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ISOLATION AND STRUCTURAL CHARACTERIZATION OF LUTESSINE, A NEW ALKALOID FROM BULBS OF *STERNBERGIA LUTEA*

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ABSTRACT.—A new alkaloid named lutessine (**2**) has been isolated, together with its deacetyl derivative (**3**), from bulbs of *Sternbergia lutea*. The structures were elucidated by spectroscopic and chemical methods, and lutessine was identified as 1-*O*-acetyl-4-methoxylycorine.

As part of our studies on the chemical and biological properties of lycorine (**1**) (1-3), we have isolated from the bulbs of *Sternbergia lutea* Ker-Gawl (Amaryllidaceae) the alkaloids sternbergine (4) and hippamine (5), in addition to the six alkaloids previously described (6). More recently, 11-hydroxyvittatine has been found in the same plant (7). The present work describes the isolation and the identification of the new alkaloid lutessine (**2**), together with its deacetyl derivative (**3**), from the bulbs.

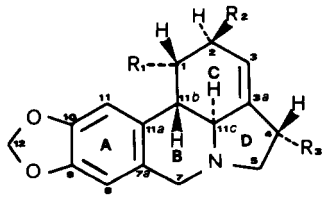
Lutessine (**2**), represents the first alkaloid that incorporates a lycorine-type skeleton but with a substituent on the D ring. It is interesting, therefore, to correlate this structural modification, in comparison with lycorine (**1**), in the inhibition of ascorbic acid biosynthesis in vivo (1). This bioassay of compound **2** and its deacetyl derivative is in progress.

The EtOAc fraction, obtained as previously reported (4) from the acid aqueous extracts of the bulbs of *S. lutea*, was shown by tlc analysis (SiO_2, $CHCl_3$-EtOAc-MeOH, 1:1:1) to contain three other products besides the known alkaloids. The three compounds all exhibited lower Rf values than lycorine.

Fractionation of the organic residue was performed by SiO_2 column chromatography, followed by purification on preparative tlc. The result was the isolation of the unknown alkaloid **2** and its deacetyl derivative **3**. Compound **2** crystallized from $CHCl_3$, while **3** was obtained as an oil.

Lutessine (**2**), showed in the mass spectrum a molecular ion peak at *m/z* 359 and significant peaks at *m/z* 358, 341, 310, 298, 279, 278, and 248. The two strongest peaks at *m/z* 279 and 278 were in agreement with the fragmentation scheme proposed for lycorine-type alkaloids (8, 9). The ir spectrum exhibited absorption frequencies at 3680 and 1730 cm^{-1}, characteristic of the presence of an hydroxyl and a carbonyl group.

A careful examination of the ^{1}H-nmr spectrum of **2** (Table 1) showed a lycorine-like pattern (10). In particular, the two singlets at δ 6.70 (H-8) and 6.44 (H-11) and the



1	R_1=OH	R_2=OH	R_3=H
2	R_1=OAc	R_2=OH	R_3=OCH_3
3	R_1=OH	R_2=OH	R_3=OCH_3
4	R_1=OAc	R_2=OH	R_3=H
5	R_1=OAc	R_2=OAc	R_3=OCH_3

TABLE 1. ^{1}H-nmr (270 MHz) of Lutessine (**2**), Its Derivatives **3** and **5** and 1-*O*-Acetyllycorine (**4**) [Chemical shifts are in ppm (δ) from TMS]

	2[a]	**3**	**4**	**5**
H-1[b]	5.60 m	4.62 m	5.50 m	5.70 m
H-2[b-c]	4.48 m	4.64 m	4.13 m	5.63 m
H-3[b-c]	5.72 m	5.59 m	5.56 m	5.94 m
H-4[c]	3.73 dd	3.77 dd	(2H) 2.59 m	3.71 dd
2H-5	3.99 ddd AX 3.50 ddd	4.06 ddd AX 3.55 ddd	3.32 ddd AX 2.35 br q	4.10 ddd AX 3.60 ddd
2H-7[b]	3.98 d AX 3.52 d	4.04 d AX 3.51 d	4.14 d AX 3.48 d	4.11 d AX 3.63 d
H-8[b-d]	6.70 s	6.65 s	6.60 s	6.70 s
H-11[b-d]	6.44 s	6.87 s	6.54 s	6.51 s
H-11b[c]	2.87 dd AX	2.69 dd AX	2.73 dd AB	2.99 dd AX
H-11c	4.07 dddd	3.83 dddd	2.84 d	3.98 dddd
2H-12	5.87 d AB 5.84 d	5.90 d AB 5.87 d	5.89 s —	5.93 d AB 5.91 d
OMe	3.45 s	3.42 s	—	3.52 s
MeCO	1.41 s	—	1.91 s	2.04 s
MeCO	—	—	—	2.00 s

J (Hz) **2, 3, 5**: 1,2=1.8; 1,4=3.1; 1,11b=1.8; 2,4=2.1; 3,5A=2.1; 3,5X=1.8; 3, 11c=2.4; 5A,5X=14.7; 5A,11c=5.8; 5X,11c=2.4; 7A,7X=12.8; 11b,11c=10.7; 12A,12BV=1.5; **4**: 1,2=1.5; 1,11b=2.2; 5A,5X=8.5; 5A,4=8.5; 5X,4=5.2; 7A,7X=14.0; 11b,11c=10.3.

[a]Run at 270 and 500 MHz.

[b]Assigned also by evidence obtained from ^{1}H-nOe difference spectra.

[c]Assignments confirmed by SFSD ^{13}C-nmr spectra.

[d]Assigned also in comparison with the data reported for lycorine (10).

two doublets of an AB system at δ 5.87 (H-12A) and 5.84 (H-12B) and comparison with the ^{1}H-nmr spectrum of the 1-*O*-acetyllycorine (**4**) (11) (Table 1), suggested the presence of an unalterated A and dioxolane ring system. In the same way, the appearance of the two doublets of an AX system at δ 3.98 (H-7X) and 3.52 (H-7A) and the multiplet of an olefinic proton at δ 5.72 (H-3) was consistent with the presence of both unchanged B and C rings. Conversely, the region of the aliphatic proton resonances showed marked modifications, with respect to that region of the spectrum of **4**. Moreover, a double doublet at δ 3.73 and a singlet at δ 3.45, assigned to H-4 and to a methoxyl group, respectively, were observed. The methoxyl group was located on the C-4 (D ring) by the evidence found in the ^{13}C-nmr spectrum of **2** (Table 2).

In fact, the carbon resonance data showed, as compared to those of **4** (Table 2), the presence of a signal at δ 78.8, attributed to a further oxygenated secondary carbon, and the absence of a signal at δ 28.5 due to an aliphatic methylene group. The signal typical for a methoxyl group appeared at δ 58.5. In addition, the signal at δ 78.8, a doublet in the single frequency off-resonance decoupled ^{13}C-nmr spectrum, collapsed into a singlet in the single frequency selective decoupled ^{13}C-nmr spectrum of lutessine, obtained by irradition of the double doublet present at δ 3.73 (H-4) in the ^{1}H-nmr spectrum.

In order to establish the relative configuration at C-4, some considerations were made on the coupling constant pattern of H-4. The long-range coupling with H-1 and H-2 ($J_{1\text{-}4}$=3.1 and $J_{2\text{-}4}$=2.1 Hz) was easily measured, but no coupling was observed between H-4 and both protons on C-5. This situation was consistent with a β configuration (*pseudo*-equatorial) of H-4 and consequently with an α configuration (*pseudo*-axial) of the methoxyl group. The dihedral angles according to this case (~80° between H-4 and each of two protons bonded to C-5) were measured by inspection of Dreiding models. The application of the Karplus relation (12) gave a set of theoretical coupling con-

TABLE 2. Carbon Shifts of Lutessine (**2**) and 1-*O*-Acetyllycorine (**4**) (Chemical shifts are in ppm (δ) from TMS)

	2	**4**
C-1	68.5 d	72.7 d
C-2[a]	67.3 d	69.4 d
C-3[a]	122.9 d	117.4 d
C-3a[b]	139.7 s	136.1 s
C-4[a]	78.8 d	28.5 t
C-5	61.8 t	56.8 t
C-7	54.4 t	53.6 t
C-7a[b]	130.1 s	129.3 s
C-8	108.1 d	107.3 d
C-9[b]	147.2 s	146.5 s
C-10[b]	145.9 s	143.7 s
C-11	104.1 d	104.9 d
C-11a[b]	128.6 s	127.2 s
C-11b[a]	39.4 d	39.2 d
C-11c	63.0 d	61.5 d
C-12	100.9 t	100.9 t
OMe	58.5 q	—
C=O	170.7 s	171.8 s
CH_3	19.9 q	20.9 q

[a]Assignments confirmed by single frequency selective decoupled spectra.

[b]Assigned by comparison with data reported in the literature for lycorine (10).

stants very close to the values experimentally obtained. Further, a β configuration of H-4 does not allow its allylic coupling (12, 13) with H-3, as may be deduced from the multiplicities of both protons. The reversed situation with an α configuration of H-4, which should raise larger coupling constants between this proton and those on C-5 and allylic coupling with H-3, was discarded.

This result was confirmed by the probable formation of a hydrogen bond between H-11c and the methoxyl group which justifies the downfield shift (14) of H-11c (Δδ 1.23), with respect to the resonace frequency observed for this proton in the spectrum of **4**.

The acetyl group was located on C-1 by the evidence obtained from the ^{1}H-nOe difference spectra (Table 3) carried out on **2**. The results a, b, and c demonstrated the spatial proximity between H-1 and both H-11 and H-11b. According to this result, an upfield shift (Δδ 0.98) for H-1 and a downfield shift (Δδ 0.43) for H-11 were measured in the ^{1}H-nmr spectrum of **3** as compared to the values observed in **2**, while the chemical shift of the reamining protons was unchanged.

The other results summarized in Table 3 also confirmed the spatial proximity between H-8 and H-7X (result d) and H-2 and H-3 (results e and f).

TABLE 3. Nuclear Overhauser Effects Measured on Lutessine (**2**)

Irradiated	Result	Observed
6.44 (H-11)	a	5.60 (H-1)
5.60 (H-1)	b	6.44 (H-11)
	c	2.87 (H-11b)
6.70 (H-8)	d	3.98 (H-7X)
5.72 (H-3)	e	4.48 (H-2)
4.48 (H-2)	f	5.72 (H-3)

On the basis of these findings, lutessine was assigned the structure of an 1-*O*-acetyl-4-methoxylycorine (**2**).

The ^{13}C-nmr data (Table 2), obtained using the proton noise decoupling (pnd), the single frequency off-resonance decoupling (sford), and the single frequency selective decoupling (sfsd) techniques, confirmed the structure assigned.

Support for the structure **2**, attributed to lutessine, was furnished from the two derivatives **3** and **5** prepared by synthesis from **2**. Lutessine, by treatment with pyridine and Ac_2O, gave the corresponding acetyl derivative **5**. Compound **5** showed a molecular ion at *m/z* 401 in the ms spectrum; its ^1H-nmr spectrum exhibited, in respect to that of **2**, the downfield shift ($\Delta\delta$ 1.15) of the multiplet due to H-2, which appeared at δ 5.63, as the only difference.

Reaction of **2** with ethanolic KOH yielded the deacetyl derivative **3**, which showed a molecular ion at *m/z* 317 in the ms spectrum; in the ^1H-nmr spectrum, as compared to that of **2**, the upfield shift (as described above) of the signal attributed to H-1 which appeared at δ 4.62 was observed.

This latter compound was identical by spectral and physical data ($[\alpha]^{25}D$, uv, ir, ms, and ^1H-nmr) with natural **3**; furthermore, the two compounds showed the same Rf value in three different tlc systems.

Compound **3** appeared to be an alkaloid naturally occurring and not an artifact formed by deacetylation of **2** during the acid extraction of bulbs of *S. lutea*. In fact, no hydrolysis was observed when **2** was treated with 1% H_2SO_4 as was followed in the extraction procedure.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected; optical rotations were measured on a Perkin-Elmer 141 polarimeter; ir spectra were recorded on a Perkin-Elmer 684 instrument for solutions in $CHCl_3$; uv spectra were measured on a Perkin-Elmer 550 S spectrophotometer on solutions in EtOH. ^1H- and ^{13}C-nmr spectra were recorded at 500 or 270 and at 67.88 MHz, respectively, on a Bruker spectrometer for solutions in $CDCl_3$. Mass spectra were performed on a Kratos MS-80 mass spectrometer with electron impact ionization at 70 eV. Analytical and preparative tlc were performed on SiO_2 plates (Merck, Kieselgel 60 F_{254}, 0.25 and 2 mm, respectively); the spots were visualized by exposure to I_2 or uv radiation. Column chromatography was carried out on SiO_2 (Merck, Kieselgel 60, 0.063-0.2 mm).

PLANT MATERIAL.—*S. lutea* was collected during the withering period near Bari and identified by Prof. O. Arrigoni of the Istituto di Botanica, Universitá di Bari, Italy (where a voucher specimen has been deposited).

EXTRACTION AND FRACTIONATION OF ALKALOIDS.—Most experimental details concerning the extraction procedure of dried bulbs of *S. lutea* have been described (4). The residue (1.680 g), left from the EtOAc extracts of the plant material (1 kg), was fractionated on a SiO_2 column. Early elution with $CHCl_3$-EtOAc-MeOH (1:1:1) afforded fractions (220 mg) yielding abundant lycorine (**1**) (15) and lutessine (**2**). The successive eluate contained a small amount of **2** but mostly 11-hydroxyvittatine and the deacetyl derivative of **2** (**3**) (43.2 mg). Further elution, with the same solvent, gave only 11-hydroxyvittatine and compound **3** (51.4 mg). The three mixtures, obtained from the homogeneous fractions by evaporation of the solvent, were further purified. Combination of column and tlc on SiO_2, using $CHCl_3$-EtOAc-MeOH (1:1:1) as eluent, afforded the following compounds all as pure oils: lutessine (29.5 mg, 0.003%), 11-hydroxyvittatine (18.6 mg, 0.0019%) and product **3** (16.4 mg, 0.0016%).

Lutessine (1-O-acetyl-4-methoxylycorine) (**2**).—Lutessine crystallized from $CHCl_3$: mp 114-118°; $[\alpha]^{25}D$ −13.4° (c 1.24, EtOH); uv λ max nm (log ϵ) 290 (3.52), 235 (3.54); ir υ max 3680, 1730, 1600, 1505, 1485, 1100 cm^{-1}; ^1H- and ^{13}C-nmr spectra are reported in Tables 1 and 2, respectively; ms *m/z* (rel. int.) 359 (M^+) (1.2), 358 (2.7), 341 (13), 310 (18), 298 (41), 279 (64), 278 (100), 248 (51), 43 (89).

Deacetylation of lutessine to **3**.—Lutessine (10.5 mg) was hydrolyzed under reflux in 5% ethanolic KOH for 0.5 h. After cooling, the mixture was cautiously neutralized with 2 N HCl. The NaCl was removed by filtration, and the filtrate was evaporated under reduced pressure. Purification of the residue by column chromatography ($CHCl_3$-EtOAc-MeOH, 1:1:1) afforded **3** as pure oil (7.9 mg, 85%): $[\alpha]^{25}D$ −42.9° (c 0.35 $CHCl_3$); uv λ max nm (log ϵ) 290 (3.71), 233 (3.78); ir υ max 3695, 1600, 1505, 1485, 1100 cm^{-1}; ^1H-nmr spectrum is reported in Table 1; ms *m/z* (rel. int.) 317 (M^+) (5.3), 316 (21), 299 (36), 278 (49),

268 (100). Compound **3** and the deacetyl derivative of **2**, naturally occurring, showed the same Rf value by analysis in three tlc systems [SiO_2, $CHCl_3$-EtOAc-MeOH, 1:1:1; $CHCl_3$-MeOH, 6:4, and on reverse phase (Stratocrom, Whatman 0.2 mm) H_2O-MeCN, 6:4] also by co-chromatography.

2-O-acetyllutessine (**5**).—Acetylation of **2** (12 mg) was performed in the usual conditions with Ac_2O (200 μl) and pyridine (200 μl). After 12 h, the reaction was stopped with MeOH and the solvent evaporated under reduced pressure. The pyridine was removed by evaporation of the azeotrope formed with C_6H_6. Purification on column chromatography ($CHCl_3$-iPrOH, 95:5) afforded crystalline **5** (8.8 mg, 73%): mp 164-168°; $[\alpha]^{25}D$ −117.8° (c 0.87, $CHCl_3$); uv λ max nm (log ε) 286 (3.69), 225 (3.95); ir υ max 1740, 1730, 1600, 1505, 1485, 1100 cm^{-1}; ^{1}H-nmr spectrum is reported in Table 1; ms *m/z* (rel. int.) 401 (M^+) (0.06), 400 (0.1), 371 (0.5), 341 (9.8), 310 (11), 278 (100), 248 (54), 43 (45).

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