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LENTICELLARINE, A MOLLUSCICIDAL ALKALOID FROM *DYSOXYLUM LENTICELLARE*

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Key Word Index—*Dysoxylum lenticellare*; Meliaceae; alkaloid; Lenticellarine; molluscicidal activity.

Abstract—A new, homoerythrina-derived alkaloid with molluscicidal activity, lenticellarine, was isolated from the leaves of *Dysoxylum lenticellare* and its structure was determined by spectroscopic methods.

INTRODUCTION

The homoerythrina alkaloids represent a growing class of natural products isolated from a number of genera [see 1, 2]. First reported in the late sixties [3–5], these natural products are closely related to the cephalotaxine alkaloid series known for their antitumour activity [6]. We have previously reported the isolation of a series of seven alkaloids, **1**–**7**, from the leaves of *Dysoxylum lenticellare* Gillespie [7, 8]. These alkaloids were of three biogenetically related structures: phenylethylisoquinoline, dibenzazocine, and homoerythrina alkaloid skeleta. During the isolation of these alkaloids we repeatedly encountered an eighth alkaloid which contained a non-aromatic carbon skeleton. From the spectroscopic data we now propose structure **8** for this new alkaloid which we have called lenticellarine. This alkaloid, most likely a transformation product of the phenolic precursors of the major alkaloid found in *D. lenticellare*, 18-methoxy-3-*epi*-schelhammericine (**2**), is another example of a homoerythrinoid alkaloid with an oxidized D-ring [9–14]. Lenticellarine proved to have moderate molluscicidal activity.

RESULTS AND DISCUSSION

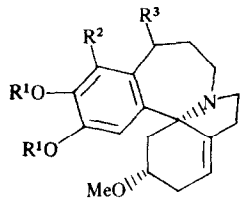
The molecular formula as established by the high resolution mass spectrum, $C_{19}H_{25}NO_5$ (Found: *m/z* 347.1759; Calcd: 347.1732), requires eight units of unsaturation. An α, β-unsaturated methyl ester conjugated with a trisubstituted double bond with the hydrogen at the α-position (IR: 1690, 1620 cm^{-1}; ^{13}C NMR: δ166.92 *s*, 153.04 *s*, 122.96 *d*, 51.66 *q*; ^1H NMR: δ6.04 1H *s*, 3.64 3H *s* UV λ^{EtOH}: 230 nm, ε 3500), a non-conjugated methyl ester (IR: 1730 cm^{-1}; ^{13}C NMR: δ173.23 *s*, 52.54 *q*; ^1H NMR: δ3.54 3H *s*), and a non-conjugated, trisubstituted double bond (IR: 1625 cm^{-1}; ^{13}C NMR: δ141.56 *s*, 118.78 *d*; ^1H NMR: δ5.52 1H *s*), account for four of the units of unsaturation. A methyl ether was also readily apparent (^{13}C NMR: δ56.45 *q*, 73.48 *d*; ^1H NMR: δ3.28 3H *s*).

The mass spectrum clearly identified **8** as possessing the C-1 to C-10 portion of the homoerythrina skeleton by the presence of the base peak at *m/z* 165.1138 (HRMS: $C_{10}H_{15}NO$). This ion loses methanol and a hydrogen atom to give the diagnostic peak at *m/z* 132 (Scheme 1). These two fragment ions are also found in the mass spectrum of **2** as well as those of other Δ^{1-6}-homoerythrina alkaloids [9, 11, 12, 14–18]. The major ion at *m/z* 192.1388 (HRMS: $C_{12}H_{18}NO$) contains the C-1 to C-12 portion of **8**, thus including two carbon atoms of the cyclopropane ring. Such an ion occurs only when the aromatic ring of the homoerythrina skeleton is no longer intact. The loss of methyl vinyl ether by a retro-Diels–Alder fragmentation confirms that the A-ring is a 3-methoxy-Δ^{1-6}-cyclohexene structure [9, 11, 12, 14–19]. Loss of methyl vinyl ether from the ions at *m/z* 288, 192, and 165 (to produce peaks at *m/z* 230, 134, and 107, respectively) was also observed.

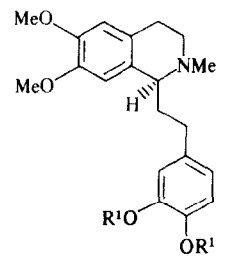
The separate, scalar coupled spin systems were distinctly delineated by a double quantum filtered, phase sensitive COSY (DQPS-COSY) experiment. The cyclohexene A-ring was thus confirmed to bear the methoxyl group at the 3-position as suggested by the mass spectrum. The H-4ax signal (δ1.37, *br t*, $J=11.7$, 12.3 Hz) showed near equal coupling (~12 Hz) to both its *gem*-partner, H-4eq (δ2.03, *dd*, $J=11.8$, 4 Hz), and H-3, (δ3.6 *m*, overlapped by–OMe *s*). This characteristic coupling confirms the α-orientation (equatorial) of the 3-methoxyl group, typical of the 3-*epi*-schelhammericine series [9, 11–18, 20].

The isolated ethylene protons of the B-ring were also readily distinguished. Allylic coupling from H-1 to the methylene protons at C-7 observed in a long range COSY (Δ=0.20 *s*) spectrum, [21, 22] confirm the connectivity between the A- and B-rings. The ^{13}C- and ^1H NMR data for **8**, Table 1, are also in accord with established values for the A- and B-rings of these types of alkaloids. [11, 12, 14, 20].

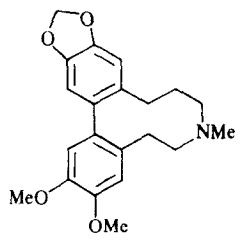
The remaining coupling network outlined by the DQPS-COSY spectrum included the relatively high-field signal at δ1.01, (*dd*, $J=6.8$, 4.9 Hz). The HETCOR spectrum showed that this proton is one of a methylene pair,



1 R^1, R^1 = —CH_2—; R^2 = R^3 = H
2 R^1, R^1 = —CH_2—; R^2 = OMe; R^3 = H
3 R^1 = Me; R^2 = R^3 = H
4 R^1, R^1 = —CH_2—; R^2 = H; R^3 = OH



5 R^1, R^1 = —CH_2—
6 R^1 = Me



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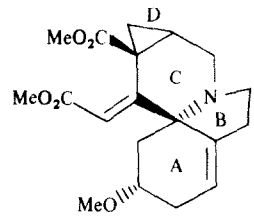

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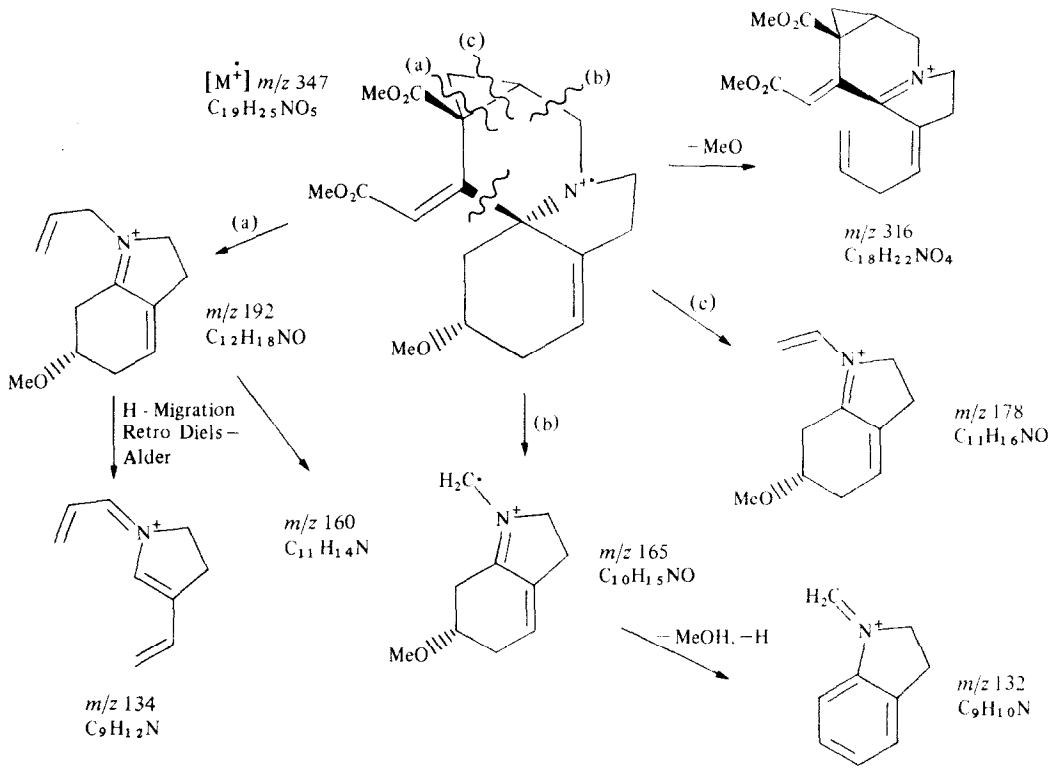

Sch. 1.

its *gem*-partner at δ2.29 (*dd*, J = 9, 4.9 Hz), with the carbon signal located at δ25.79. The *gem* coupling between these two protons of only 4.9 Hz can only be explained by a cyclopropyl methylene structure [23]. The vicinal partner of these methylene protons at δ1.58 (*m*), which must also be part of the cyclopropyl D-ring, is also coupled to the C-10 methylene protons of the C-ring which in turn are adjacent to the nitrogen (^{13}C NMR: δ42.71, *t*; ^{1}H NMR: δ3.02 *dd*, J = 15.2, 5.2 Hz; 3.41 *dd*, J = 15.2, 8.6 Hz). Thus, the cyclopropyl ring must be

Table 1. Chemical shifts of **8** (93.93 kG, CD_2Cl_2. ^{13}C NMR: 100 MHz,* ^1H NMR: 400 MHz)

Position	^{13}C	^1H
1	118.78 *d*	5.52, *br s*
2 (ax)	31.76 *t*	1.94, *m*, $J_{AB}=16.4$, $J_{2ax,3}=8.6$ Hz
2 (eq)		2.65, *br d*, $J_{AB}=16.4$, $J_{2eq,3}<2$ Hz
3	73.48 *d*	3.6, *m*, overlapped by −OMe
4 (ax)	38.80 *t*	1.37, *br t*, 12.3, 11.7 Hz
4 (eq)		2.03, *dd*, 12.3, 4 Hz
5	64.89 *s*	
6	141.56 *s*	
7	27.43 *t*	2.38, *m*, $J_{AB}=15.3$ Hz
		2.40, *m*, $J_{AB}=15.3$ Hz
8	48.24 *t*	2.95, 2H *m*
10a	42.71 *t*	3.02, *dd*, 15.2, 5.2 Hz
10b		3.41, *dd*, 15.2, 8.6 Hz
11	21.89 *d*	1.58, *dddd*, 9, 8.6, 6.8, 5.2 Hz
12a	25.79 *t*	1.01, *dd*, 6.8, 4.9 Hz
12b		2.29, *dd*, 9, 4.9 Hz
13	26.23 *s*	
14	153.03 *s*	
15	122.96 *d*	6.04, *s*
16	166.92 *s*	
17	173.23 *s*	
3–OMe	56.45 *q*	3.28, *s*
16–OMe†	51.66 *q*	3.64, *s*
17–OMe†	52.54 *q*	3.54, *s*

*Multiplicities were assigned with a DEPT experiment.
†Signals assigned by selective INEPT [24, 25].

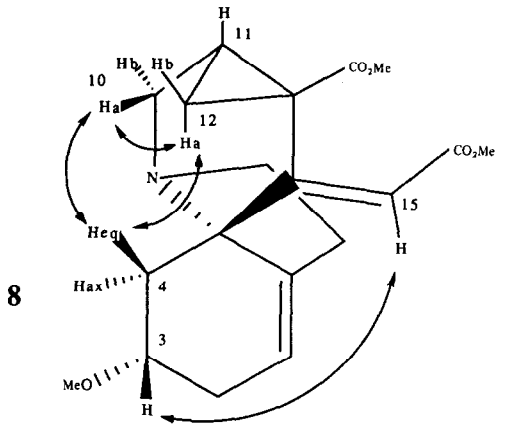

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Fig. 1. Nuclear Overhauser enhancements used to determine stereochemistry of the cyclopropane ring and the Δ^{14-15} double bond **8**, from 2D-NOE spectrum.

located from C-11 through C-13 with the methine at C-11, methylene at C-12, and a quaternary carbon (^{13}C NMR: 26.23, *s*) at C-13.

A heteronuclear 2D-*J* experiment enabled determination of all $^1J_{C-H}$ coupling constants. The $^1J_{C-H}$ values for C-12 (*t*, 177 Hz) and C-11 (*d*, 178 Hz) confirm the presence of the cyclopropane ring [24]. The tetracyclic skeleton thus accounts for the remaining four units of unsaturation. The location of the cyclopropyl D-ring at C-11 through C-13 requires that the exocyclic vinyl group be at C-14 of the C-ring.

The remaining structural uncertainties, the stereochemistry of the $\Delta^{14,15}$-double bond and the cyclopropyl stereocenters, C-11 and C-13, were resolved with a 2D-NOE experiment. Thus, dipolar coupling between H-15 and H-3 confirmed the orientation of the exocyclic $\Delta^{14,15}$-double bond [13, 25], as would be predicted from biosynthetic considerations. Similarly, dipolar couplings from H-12a to H-4eq and H-10a, as well as couplings from H-12b to H-11 enable the assignment of the stereochemistry of the cyclopropyl D-ring as indicated (Fig. 1). The C-17 ester group thus deshields H-12b, thereby accounting for the relatively low field shift (δ 2.29) for this cyclopropyl hydrogen. Under the conditions of the 2D-NOE (mixing time of 0.8 *s*), dipolar couplings were not observed between H-12b and H-10b, nor between H-12a and H-11. Other observed NOE's allowed assignment of all the proton resonances, and confirmed the structure of lenticellarine as **8**.

The carbon assignments, Table 1, were made in accord with known compounds of the homoerythrina structure [11, 12, 14, 20] and were confirmed using the DEPT and HETCOR spectra. The assignment of the methyl signals of the two ester groups, however, could not be distinguished using these techniques. Since the carbonyl carbon of the α,β-unsaturated ester can be distinguished from the non-conjugated ester carbonyl by its relative upfield shift in the ^{13}C NMR spectrum, these methyl signals (both ^1H- and ^{13}C-signals) were distinguished by a selective INEPT experiment [26, 27]. Irradiation of the methyl singlet at δ 3.54 caused a polarization transfer to the downfield carbonyl signal, δ 173.23, thereby confirming that the higher field (^1H NMR: δ 3.54) methyl ester resonance corresponds to the non-conjugated methyl ester unit, ^{13}C NMR: δ 52.54 (HETCOR). The lower field resonance (^1H NMR: δ 3.64) thus belongs to the α,β-unsaturated methyl ester, ^{13}C NMR: δ 51.66 (HETCOR). These methyl assignments were subsequently confirmed by a long range COSY spectrum ($\Delta=0.40$ *s*) whereby 5-bond coupling between H-15 and the methyl group of the α,β-unsaturated ester at C-16 was detected.

Conceivably **8** and **2** could share a common precursor with two methoxyl groups on the phenolic ring. Closure to the methylenedioxy unit would thus form **2**. The absolute stereochemistry of **8** would therefore retain that of its precursor and be the same as in **2**, (3*S*, 5*S*) [8]. The two methyl esters could derive from the aryl methyl ethers and would not be an artefact of the original methanolic extraction.

Lenticellarine proved to be moderately molluscicidal against the snail *Biomphalaria glabrata*, $LC_{75}=100$ ppm, $LC_{50}=40$ ppm within 24 hr. Thus, **8** is not as active as **1**, $LC_{100}=8$ ppm, or **2**, $LC_{100}=6$ ppm within 24 hr; nor is **8** as active as the crude methanolic extract of leaves, $LC_{100}=100$ ppm within 24 hr. These compounds represent the first alkaloids to show this level of molluscicidal activity. The only previous report of a molluscicidal alkaloid, to the best of our knowledge, is the quinolizine alkaloid 2,3-dehydro-*O*-(2-pyrrolylcarbonyl)-virgiline, isolated from the leaves of *Calpurnia aurea* (Leguminosae), with a reported $LC_{100}=130$ ppm within 48 hr, also against *B. glabrata* [28].

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra, recorded at 93.93 kG (400 MHz for ^{1}H, 100 MHz for ^{13}C) in CD_2Cl_2 unless otherwise stated, residual CH_2Cl_2 as int. standard. Standard acquisition and data transformation parameters were employed for the two-dimensional spectra. For the selective INEPT experiment, an interval (τ) of 0.10 sec was employed, corresponding to coupling of 5 Hz [26, 27]. For the long range COSY spectrum delays (Δ) of 0.20 and 0.40 sec were employed to detect couplings between H-2 and H-7, and between H-15 and the methoxy ester at C-16, respectively [21, 22]. The 2D-NOE spectrum utilized a mixing time of 0.8 sec. Specific rotations were measured in EtOH with a 1 dm cell. IR spectra were recorded as films on NaCl plates.

Extraction and isolation [7]. The dried leaves (2.8 kg) of *D. lenticellare* were extracted with MeOH, and the extract partitioned with pentane, then with $CHCl_3$. The $CHCl_3$ extract (36 g) was chromatographed on alumina, using cyclohexane, then cyclohexane–EtOAc (9:1) as eluant. The latter eluant gave the alkaloid fractions as previously described [7]. The fraction containing **8** was subjected to prep TLC on alumina with cyclohexane–EtOH (19:1) as eluant, yielding **8** (324 mg, 0.012%).

Lenticellarine (**8**). Gum which darkens upon standing; $[\alpha]_D^{18}$ +16 (abs. EtOH; *c* 0.165); UV λ_{max}^{EtOH} nm: 230 (ε 3500); IR ν cm^{-1}: 2960, 2930, 2840, 1730, 1690, 1625 sh, 1620, 1515, 1480, 1438, 1370, 1336, 1260, 1205, 1150, 1100, 1080, 933, 755; HRMS *m/z*: Found 347.1759 [M$^+$], Calcd: 347.1732 for $C_{19}H_{25}NO_5$; EIMS *m/z* (%, formula from HRMS): 347 (39, $C_{19}H_{25}NO_5$), 316 (69, $C_{18}H_{22}NO_4$), 289 (8, $C_{16}H_{19}NO_4$), 288 (18, $C_{17}H_{22}NO_3$), 284 (22, $C_{17}H_{18}NO_3$), 261 (19, $C_{15}H_{19}NO_3$), 256 (19, $C_{16}H_{16}NO_2$), 230 (24, $C_{14}H_{16}NO_2$), 196 (16, $C_{10}H_{12}O_4$), 192 (68), 178 (14), 165 (100, $C_{10}H_{15}NO$), 160 (45, $C_{11}H_{14}N$), 134 (33, $C_9H_{12}N$), 132 (56, $C_9H_{10}N$), 120 (35, $C_8H_{10}N$), 107 (12, C_7H_9N). ^{1}H NMR ($CDCl_3$, 300 MHz): δ6.07 (1H, *s*, H-15), 5.52 (1H, *br s*, H-1), 3.69 (3H, *s*, –OMe), 3.66 (1H, *m*, H-3), 3.62 (3H, *s*, –OMe), 3.44 (1H, *dd*, *J* = 15, 9 Hz, H-10b), 3.33 (3H, *s*, –OMe), 3.04 (1H, *dd*, *J* = 15, 5 Hz, H-10a), 2.97 (1H, *m*, H-8a), 2.95 (1H, *m*, H-8b), 2.67 (1H, *br d*, *J* = 15, H-2eq), 2.49 (1H, *m*, H-7a), 2.47 (1H, *m*, H-7b), 2.37 (1H, *dd*, *J* = 9, 5 Hz, H-12b), 2.06 (1H, *dd*, *J* = 12, 4 Hz, H-4eq), 2.00 (1H, *br*, H-2ax), 1.60 (1H, *m*, H-11), 1.37 (1H, *t*, *J* = 12, H-4ax), 1.08 (1H, *dd*, *J* = 6.8, 5 Hz, H-12a). ^{13}C and ^{1}H NMR in CD_2Cl_2: See Table 1.

Molluscicidal bioassay. The bioassays for the crude methanolic extract and alkaloids **1**, **2**, and **8** were performed as previously described using *Biomphalaria glabrata* [28].

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