Samoquasine A, a Benzoquinazoline Alkaloid from the Seeds of *Annona squamosa*

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Received July 10, 2000

A benzooxyquinazoline alkaloid, samoquasine A (**1**), has been isolated from the seeds of *Annona squamosa*, and its structure was elucidated by spectroscopic and chemical methods.

Annona squamosa L. (Annonaceae), commonly known as custard apple is a tropical fruit tree distributed mainly in the Americas and in southeast Asia. The seeds are well-known to contain both many acetogenins with antifeedant, antimalarial, cytotoxic, and immunosuppressive activities and waxy substances containing long-chain fatty acids.[1] On the other hand, the leaves and wood are known to be a rich source of aporphine alkaloids.[2] Recently, we have isolated seven new cyclic peptides, cyclosquamosins A−G, from the seeds of *A. squamosa* collected in Malaysia.[3] Further investigation on extracts of the seeds of the same plant resulted in isolation of samoquasine A (**1**), a benzooxyquinazoline alkaloid. Herein we describe the isolation and structure elucidation of **1**.

The EtOAc-soluble fraction of the methanolic extract of the seeds was partitioned between 3% tartaric acid and EtOAc. The 3% tartaric acid-soluble extract was adjusted to pH 9.0 with saturated Na_2CO_3 solution followed by partitioning with $CHCl_3$. The $CHCl_3$-soluble materials were subjected to a Si gel column ($CHCl_3$/MeOH, 15:1), followed by C_{18} HPLC (MeOH/MeCN/H_2O, 1:1:3), to afford samoquasine A (**1**, 0.003%), together with a known aporphine alkaloid, liriodenine.[4]

Samoquasine A (**1**) has a molecular formula of $C_{12}H_8N_2O$, as revealed by HRFABMS (*m*/*z* 197.0728, $[M + H]^+$, Δ +1.3 mmu). IR absorptions at 3422, 1663, and 1626 cm^{-1} demonstrated the presence of hydroxyl and conjugated carbonyl groups, respectively. ^1H and ^{13}C NMR data (Table 1) indicated that the molecule possesses a conjugated ketone carbonyl and 11 olefinic carbons, one of which is included in an imino group. Because 7 out of 10 unsaturations were accounted for, compound **1** was inferred to contain a naphthalene ring system and a pyrimidine ring. Interpretation of the $^1H-^1H$ COSY and HOHAHA spectra revealed proton connectivities from H-5 to H-6 and from H-7 to H-10. The connections between the above units and two sp^2 carbons (C-6a and C-10a) were assigned on the basis of $^1H-^{13}C$ long-range correlations in the HMBC spectrum. (See Figure 1.) HMBC correlations of H-9 to C-10a (δ_C 148.5), H-8 to C-6a (δ_C 123.6), and H-5 to C-6a suggested a connectivity from C-5 to C-10 through C-6a and C-10a. In addition, connectivities indicated by HMBC correlations of H-10/C-6a, H-7/C-10a, H-7/C-10b, H-5/C-10b, and H-6/C-4a confirmed a 1,2-disubstituted naphthalene skeleton. An amide carbonyl carbon, C-4 (δ_C 164.0), and C-2 (δ_C 150.5), assignable to the carbon between two nitrogen atoms, were included as part of a pyrimidine ring, together with the remaining two olefinic carbons, C-4a and C-10b (δ_C 118.7 and 144.3). Thus, the singlet olefinic proton (H-2) at δ_H 9.60, which was attached to C-2 (δ_C 150.5), showed long-range correlations to C-4a, C-10a (δ_C 148.5), and C-10b, while H-5 (δ_H 7.55) showed long-range correlations to C-10b (δ_C 144.3) and the carbonyl carbon at C-4 (δ_C 164.0), completing the benzoquinazoline skeleton. Accordingly, the structure of samoquasine A (**1**) was elucidated as 3,4-dihydrobenzo[*h*]quinazolin-4-one.

Treatment of samoquasine A (**1**) with trimethylsilyldiazomethane afforded the *O*-methyl derivative (**2**), of which the ^1H and ^{13}C NMR spectra were almost the same as those of **1**, except for the existence of a methoxyl signal (δ_H 3.72)

Table 1. ^1H and ^{13}C NMR Data of Samoquasine A (**1**) in $CDCl_3$

position	δ_H (*J*, Hz)	δ_C	HMBC (^1H)
2	9.60 s	150.5	
4		164.0	5
4a		118.7	2, 6
5	7.55 d (7.3)	136.0	6
6	7.30 d (7.3)	101.6	
6a		123.6	5, 8, 10
7	8.40 dd (1.0, 8.3)	125.0	9
8	7.71 dt (1.0, 8.3)	128.7	10
9	7.87 dt (1.0, 8.3)	132.8	
10	8.12 dd (1.0, 8.3)	130.4	8
10a		148.5	2, 7, 9
10b		144.3	2, 5, 7

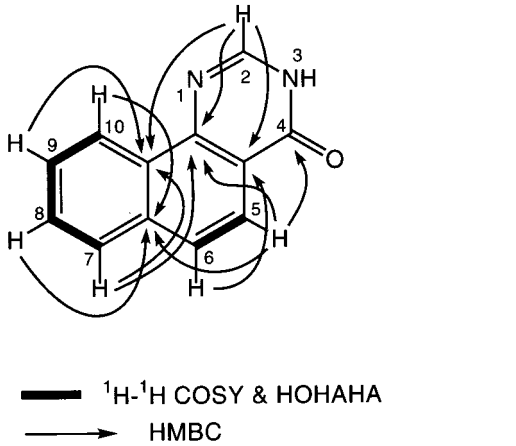

Figure 1. 2D NMR correlations fo samoquasine A (**1**).

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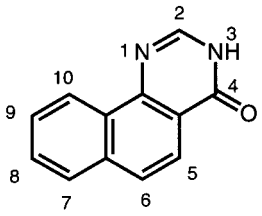
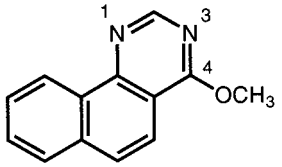
10.1021/np000342i CCC: $19.00
Published on Web 11/08/2000

in **2**. The cross-peak for H_3−OMe/H-5 in the NOESY spectrum indicated that the methoxyl group was located at C-4. These data supported the proposed structure of samoquasine A (**1**).



1



2

This is the first isolation of a benzo[*h*]quinazoline alkaloid from a plant in the Annonaceae, although many aporphine alkaloids such as liriodenine have been reported from this family.[2] Samoquasine A (**1**) exhibited significant cytotoxicity against murine lymphoma L1210 cells, with an IC_{50} value of 0.38 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were measured on a micromelting point apparatus and are uncorrected. UV spectra were obtained on a Shimadzu UV-1600PC spectrophotometer. IR spectra were run on a JASCO FT/IR-230 infrared spectrometer recorded as KBr pellets. ^{1}H and 2D NMR spectra were recorded in $CDCl_3$ on a 600 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured at 125 MHz (Bruker AMX600 and ARX500). Chemical shifts were reported using residual $CDCl_3$ peaks (δ_H 7.26 and δ_C 77.03) as internal references. Standard pulse sequences were employed for the 2D NMR experiments. HMBC spectra were recorded using a 50-ms delay time for long-range C−H coupling with *z*-axis PFG. NOESY spectra were measured with a mixing time of 800 ms. FABMS were measured using a glycerol matrix.

Plant Material. The seeds of *A. squamosa* were collected at Penang, Malaysia, in December 1997. The botanical identification was made by one of us (K.-L.C.). A voucher specimen (no. 971201) has been deposited in the herbarium of this institute.

Extraction and Isolation. The seeds of *A. squamosa* (1.36 kg) were crushed and extracted with MeOH (3 L) three times to give an extract (225 g), which was treated with hexane, EtOAc, *n*-BuOH, and H_2O, successively. The EtOAc-soluble fraction (18.8 g) was partitioned by 3% tartaric acid to give a water-soluble fraction, which was adjusted to pH 9.0 with saturated Na_2CO_3 solution. The alkaline fraction was partitioned with $CHCl_3$, and the $CHCl_3$-soluble materials were subjected to Si gel column chromatography ($CHCl_3$/MeOH). A fraction that eluted with $CHCl_3$/MeOH (15:1) was subjected to C_{18} HPLC (Develosil ODS−HG-5, MeOH/CH_3CN/H_2O, 1:1:3) to give samoquasine A (**1**, 0.003%) and liriodenine (0.0007%).

Samoquasine A (1): colorless needles; mp >300 °C; UV (MeOH) λ_{max} (log ϵ) 244 (ϵ 3.7), 251 (3.6), 322 (3.1), 337 (3.1), 353 (3.1) nm; (MeOH + 1 N NaOH) λ_{max} 240 (ϵ 3.8), 287 (3.1), 325 (3.0), and 368 (3.2) nm; IR (KBr) ν_{max} 3422, 2918, 2849, 1663, 1626, 1463, 1384, and 1127 cm^{-1}; FABMS *m*/*z* 197 [M + H]$^+$; HRFABMS *m*/*z* 197.0728 [M + H] (calcd for $C_{12}H_9N_2O$, 197.0715); ^{1}H and ^{13}C NMR data, see Table 1.

Methylation of 1. To a hexane solution (100 μL) containing 2.0 M trimethylsilyldiazomethane was added a methanol solution (200 μL) of **1** (1 mg), which was stirred at room temperature for 10 min. The solution was evaporated under reduced pressure, and the residue was chromatographed on a Si gel column ($CHCl_3$/MeOH, 20:1) to give the methyl derivative **2** (0.8 mg) as a colorless powder: ^{1}H NMR ($CDCl_3$) δ 3.72 (3H, s, OMe, C-4), 7.20 (1H, d, J = 7.4, H-6), 7.54 (1H, d, J = 7.4, H-5), 7.69 (1H, t, J = 8.0, H-8), 7.86 (1H, t, J = 8.0, H-9), 8.22 (1H, d, J = 8.0, H-10), 8.33 (1H, d, J = 8.0, H-7), and 9.78 (1H, s, H-2); FABMS *m*/*z* 211 [M + H]$^+$.

Cytotoxic Assay. Assays were carried out by the method described previously.[5]

Acknowledgment. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

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NP000342I