Journal of Natural Products
Vol. 58, No. 3, pp. 459-462, March 1995

CYATHOCALINE, AN AZAFLUORENONE ALKALOID FROM *CYATHOCALYX ZEYLANICA*

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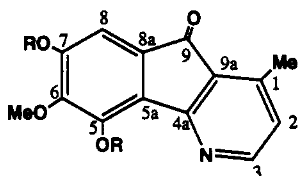
ABSTRACT.—Bioactivity-guided fractionation of a MeOH extract of the stem bark of *Cyathocalyx zeylanica* afforded a moderately bioactive new azafluorenone alkaloid, cyathocaline, the structure of which was established as 5,7-dihydroxy-6-methoxy-1-methyl-4-azafluoren-9-one [**1**] with the aid of spectroscopic data.

As a part of our continuing efforts to discover molecules of biological interest from the Sri Lankan flora, we have initiated the screening of Sri Lankan plants in the family Annonaceae for potential cytotoxic compounds employing the brine shrimp toxicity assay (1). An extract of *Cyathocalyx zeylanica* Champ. ex Hook. f. & Thoms. showing a positive response to this bioassay was selected for further investigation. In Sri Lanka, the genus *Cyathocalyx* is represented by a single species, *C. zeylanica,* which is endemic to the country (2). There is no reported work on this genus. A MeOH extract of *C. zeylanica,* which showed a positive response to the brine shrimp assay (3), was also found to be active in our mechanism-based yeast bioassay for DNA-modifying agents (4). Bioactivity-guided fractionation utilizing the latter bioassay resulted in the isolation of a moderately bioactive new azafluorenone alkaloid, named cyathocaline. In this paper we present the structure elucidation and biological activity of cyathocaline [**1**].

The stem bark of *C. zeylanica* was exhaustively extracted with hot MeOH. This extract was active in both brine shrimp cytotoxicity (3) and mechanism-based yeast (4) bioassays. Bioactivity-guided fractionation involving solvent-solvent partitioning and Si gel cc afforded cyathocaline as an orange-colored crystalline solid, mp 222–224°, showing a positive response to alkaloid tests on tlc. The structure of this alkaloid, named cyathocaline, was established as 5,7-dihydroxy-6-methoxy-1-methyl-4-azafluoren-9-one [**1**] with the aid of spectral data as described below.

Cyathocaline [**1**], $C_{14}H_{11}NO_4$ (hreims), showed in its ^{1}H-nmr spectrum the presence of a Me on an aromatic ring (δ 2.53, br s), an OMe (δ 3.98, s), an aromatic 1H singlet at δ 6.75, and an AB pair (J=5.5 Hz) of aromatic protons at δ 6.97 and 8.24; the former signal showed additional coupling (J=0.6 Hz). These data along with ir and uv data were reminiscent of an azafluorenone skeleton (5). Acetylation (Ac_2O/pyridine) afforded the diacetate [**2**] (δ_H 2.35 and 2.49; δ_C 168.3, 168.8, 20.6, and 20.9) indicating



1 R=H
2 R=$COCH_3$

the presence of two OH groups in cyathocaline. Thus, it remained to locate these two OH groups and the OMe group on the carbocyclic ring of the azafluorenone. In the long-range selective proton decoupling (LSPD) nmr spectrum of cyathocaline, irradiation of the proton signal at δ 6.75 led to an enhancement of the carbon signals at δ 192.3, 154.9, 141.6, and 120.1. Because the signal at δ 192.3 was due to the CO carbon, the ^{1}H-nmr singlet at δ 6.75 was assigned to the C-8 proton, suggesting a 5,6,7-trioxygenated carbocyclic ring for cyathocaline [**1**]. In the difference nOe nmr spectrum of the diacetate [**2**], irradiation of the ^{1}H-nmr signal at δ 2.35 caused an enhancement of the signals at δ 3.95 (OMe) and 7.34 (H-8). Therefore, the OAc group indicated by the singlet at δ 2.35 was placed at C-7 and the OMe group at C-6, leaving the remaining OH group to be placed at C-5. The ^{13}C-nmr spectrum of cyathocaline [**1**], analyzed with the aid of its DEPT spectrum, showed the presence of 9 singlets, 3 doublets, and 2 quartets. Assignments were made by comparison with those reported for related azafluorenones (5–8). ^{1}H- and ^{13}C-nmr assignments for cyathocaline [**1**] and its diacetate [**2**] are given in Table 1, and are consistent with the assigned structure.

Cyathocaline [**1**] exhibited moderate but selective activity in our mechanism-based yeast bioassay for DNA-modifying agents (4,9) with the following IC_{12} values: RS 322 YK (*rad* 52Y), 90 μg/ml; RS 321 N, 87 μg/ml; RS 188 N (RAD^{+})>400 μg/ml; inactive in the RS 167 N (*rad* 6) strain. In a cytotoxicity test against the A-549 human lung carcinoma cell line it had an IC_{50} value of 8.5±0.07 μM. Thus far, only two papers have appeared on the biological activity of azafluorenone alkaloids. In a recent paper, Wu and his co-workers reported that darienine, polyfothine, and isocondine were all inactive in their cytotoxicity assay (8). Onychine has been reported to have anticandidal activity (10).

The sixteen natural azafluorenones

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for Cyathocaline [**1**] and its Diacetate [**2**].

Position	Compound			
	1		**2**	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1	—	148.4 s	—	150.5 s
2	6.97 dd (*J*=5.5,0.6)	125.5 d	6.92 d (*J*=5.2)	125.5 d
3	8.24 d (*J*=5.5)	152.4 d	8.37 d (*J*=5.2)	152.8 d
4a	—	167.5 s	—	163.4 s
5	—	148.6 s	—	147.2 s
5a	—	126.4 s	—	126.4 s
6	—	141.6 s	—	139.1 s
7	—	154.9 s	—	152.8 s
8	6.75 s	105.9 d	7.34 s	116.7 d
8a	—	120.1 s	—	130.0 s
9	—	192.3 s	—	190.7 s
9a	—	131.2 s	—	133.6 s
Me-1	2.53 s	17.4 q	2.59 s	17.3 q
OAc-5	—	—	2.49 s	168.3 s[c], 20.6 q[d]
OAc-7	—	—	2.35 s	168.8 s[c]. 20.9 q[d]
OMe-6	3.98 s	61.3 q	3.95 s	61.5 s

[a]*J* in Hz.
[b]Multiplicity deduced from a DEPT experiment.
[c,d]Values with the same superscript in the same column may be interchanged.

thus far known are restricted to the plant family Annonaceae, which is known to elaborate a variety of alkaloids including isoquinolines, aporphines, dioxoaporphines, oxoaporphines, diazafluoranthenes, azaanthraquinones, and azafluorenones. Cavé and his co-workers have recently postulated a possible biogenetic relationship between the latter four classes of alkaloids (11). The proposed pathway from azaanthraquinones to azafluorenones involve a decarbonylation step for which they have suggested catalysis by a metalloenzyme producing a high-energy carbocationic species. For this biogenetic transformation we wish to postulate a Favorski-type intermediate [**3**] derived from an azaanthraquinone which could undergo a ring opening followed by a decarboxylation leading to the azafluorenone system (see Scheme 1).

EXPERIMENTAL

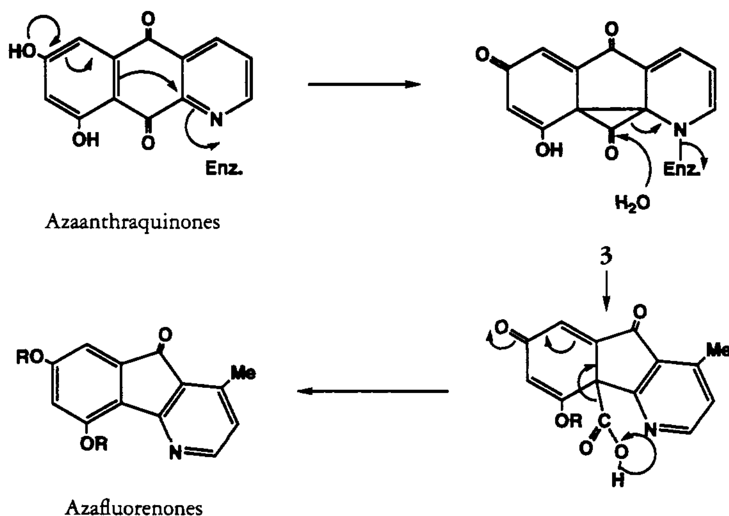
GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler hot-stage apparatus and are uncorrected. Ir spectra were recorded on a Shimadzu IR-470 spectrometer and uv spectra on a Shimadzu UV-160A uv/vis spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a JEOL JNM-GX 400 nmr spectrometer at 400 and 100 MHz, respectively, chemical shifts are reported in ppm (δ) downfield from internal TMS.

PLANT MATERIAL.—The plant material used in this study was collected in Kanneliya rainforest in the southern province of Sri Lanka. It was identified by Mr. D.A.S. Wijesundera of the Peradeniya Royal Botanic Gardens of Sri Lanka. A voucher specimen is deposited at the Medical Research Institute Herbarium.

EXTRACTION AND ISOLATION.—The dried and powdered stem bark of *C. zeylanica* (3.5 kg) was exhaustively extracted with hot MeOH. The bioactive MeOH extract was partitioned between CH_2Cl_2 and H_2O. The bioactive CH_2Cl_2 fraction was then partitioned between hexane and 10% aqueous MeOH. The latter fraction (70 g) which was found to be bioactive was evaporated and a portion (50 g) of it was chromatographed on a column of Si gel (mesh 70–230, 700 g) made up in CH_2Cl_2 and eluted with CH_2Cl_2 containing increasing amounts of MeOH.

Cyathocaline [**1**].—The fraction eluted with 2% MeOH in CH_2Cl_2 followed by flash chromatography afforded an orange crystalline solid (47 mg, 1.9×10^{-1}%), mp 222–224°; uv (EtOH) λ max (log ε) 224 (3.40), 261 (3.78), 313 (3.28), 348 (3.26) nm; uv (EtOH+NaOH) λ max (log ε) 232 (3.42), 274 (3.60), 354 (3.34), 448 (3.30) nm; ir (KBr) ν max 3465, 2920, 1743, 1708, 1075 cm^{-1}; hreims *m/z* [M^{+}] 257.0687 ($C_{14}H_{11}NO_4$ requires 257.0688) (54), 239 (100), 228 (14), 214 (41), 195 (9), 183 (14), 158 (11), 130 (10), 28 (8); ^{1}H- and ^{13}C-nmr data, see Table 1.

Diacetylcyathocaline [**2**].—Cyathocaline [**1**] (10 mg) was treated with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temperature for 24 h and the reaction mixture was worked up in the usual



SCHEME 1. Proposed pathway for the biogenetic conversion of azaanthraquinones to azafluorenones.

way to give the diacetate **2** (11 mg), mp 107–108°, ir (KBr) ν max 2920, 1736, 1710, 1620, 1065 cm^{-1}; eims *m/z* [M^+] 341 (3), 299 (29), 257 (22), 239 (100), 228 (28), 214 (25), 157 (22), 128 (21), 50 (6); ^1H- and ^{13}C-nmr data, see Table 1.

ACKNOWLEDGMENTS

This work was supported by the Japan International Cooperation Agency (JICA) and a National Cooperative Drug Discovery Group Grant awarded to the University of Virginia (1U01 CA 50771) (Dr. S. Hecht, principal investigator). We thank Mr. D.A.S. Wijesundera (Royal Botanic Gardens, Peradeniya, Sri Lanka) for the identification of plant material and Dr. Randall K. Johnson and Dr. Francis L. McCabe (SmithKline Beecham Pharmaceuticals, Philadelphia) for the yeast strains used in this work and the cytotoxicity determination.

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Received 29 August 1994