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A Novel Alkaloid from the Indo-Pacific Sponge *Clathria basilana*

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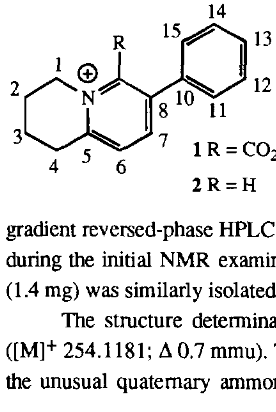
Abstract: One Indonesian collection of the massive orange marine sponge *Clathria basilana* yielded a new tetrahydroquinolizinium ion, clathryimine A (**1**),which on heating lost CO_2 affording B (**2**).

Inexplicably, orange Indo-Pacific sponges differing at the highest taxonomic levels have provided us with several unusual nitrogen containing compounds. Interesting structures we have reported from such sponges range from polypeptides and ketide-amino acids to nitrogenous terpenoids.[1] A very distinctive and massive orange sponge, *Clathria basilana* (Family: Microcionidae, syn. Clathriidae; Order: Poecilosclerida), is included in Indo-Pacific field guide books[2] but it has not been a subject of published chemical studies. In addition to *basilana* there are eleven other common Indo-Pacific species known in this genus.[2] Their entire chemical knowledge is limited to reports on *Clathria* sp., which affords clathrynamides A-C[3] and *C. pyramida,* a source of 5-thio-D-mannose.[4] Several years ago we began the chemical study of *C. basilana* and in this communication outline that one collection contained a novel quinolizine alkaloid, clathryimine (**1**).



1 R = CO_2H

2 R = H

Five different specimens of *C. basilana*[5] were processed according to our standard procedures.[1c] However, only one specimen (coll. no. 94563, 388 g dry wt) afforded a crude CH_2Cl_2 (0.42 g) extract partition fraction appearing to contain unusual constituents. Additionally, this oil was toxic to brine shrimp (0.2 mg/ml, 96% mortality) so it was fractionated by gradient Silica flash chromatography (CH_2Cl_2:MeOH). A fraction (41 mg, elution at approx. 5% MeOH), although devoid of brine shrimp activity, was enriched in clathryimine A (**1**).[6] Final purification by gradient reversed-phase HPLC (ODS, MeOH:H_2O 50:50, with MeOH increasing at 1%/min) gave pure **1** (6.2 mg) which, during the initial NMR examination ($CDCl_3$) underwent partial decarboxylation to afford **2**.[7] An additional sample of **1** (1.4 mg) was similarly isolated from the original crude aq. MeOH extract (yield 0.10 g).

The structure determination commenced with the molecular formula of $C_{16}H_{16}NO_2$ established by HRFABMS ($[M]^+$ 254.1181; Δ 0.7 mmu). The unsaturation equivalence of 10 was established after it was recognized that **1** contained the unusual quaternary ammonium functionality. The ^1H-^1H COSY NMR data summarized in Table 1 revealed three

Table 1. NMR data for **1** and **2** at 62.9 (^{13}C, $CDCl_3$) and 500 (^1H, COSY, HMBC) MHz.

	1 ($CDCl_3$)			**1 (CD_3OD^b)**			**2 ($CDCl_3$)**	
Atom #	^{13}C	^1H (*J*, Hz)	HMBC//COSY	^{13}C	^1H (*J*, Hz)	HMBC//COSY	^{13}C	^1H (*J*, Hz)
1	52.6	4.84 t, 6.7	H2,H3//H2	52.0	4.61 t, 6.5	-//H2	56.4	5.19 bs
2	21.6	2.20 p, 6.7	H1,H3,H4//H1,H3	21.5	2.17 p, 6.5	H1,H4//H1,H3	21.3	2.26 p, 6.5
3	17.5	2.03 p, 6.7	H1,H2,H4//H2,H4	16.0	2.02 p, 6.5	H1,H4//H2,H4	17.8	2.09 p, 6.5
4	29.1	3.20 t, 6.7	H2,H3,H6//H3	28.0	3.25m	H2,H3,H6//H3,H6	28.4	3.31 p, 6.5
5	150.9		H1,H3,H4,H6,H7//-	153.9		H1,H3,H4,H6,H7//-	153.4	
6	124.1	7.46 m	H4//H7	125.5	7.78 d, 8.5	H4//H4,H7	128.2	7.79 d, 8.5
7	144.5	8.10 d, 8.0	- //H6	-	8.31 d, 8.5	-//H6	141.5	8.42 dd, 8.5, 0.8
8	135.4[a]		H6,H11=15//-	134.5		H6//-	132.7[a]	
9	156.4		H1,H7//-	153.2		H1,H7//-	144.1	9.92 bs
10	134.9[a]		H7,H12=14//-	135.1		H7,H12=14//-	138.6[a]	
11=15	128.7	7.71 dd, 8.0, 1.5	H15=11//H12=14,H13	-	7.61 m	-//H12=14,H13	127.6	7.92 d, 7.5
12=14	129.0	7.46 m	H14=12//H11=15	-	7.46 m	-//H11=15	129.8	7.54 d, 7.5
13	129.4	7.45m	H11=15//H11=15	-	7.46 m	-//H11=15	130.3	7.48 d, 7.5
CO_2H	162.4			-				

[a] These shifts are interchangeable based solely on $CDCl_3$ data. [b] ^{13}C shifts in CD_3OD are those which were available from HMBC data.

separate spin systems consisting of a monosubstituted aromatic ring, a disubstituted double bond and four contiguous CH_2's. Further consideration of the HMBC correlations, especially those from C9 to H1 and H7, from C6 to H4, and from C5 to H7, eventually allowed construction of a tetrahydroquinolizinium ion core with disubstitution at C8 and C9. Further confirmation came from comparing the observed ^{13}C and ^{1}H shifts (Table 1) to those of previously synthesized compounds of this class.[8] The C8/C9 substituents consisting of the carboxylate group (δ 162) and the phenyl ring (Table 1) were further confirmed by LRFABMS fragments at 210 $[M-CO_2]^+$ and 177 $[M-C_6H_5]^+$. The regiochemistry of these two groups could not be assigned solely on the NMR data obtained in $CDCl_3$ because (a) C8 and C10 could not be unequivocally assigned and (b) no HMBC correlations were observed to the carboxyl carbon. Fortunately, in CD_3OD H6 was observed as an individual resonance and an HMBC correlation observed from C8 to H6 allowed unambiguous assignment of C8 and C10. Further, this allowed proper interpretation of an important correlation from C10 to H7, fixing the phenyl ring at C8.

During the NMR experiments performed on **1** in $CDCl_3$ this sample partially converted to **2**.[7] This reaction was driven to completion simply by heating the NMR sample of **1** overnight at 40° C which afforded **2** exhibiting a LRFAB *m/z* of 210, $[M]^+$. The progress of this reaction could be assessed by observing the disappearance of a distinctive IR absorption band (C=O stretch, 1640 cm^{-1}) and the appearance of a new ^{1}H NMR peak at δ 9.92. Also, as expected based on the structure of **2**, the δ 9.92 resonance exhibited a long range COSY correlation to δ 8.42, H7.

Our comparative observations on the five different *basilana* collections[5] reveal an interesting situation. Only the specimen reported on above contained clathryimine A or its product B, whereas another sample (coll. no. 95510) appeared to be rich in a mixture of halitoxins.[9] The structure of clathryimine A (**1**) provides the first example of a quinolizinium metabolite from a marine sponge. The best analogies to **1** among sponge derived alkaloids are not very similar because they have quite different bicyclic nitrogen containing rings. These compounds include stelletamide A,[10] the sarain family,[11] and the petrosins.[12]

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5. These collections include specimens from Papua New Guinea (coll. no. 90164) and Indonesia (coll nos. 92559, 92560, 94563, 95510).
6. Clathryimine A (**1**): $C_{16}H_{16}NO_2$ amber viscous oil, UV (MeOH) λ_{max} (ε) 291(4500), 241(5500); IR (neat), υ 3423, 3059, 2951, 1640, 1478, 1370, 1335, 844, 785, 701, cm^{-1}; HRFABMS (*m/z* 254.1181, M^+, Δ 0.7 mmu of calcd).
7. Clathryimine B (**2**): $C_{15}H_{16}N$, brown viscous oil, UV (MeOH) λ_{max} (ε) 256(5800), 238(6100); IR (neat), υ, 3062, 2960, 2924, 2850, 1631, 1452, 768, 699, cm^{-1}; LRFABMS (*m/z* 210.1, M^+).
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