHCl_3$ extract was adjusted to pH 9 with NH_4OH and then extracted with $CHCl_3$ to give a crude alkaloid mixture (2 g), which was purified by CC (SiO_2; $CHCl_3$/MeOH mixtures of increasing polarity): five fractions. The first (eluted with $CHCl_3$) was purified repeatedly by CC (SiO_2) to afford **5** (8 mg) and **8** (9 mg).

The remaining aqueous extract (from the NH_4OH treatment) was subjected to HPLC (*Diaion HP-20* column; H_2O/MeOH mixtures of increasing polarity) to give five fractions. The third (eluted with H_2O/MeOH 1:1) was partitioned between $CHCl_3$ and H_2O. The org. layer was subjected to RP-HPLC (*Develosil ODS-10*; MeCN/H_2O 2:3) to give **4** (10 mg).

*(10′b*R*)-1′,5′,6′,10′b-Tetrahydro-9′-hydroxy-7′,8′-dimethoxyspiro[cyclohexa-2,5-diene-1,2′-pyrrolo[2,1-*a*]isoquinoline]-3′,4-dione* (= *Annosqualine*; **1**). Syrup. $[\alpha]_D^{27} = +56.03$ ($c = 0.19$, MeOH). IR: 3408, 1677, 1654, 1453, 1414, 1356. EI-MS: 341 (14, M^+), 340 (67, $[M-1]^+$), 325 (18, $[M-CH_4]^+$), 310 (42, $[M-MeO]^+$), 253 (30), 225 (22), 208 (100), 177 (25). ^1H- (400 MHz) and ^{13}C-NMR (125 MHz): see *Table 1*. HR-FAB-MS: 342.1349 ($[M+1]^+$, $C_{19}H_{20}NO_5^+$; calc. 342.1341).

3-(4-Hydroxy-3,5-dimethoxyphenyl)-N-*[2-(4-hydroxyphenyl)ethyl]propanamide* (*Dihydrosinapoyltyramine*; **2**). Wax. IR: 3356, 2937, 1641, 1613, 1516, 1460, 1217, 1114. ^1H- (200 MHz) and ^{13}C-NMR (50 MHz): see *Table 2*. EI-MS: 345 (7, M^+), 344 (23, $[M-1]^+$), 224 (100), 180 (82). HR-FAB-MS: 346.1660 ($[M+1]^+$, $C_{19}H_{24}NO_5^+$; calc. 346.1654).

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