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THE STRUCTURE OF 10-*EPI*-LYFOLINE, A NOVEL ALKALOID FROM *HEIMIA MONTANA*

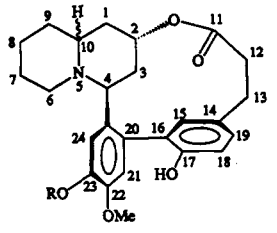
XIANG-QUN XIE, ANA ROTHER, and J.M. EDWARDS*

Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, Connecticut 06269

ABSTRACT.—The structure of 10-*epi*-lyfoline [**6**], a biphenylquinolizidine alkaloid isolated from *Heimia montana*, has been established by nmr studies. The presumed structure of lyfoline [**4**] has been confirmed.

Heimia montana (Griseb.) Lillo (Lythraceae) has been shown to elaborate several related biphenylquinolizidine alkaloids that are also found in other plants of the genus (1). Vertine [**1**], heimidine [**2**], lythrine [**3**], lyfoline [**4**], and lythridine [**5**], have all been identified from the plant, and, as can be seen from the structures, with the exception of **4**, the alkaloids exist as diastereomeric pairs differing only in the stereochemistry at C-10. We wish to report herein on the structure of the previously unreported 10-*epi*-lyfoline [**6**], and to present evidence which confirms the long-assumed structure of lyfoline [**4**].

10-*epi*-Lyfoline[1] [**6**] was first isolated from *H. montana* in 1986. The alkaloid has never been identified from other *Heimia* species, and its occurrence in *H. montana* has been sporadic. Only a small amount of the alkaloid has been available, precluding fractional crystallization; the alkaloid was purified by repetitive tlc (1). The molecular formula ($C_{25}H_{27}NO_5$) was established by ms, and showed the alkaloid to be an isomer of **4**. The ^{1}H-nmr spectrum, especially with respect to the H-2 and H-4 resonances (2), suggested that the H-2 stereochemistry was consistent with that of the known Lythraceae alkaloids and that the alkaloid belonged to the series with a *cis*-quinolizidine ring junction.



	H-10	12–13	R
1	β	CH=CH	Me
2	β	CH_2-CHOH	Me
3	α	CH=CH	Me
4	α	CH=CH	H
5	α	CH_2-CHOH	Me
6	β	CH=CH	H

These deductions were supported by ms and tlc analysis of the two products formed upon methylation (1). Ms showed that **6** gave both a monomethyl- and dimethyl ether. Tlc comparison with known biphenylquinolizidines unequivocally showed that the mono- and dimethyl ethers derived from **6** differed from the *trans*-quinolizidines **3** and *O*-methyllythrine, and that they were chromatographically and chromogenically identical, respectively, to the *cis*-biphenylquinolizidine lactones **1** and *O*-methylvertine.

It was concluded therefore that the new *Heimia* alkaloid was demethylvertine. The placement of the OH and OMe groups at C-22 and C-23 or vice versa still had to be established.

In order to resolve the problem of the relative positions of the OH and OMe groups, and to establish the conformation of **6**, an investigation using both nmr and computer modeling was under-

[1]We have chosen this name rather than demethylvertine used by Rother (1); the latter name is equivocal because the position of the OMe group is not defined.

taken. The well-established chemical shifts of the H-2 and H-4 protons of the quinolizidine ring in this series of alkaloids (2) were used to assign the spectral data through the coupling connectivities seen in the homo-COSY nmr spectrum. The results are presented in Table 1. The distinction between the two aromatic singlets due to H-21 (δ 6.96) and H-24 (δ 7.23) was established by the observation of nOe cross-peaks between H-24 and H-6e of the quinolizidine ring. H-21, on the other hand, showed no nOe interaction with protons of the quinolizidine system, but did show an nOe with the OMe group at C-22. Analysis of the ^{1}H-nmr spectrum of the quinolizidine ring is based upon the previously known chemical shifts of H-2e and H-4a (2), and from that of H-6e identified in the nOe study. The indicated conformation of **6** was also supported by nOe interactions observed between the aromatic proton H-15 and H-4a and H-3e of the heterocyclic ring.

The observation of an nOe interaction between H-4a and H-9a of epilyfoline explains the observed downfield shift of H-4a in the cis alkaloids of this series. The deshielded nature of H-4a has been attributed to the fact that the bonds between C-6–C-7 and C-9–C-10 in the cis compounds are parallel to the H-4 bond (3), but it can better be attributed to the van der Waal's effect between H-4a and H-9a which are in close steric proximity. The expected nOe between H-7a and H-9a was imbedded in the unsuppressed *J* cross-peaks.

A similar nmr study was undertaken to confirm the long assumed, but not proven, structure [**4**] for lyfoline. The relative configuration and structure of **4**, apart from the placement of the OMe and OH groups at C-22 and C-23, were determined by correlation (4) of its structure with that of lythrine [**3**], which has been established by chemical degradation and X-ray crystallography (5). The OH and OMe groups were initially positioned as shown in **4** by Ferris "on the basis of current knowledge of biphenyl coupling in alkaloid biosynthesis" (2); it is of some interest to note that *O*-methy-

TABLE 1. ^{1}H-Nmr Data for **4** and **6**.

Proton(s)	Compound					
	4			**6**		
	δ_H	COSY $^2J,^3J$	COSYLR ^4J	δ_H	COSY $^2J,^3J$	Observed nOe
H-1a	1.72 br t	1e, 10a		1.67 m	1e, 2e, 10	
H-1e	1.75 br d	1a, 2e	3e	2.27 m	1a, 2e, 10	
H-2e	5.34 m	1e, 3a, 3e		5.36 br m	1a, 3a, 3e	
H-3a	2.03 td	2e, 3e, 4a		2.06 m	2e, 3e, 4a	H-24
H-3e	2.24 br d	2e, 3a, 4a		2.27 br d	2e, 3a, 4a	H-15
H-4a	3.61 dd (10, 1.1 Hz)	3a, 3e		4.56 d (11.1 Hz)	3a, 3e	H-15, 9a
H-6a	1.14 m	6e, 7a		2.47 td	6e, 7a, 7e	
H-6e	2.69	6a	8e	2.90 b rd	6a, 7a, 7e	H-24
H-7a	1.40	6a, 7e, 8a		0.57 tt	6a, 7e	
H-7e	1.48	7a		0.83 d	7a, 6e	
H-8a	1.18	7a, 8e, 9e		1.11 m	7e, 8e	
H-8e	1.61	8a, 9a, 9e		1.28 m	8a	
H-9a	1.27-1.30 dd	8a, 9e, 10a		1.58	10, 9e	4a
H-9e	1.40-1.43 m	8a, 8e, 9a, 10a		1.42	9a, 10	
H-10	1.96 br t	1a, 9a, 9e		3.21 b	1a, 1e, 9a, 9e	
H-12	5.87 d (12.5)	13		5.86 d (12.5 Hz)	13	
H-13	6.79 d (12.5)	12		6.80 d (12.5 Hz)	12	
H-15	7.12 d (2.2)	19	13	7.15 d (2.1 Hz)	19	4a, 3e
H-18	7.00 d (8.3)	19		7.01 d (8.29 Hz)	19	
H-19	7.19 dd (8.3, 2.2)	18, 15	13	7.21 dd (8.29, 2.1 Hz)	15, 18	
H-21	6.93 s			6.96 s		OMe
H-24	7.18 s		4a	7.23 s		6e, 3a
22-OMe ...	3.90 s			3.91 s		H-21

lation at C-22 is now thought to take place subsequent to the coupling of the aromatic rings (6,7), so that Ferris' suggestion, though correct, could not now have been made using the same logic.

The results of the analysis of the nmr spectrum of **4** are set out in Table 1. The relative positions of the OH and OMe groups were established again from the nOe interactions between H-24 (δ 7.18) and, in this case, both H-3a and 6e of the quinolizidine system, and between H-21 and the OMe group. Additional nOe interactions were again seen between H-15 (δ 7.12) and both H-3e and H-4a of the basic ring, confirming the conformation shown for **4**.

Molecular dynamics calculations were used to search for the most energetically favored conformations of both **4** and **6**, and the resulting minimum energy conformations were again minimized using semi-empirical MOPAC/AM1 calculations. The most favored conformation for both molecules, shown in Figure 1, is one in which the two aromatic rings form part of a right-handed helix: the dihedral angle C-17-C-16-C-20-C-21 is +63.9°; and in which the aromatic ring at C-4 is almost at right angles to the plane of the *trans*-quinolizidine system in **4**, and has a similar relative position in **6**: the angle C-24-C-25-C-4-N-5 is −50°. This conformation corresponds to the absolute stereochemistry determined previously for lythridine [**5**] by X-ray measurements (8) and from ord/cd work (9). The interatomic distances deduced from the modeling studies for the protons showing nOes in the nmr were: H-15–H-3e, 2.5 Å; H-15–H-4a, 2.7 Å; H-24–H-6e, 2.8 Å; H-24–H-3a, 2.4 Å; and H-21–OMe-22, 2.9 Å. The observed nOe interactions are sufficient to determine the conformations of both **4** and **6**, and although additional support could have been obtained, e.g., from a determination of the long-range benzylic couplings (H-4–H-24), we feel that the relative orientation of the rings is fully established.

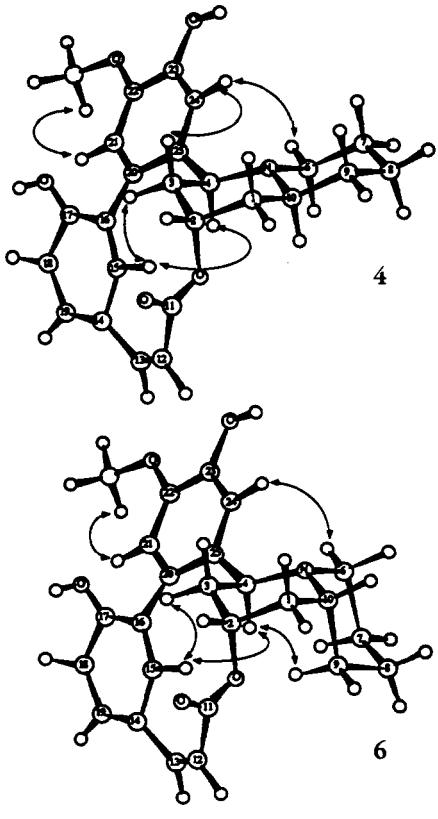


FIGURE 1. Conformations and nOe interactions for lyfoline [**4**] and 10-*epi*-lyfoline [**6**] (arrows indicate some important nOe interactions).

The observation that the monomethoxy-biphenylquinolizidine lactones, **4** and **6**, differ solely in the stereochemistry at C-10, and thus that they vary from the dimethoxybiphenylquinolizidine lactones, **1** and **2**, in the lack of methylation of OH-23, points to a possible reason for the observed variation in alkaloid accumulation in *H. montana* (1). The plant samples analyzed were either high-, low-, or non-yielding of **4** or **6**. Whereas *H. montana* of good content of **4** and **6** was a comparatively poor producer of the epimeric pair **1** and **3**, the inverse relation was observed with the *H. montana* samples of low- or undetectable-monomethoxy alkaloid content (1). Biosynthetic studies with *H. salicifolia* have shown that the *cis*- and the *trans*-

biphenylquinolizidines originate from a common metabolic pool and that **3** is derived from **4** (7, and references cited therein). Thus, the observed shift in alkaloid content from **6** and **4**, to **1** and **3**, might be due to one *O*-methyl transferase activity. A similar argument might be made to explain the lack of any observed accumulation of **6** in either *H. myrtifolia* or *H. salicifolia* (1).

EXPERIMENTAL

STANDARD COMPOUNDS.—10-*epi*-Lyfoline [**6**] was isolated from *H. montana* as described previously (1). The samples remaining after the preliminary work (1) were pooled and rechromatographed twice [Si gel 60 F_{254} $CHCl_3$ saturated with NH_4OH-MeOH (15:3)]. Lyfoline [**4**] was isolated from *H. salicifolia* in 1977; mp 164–166° (fine needles) from $CHCl_3$, [lit. (3), 223–224°, from cyclohexane]. (We have no reasonable explanation for the difference in mp).

NMR SPECTRA.—The high-resolution 1D and 2D nmr spectra were obtained in $CHCl_3$ solution on a Bruker WP-200SY spectrometer equipped with a $^1H/^{13}C$ dual probe and adequate software to perform 2D experiments.

COMPUTER MOLECULAR MODELING.—Molecular modeling and graphic display were performed on a Silicon Graphics Iris Indigo 2 workstation using the Tripos SYBYL molecular modeling package. The structure of lyfoline [**4**] was built at standard bond lengths and bond angles using the SYBYL software. Molecular mechanics/dynamics calculations were carried out to search the minimum energy conformation of the lyfoline [**4**] molecule, using time steps of 1 fsec for 300 psec with atomic coordinate trajectories recorded every 1 psec. The 300 frames recorded during the dynamics run were retrieved and minimized until the maximum derivative was less than 0.001 kcal $mole^{-1}$. The calculations were carried out in a vacuum condition. The low-energy conformations were then minimized using the AM1 Hamiltonian of MOPAC as implemented in the SYBYL program.

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