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FURTHER EXPERIMENTS ON STRUCTURE–ACTIVITY RELATIONSHIPS AMONG THE LYCORINE ALKALOIDS

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Key Word Index—*Sternbergia lutea*; Amaryllidaceae; ascorbate biosynthesis inhibition; phenanthridine alkaloids; lycorine; narciclasine; structure–activity relationships.

Abstract—Synthetic lycorine analogues, five Amaryllidaceae alkaloids and narciclasine, all structurally related to lycorine, were tested for their ability to inhibit ascorbic acid biosynthesis *in vivo*. The highest potency observed was displayed by narciclasine followed by compounds having an aromatic C-ring. Derivatives modified at C-1 and/or C-2 were inactive, while the compound with a double bond between these positions is a weak inhibitor. Also lutessine and its deacetyl derivative having an α-methoxyl group bonded to C-4 of the D-ring appeared completely inactive. These results confirm that the presence of an appropriately substituted C-ring is a necessary requirement for optimal 'response-triggering' contact between the lycorine derivatives and the specific receptor. Functional groups jutting out from the α-side of the molecule do not allow a good fit with the binding sites.

INTRODUCTION

Among the phenanthridine alkaloids [1], lycorine (**1**) [2] can be considered the most important because of its biological activities such as inhibition of growth and cell division in higher plants and algae [3], its inhibition of cyanide-resistant respiration [4] and of peroxidase enhancement [5]. All the effects of lycorine on physiological processes have been ascribed to its ability to inhibit ascorbic acid (AA) biosynthesis *in vivo* [6].

In previous work, some lycorine derivatives and related alkaloids were tested as inhibitors of AA biosynthesis in potato tubers [7]. It was observed that the structural features essential to preserve the inhibitory activity of AA biosynthesis were an integral A and B-ring system, a β-configuration of the D-ring when the C/D ring junction changes and the presence of an electrophilic site located between C-1 and C-2 of the C-ring [7].

The preliminary screening data, obtained with 23 alkaloids with appropriate structural modifications, have prompted the examination of an additional 17 compounds for their inhibitory effect on AA biosynthesis. This paper reports the results of the bioassay of 11 lycorine derivatives, prepared from **1**, five Amaryllidaceae alkaloids and narciclasine (**18**) [8, 9], a strongly antimitotic substance; all the compounds tested were structurally related to **1**.

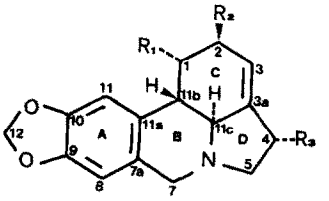
RESULTS AND DISCUSSION

In the previous study [7] on the inhibition of ascorbic acid biosynthesis by lycorine derivatives and Amaryllidaceae alkaloids, it was found that the inhibitory effect was preserved when the A and B-rings were unchanged, while the dioxole ring was not involved. Moreover, derivatives possessing an electron donor group at C-1 and C-2 were good inhibitors, whereas those having modifications of the glycol system lacked this activity. A large decrease in activity was also observed when alkaloids with an α-configuration of the D-ring associated with a modification of the C/D ring junction were tested.

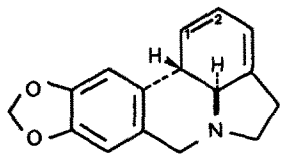
The present investigation was undertaken in order to clarify further the structural features promoting the inhibition of AA biosynthesis *in vivo*. The inhibitory activity was examined of lycorine derivatives, prepared by synthesis from lycorine, five Amaryllidaceae alkaloids and narciclasine (**18**), this last being a neutral substance extracted from various *Narcissus* species and structurally related to **1** [8, 9]. In addition, compounds **10** and **11**, obtained from lycorine by microbiological degradation [10], were screened. These compounds, as well as the hydrochloride of **10** (**12**) and its structural isomer **13** [11], were prepared by synthesis from **1**.

In order to define further the role of the functional groups linked to C-1 and C-2 of the C-ring, six alkaloids were tested. They showed, when compared to **1**, the following modifications: dehydroxylation or chlorination at C-2, dehydroxylation at both C-1 and C-2, methylation of the hydroxyl group at C-2, and the presence of an epoxy group or a double bond between C-1 and C-2. The role of the D and dioxole rings was investigated by assaying alkaloids having either an α-substituent on the D-ring or an open dioxole ring carrying a methoxyl group at C-9 and a hydroxyl group at C-10 or vice versa. Furthermore, derivatives having the C-ring or both B and C-rings aromatized were tested, as was narciclasine which contains a more highly hydroxylated C-ring than **1**.

Inhibition by natural and chemically modified compounds was measured over the range 5–20 μM; in each case, dose–response effects were observed. Inhibitory activity was compared at a standard concentration of 20 μM (Table 1). The removal of the hydroxyl group from C-2, as in caranine (**2**) [12], and from C-1 and C-2, as in lycorene (**4**) [12], caused total loss of activity. Methylation



1	R_1=R_2=OH,	R_3=H		**5**	R_1=OH,	R_2=OMe,	R_3=H
2	R_1=OH,	R_2=R_3=H		**6**	R_1=OAc	R_2=OH,	R_3=OMe
3	R_1=OH,	R_2=Cl,	R_3=H	**7**	R_1=R_2=OH,		R_3=OMe
4	R_1=R_2=R_3=H						



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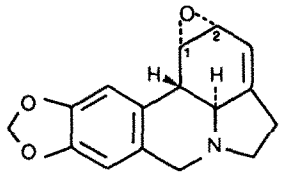

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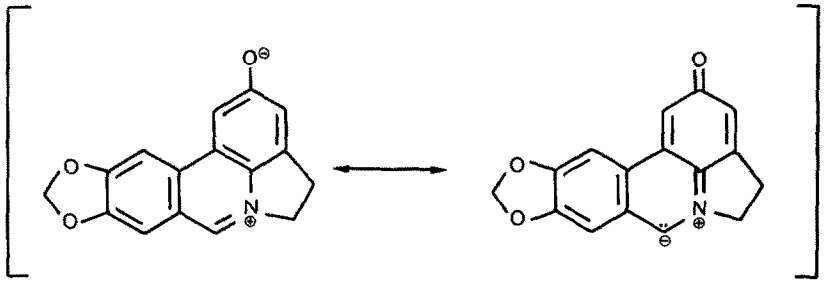

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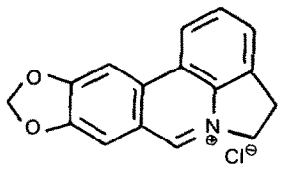

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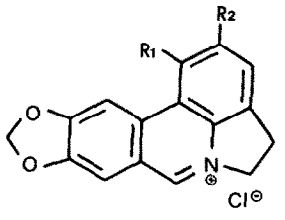
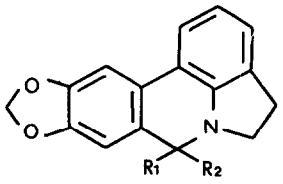
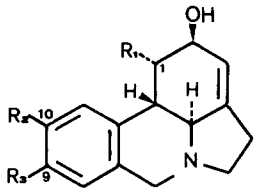


12 R_1=H, R_2=OH
13 R_1=OH, R_2=H



14 $R_1=R_2=H$
15 $R_1+R_2=O$



16 R_1=OAc, R_2=OMe, R_3=OH
17 R_1=OH, R_2=OH, R_3=OMe

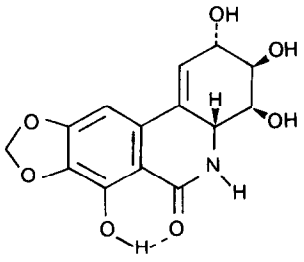


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Table 1. Inhibition of ascorbic acid biosynthesis by lycorine derivatives, naturally occurring alkaloids of the Amaryllidaceae and narciclasine*

Compounds	% Inhibition of ascorbic acid biosynthesis		
	5 μM	10 μM	20 μM
1	18	45	88
2	0	0	9
3	0	13	23
4	0	0	0
5	0	40	53
6	0	0	9
7	0	0	0
8	7	24	27
9	7	25	41
10	61	96	—
11	67	100	—
12	31	35	34
13	52	78	93
14	51	97	—
15	41	45	63
16	0	57	57
17	36	49	66
18	100	—	—

*The relative inhibition given for each compound, under standard conditions, is reported. The data are the mean of at least three experiments run in triplicate.

of the C-2 hydroxyl group, as in hippamine (**5**) [13], decreased activity slightly, whereas the substitution of the same group by the electron-attracting chlorine produced a large decrease in activity, as observed in the bioassay of lycorine chlorohydrin (**3**) [14]. These results confirmed the importance of the glycol system located between C-1 and C-2 for activity.

A large decrease in activity was observed when 1,2-α-epoxylycorine (**8**) was tested [15]. Since this functional group is similar to that of the glycol system, this result could be due to the α-configuration of the epoxy ring. The low inhibition recorded when testing derivative **9** [14] indicated that the presence of a double bond between C-1 and C-2 satisfied in part the requirement for an electrophilic site in this position. Therefore, the high activity possessed by buphanisine, another Amaryllidaceae alkaloid with a double bond at the 1,2-position of the C-ring [7], was attributed to the following structural features: (a) a conformational freedom in ring C greater than that shown in **9**, due to the absence in buphanisine of the 3,3a-double bond and by the different C/D ring junction; and (b) the β-configuration assumed by the D-ring.

Derivatives containing an aromatic C-ring, i.e. anhydrolycorine (**14**) [16] and compounds **10** [10, 11] and **11** [10, 14], showed a great increase in activity. The presence of an azomethyne group (N=CH-7) in **10** and **11** did not affect the activity as compared to that of **14**. Conversely, the presence of a carbonyl group located at the same position of the B-ring (O=C-7), associated with an amide nitrogen atom, as in anhydrolycorine lactam (**15**) [16], caused a substantial decrease in activity. A similar result was previously observed when the activity of dihydrolycorine lactam was compared with that of the corresponding tertiary base [7].

These data indicate that aromatization of the C-ring, enhancing its electrophilic nature, greatly increases the activity. But when a phenolic group is substituted in the aromatic C-ring, the inhibition is only preserved when it is in the phenate form, as the betaine **10**. In fact, an undissociated phenolic group, as in **12** [10] and its structural isomer **13** [10], leads to a marked decrease in activity. Thus it appears that an electrostatic interaction occurs between the phenate ion or an equivalent electron-donating group and a positively charged group of the receptor.

The effect of substituents in the D-ring was tested with the new alkaloid lutessine (**6**) [17] and the corresponding deacetyl derivative **7** [17]. Both alkaloids lacked activity; this result was not due to the presence of a methoxyl group on C-4, but to its α-configuration. This deduction was corroborated by the low activity previously displayed by haemanthamine and haemanthidine [7], both having the D-ring with an α-configuration associated with a modification of the C/D ring junction. Thus inhibitory activity requires that the α-side of the molecule is unsubstituted, allowing a good fit with the binding sites of the specific receptor.

A methylenedioxy ring at C-9 and C-10 is not essential for activity since the new alkaloid sternbergine (**16**), extracted from *Sternbergia lutea* [18], and its deacetyl structural isomer pseudolycorine (**17**) [19] are both active. The acetyl group at C-1 present in **16** does not affect its activity since this alkaloid is easily hydrolysed in weak acid aqueous solution [18].

Narciclasine (**18**), although it does not belong to the alkaloid family, was also tested. This compound, which occurs in various members of the Amaryllidaceae, is not a base but it is structurally related to **1** and is biologically active [8, 9]. Of all the compounds tested, **18** is the strongest inhibitor of AA biosynthesis. This surprising result is due to the presence in **18** of a trihydroxylated C-ring which has greater conformational freedom larger than that exhibited in **1**. This steric situation in **18** is determined by the absence of the D-ring and by the different position of the double bond located in **1** between C-3 and C-3a. The presence of an amide group on the B-ring and of a substituent group on the A-ring lowered the activity of lycorine analogues [7]; these effects were probably not evident in narciclasine owing to the high activity exhibited by this compound. On the other hand, the hydrogen bond formed between the carbonyl and the phenolic hydroxyl group might also mask their specific chemical nature.

In conclusion, these data confirm that the inhibitory activity on AA biosynthesis is determined by the presence of an appropriately substituted C-ring and by a free α-side of the molecule. Moreover, narciclasine, which seems to possess all the structural features necessary for activity, appears to be a stronger inhibitor than lycorine.

EXPERIMENTAL

General methods. Optical rotations were measured in $CHCl_3$ unless otherwise noted. Mps are uncorr.; IR and UV spectra were recorded in $CHCl_3$ and EtOH soln, respectively; ^{1}H NMR spectra were recorded at 270 MHz in $CDCl_3$, using TMS as internal standard. MS were recorded by electron impact at 70 eV. Analytical and prep. TLC was carried out on silica plates (Merck, Kieselgel 60, F_{254}) 0.25 and 2.0 mm, respectively; the spots were visualized by exposure to I_2 vapour or UV radiation. CC was performed on silica gel (Merck, Kieselgel 60, 0.063–0.20 mm). Solvent systems: (A) $CHCl_3$–*i*-PrOH (19:1); (B) $CHCl_3$–EtOAc–MeOH (2:2:1); (C) $CHCl_3$–*i*-PrOH (9:1).

Inhibition in vivo of ascorbic acid biosynthesis. The activities of the compounds were assessed by their ability to inhibit ascorbic acid biosynthesis *in vivo* as previously described for lycorine and related compounds [7].

Chemicals. Lycorine (**1**) was obtained from dried bulbs of *Sternbergia lutea* Ker Gawl by extraction carried out according to the ref. [20]. The alkaloids hippamine (**5**), lutessine (**6**) and its deacetyl derivative (**7**), and sternbergine (**16**) were obtained according to the procedures reported in refs. [13], [17] and [18], respectively. Compounds **10**, **11**, **12**, **13**, anhydrolycorine (**14**) and anhydrolycorine lactam (**15**) were prepared from lycorine according to the methods described in our paper and in the refs. therein [10].

Caranine (**2**) *and lycorene* (**4**). Lycorine (1 g) in *n*-amyl alcohol (50 ml) was converted into caranine and lycorene according to the procedure of Fales and Wildman [12]. The crude oil (468 mg), obtained after work-up of the reaction mixture, was purified by CC. Elution with solvent B gave fractions containing only lycorene (**4**), which crystallized by evaporation of the solvent (101 mg): mp 112–116° (lit. [12]: mp 118–120°); UV λ_{max} nm: 290 and 237 (log ε 3.67 and shoulder, respectively); IR ν_{max} cm^{-1}: 1605, 1505, 1485 (C=C); ^{1}H NMR: see Table 2; MS *m/z* (rel. int.): 255 $[M]^+$ (43), 254 (100), 252 (26), 250 (15), 227 (21) and 226 (35). The fractions, collected successively, afforded on evaporation of the solvent a crude solid (36.4 mg). Caranine (**2**), crystallized from Me_2CO, mp 176–178° (lit. [12]: mp 176–180°); UV λ_{max} nm: 291, 280 and 237 (log ε 3.44, 3.49 and 3.49, respectively); IR ν_{max} cm^{-1}: 3680 (3.28 μ, OH), 1605, 1505, 1485 (C=C) (lit. [14]): UV λ_{max}^{EtOH} nm: 291 and 240 (log ε 3.67 and 3.55, respectively); IR ν_{max}: 3.13 μ (OH); ^{1}H NMR: see Table 2; MS *m/z* (rel.

Table 2. ^{1}H NMR spectral data of alkaloids **2**, **3**, **4**, **8** and **9** [chemical shifts are in ppm (δ) from TMS]

H	2	3		4	8	9
1	4.70 *m*	4.78 *dd*	H-1A	2.54 *m*	4.64 *dd*	6.11 *br s*
			H-1B	2.37 *m*		
2	2.59 *m*	4.65 *m*	H-2A	2.54 *m*	5.01 *m*	6.11 *br s*
			H-2B	2.30 *m*		
3	5.41 *m*	5.56 *m*		5.44 *m*	5.45 *m*	5.82 *m*
4 (2H)	2.59 *m*	2.63 *m*		2.35 *m*	2.62 *m*	2.70 *m*
5X	3.32 *ddd*	3.35 *ddd*		3.23 *ddd*	3.36 *ddd*	3.35 *ddd*
5A	2.33 *br q*	2.37 *br q*		2.32 *br q*	2.40 *br q*	2.43 *br q*
7X	4.13 *d*	4.13 *d*		4.11 *d*	4.12 *d*	4.10 *d*
7A	3.52 *dd*	3.51 *d*		3.54 *dd*	3.52 *d*	3.60 *dd*
8*	6.58 *s*	6.59 *s*		6.58 *s*	6.56 *s*	6.59 *s*
11*	6.82 *s*	6.79 *s*		6.77 *s*	6.79 *s*	6.88 *s*
11b	2.41 *ddd*	2.85 *dd*		2.45 *ddd*	3.11 *dd*	2.80 *dd*
11c	2.78 *dd*	2.99 *d*		2.43 *dd*	2.82 *d*	3.42 *d*
12A	5.92 *d*	5.93 *d*		5.92 *d*	5.93 *d*	5.93 *d*
12B	5.90 *d*	5.91 *d*		5.90 *d*	5.90 *d*	5.91 *d*

J (Hz): **2**, **3**, **4**, **8**, **9**: 7A, 7X = 14.0; 12A, 12B = 1.5; **2**, **3**, **4**, **8**: 1, 11b = 2.0; 5A, 5X = 9.2; 11b, 11c = 10.7; **2**: 3, 11c = 2.6; 7A, 11b = 2.2; **3**: 1, 2 = 2.2; 2, 3 = 3.2; **4**: 3, 11c = 7A, 11b = 2.2; **8**: 1, 2 = 3.7; 2, 3 = 2.9; **9**: 5A, 5X = 8.0; 7A, 11b = 1.8; 11b, 11c = 17.7.

*Attributed by comparison with the data reported for lycorine [21].

int.): 271 $[M]^+$ (91), 270 (55), 252 (64), 227 (64) and 226 (100).

Lycorine chlorohydrin (**3**) *and* 1,2-α-*epoxylycorine* (**8**). Compounds **3** and **8** were prepared by treating lycorine (0.5 g) with $POCl_3$ [14]. The crude residue (274 mg), obtained by work-up of the reaction mixture, was fractionated by CC (eluant A). The first substances eluted were anhydrolycorine (**14**) and the corresponding lactam (**15**). Further elution with the same solvent gave fractions containing only compound **8**. Evaporation of the solvent afforded **8** as a pure oil (6.4 mg), resistant to crystallization: $[\alpha]_D^{25} - 194.4°$ (*c* 0.09); UV λ_{max} nm: 292, 281 and 235 (log ε 3.75, 3.79 and shoulder, respectively) (lit. [15]: $[\alpha]_D^{25} - 192°$ (*c* 0.76; $CHCl_3$); UV λ_{max}^{EtOH} nm: 290 and 235 (log ε 3.74 and shoulder respectively); IR ν_{max} cm^{-1}: 1620, 1505, 1485 (C=C); ^1H NMR: see Table 2; MS *m/z* (rel. int.): 269 $[M]^+$ (2), 265 (12), 250 (100), 227 (1.8) and 226 (2.0). The successive eluate contained only **3**, which was obtained as a pure oil (11.2 mg) by evaporation of the solvent. It had UV λ_{max} nm: 290 and 280 (log ε 3.54 and 3.60, respectively) (lit. [14]: UV λ_{max}^{EtOH} nm: 292 (log ε 3.68)); IR ν_{max} cm^{-1}: 3520 (OH), 1625, 1505, 1485 (C=C); ^1H NMR: see Table 2, MS *m/z* (rel. int.): 307 $[M+2]^+$ (3.6), 305 $[M]^+$ (10), 270 (20) 250 (100), 227 (8.7) and 226 (11).

Compound **9**. Compound **9** was prepared from crude lycorine chlorohydrin (**3**) (270 mg) by reflux with Zn in HOAc [14]. The crude residue (264 mg), obtained by work-up of the reaction mixture, was fractionated by CC. The first product to be eluted with solvent C was **9**. Evaporation of the solvent gave **9** as a pure oil (7.2 mg): $[\alpha]_D^{25} - 284°$ (*c* 0.30; EtOH); UV λ_{max} nm: 290, 278 and 235 (log ε 3.73, 3.73 and 3.85, respectively) (lit. [14]: $[\alpha]_D^{25} - 349.8°$ (*c* 1.423; EtOH); UV λ_{max} nm: 292 and 235 (log ε: 3.82 and 3.78, respectively); ^1H NMR: see Table 2; MS *m/z* (rel. int.): 253 $[M]^+$ (21), 252 (52), 250 (100) and 227 (22).

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