Phytochemistry, Vol. 22, No. 10, pp. 2193–2196, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00
Pergamon Press Ltd.

LYCORINE STRUCTURE–ACTIVITY RELATIONSHIPS*

ANTONIO EVIDENTE, MARIA ROSARIA CICALA, GIACOMINO RANDAZZO, RODOLFO RICCIO, GIUSEPPE CALABRESE†, ROSALIA LISO† and ORESTE ARRIGONI†

Istituto di Chimica Organica e Biologica, Università di Napoli, Via Mezzocannone 16, 80134 Napoli, Italy; †Istituto di Botanica, Università di Bari, Via Amendola 175, 70126 Bari, Italy

(*Received* 21 *January* 1983)

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

Abstract—Twenty three lycorine derivatives and naturally occurring alkaloids, structurally related to lycorine, were tested for their ability to inhibit ascorbic acid biosynthesis in potato tubers. The following relationships between structure modification and activity were observed: (a) cleavage of the acetalic bonds on the dioxole ring had no effect on activity; (b) derivatives with a methoxy group on C-8 (A ring) were inactive; (c) oxidation of NCH_2-7 to an amide group (B ring) caused loss of activity; (d) modification of the C/D ring junction had no effect on activity when the D ring assumed a β configuration whereas a great decrease of activity was observed when that ring assumed an α configuration; (e) selective or complete acetylation of hydroxyl groups of the C ring, epimerization or oxidation of the hydroxyl group on C-2 led to a loss of activity; (f) a compound with a double bond located in the 1,2 position showed activity almost identical to lycorine; (g) stereoselective hydrogenation of the double bond of the C ring induced a considerable increase of the activity; (h) protonation of the nitrogen atom had no effect on activity.

INTRODUCTION

The effects of lycorine **1** [1], an alkaloid extracted from various Amaryllidaceae [2], on several important physiological processes such as inhibition of growth and cell division in higher plants, algae and yeasts [3], inhibition, at low concentration (10^{-6} M) of ascorbic acid biosynthesis [4], prevention of cyanide-insensitive respiration [5], have made this substance a valuable tool for studying a number of biological effects. Thus, investigations on the mechanism of action of lycorine *in vivo* and *in vitro* are actively pursued.

This paper describes the results of a systematic investigation of the inhibitory activity of synthetic lycorine derivatives and naturally occurring Amaryllidaceae alkaloids on ascorbic acid biosynthesis.

RESULTS AND DISCUSSION

Studies on structure–activity relationships in this family of alkaloids were feasible as a large number of chemically modified lycorine derivatives had been prepared and several naturally occurring Amaryllidaceae alkaloids were available.

In order to clarify the structure–activity relationships the following structural modifications were tested: cleavage of acetal bonds on to the dioxole ring; substitution by 8-methoxylation of the aromatic ring (ring A) and 7-oxidation of the B ring; change of the C/D ring junction combined with variation of the configuration of the D ring; 1 and/or 2-acetylation, 2-oxidation, 2-epimerization and the presence, in the 1,2-position, of a double bond or an epoxy group on the C ring; α and β hydrogenation of the double bond located in the 3,3a-position; protonation of the nitrogen atom.

Twenty-three compounds representing the above modifications were tested for their inhibitory effects on ascorbic acid biosynthesis in potato tubers [4]. The structural variations were tested, routinely, in turn but sometimes simultaneously. Inhibition by natural and chemically modified alkaloids were determined over the range 5–40 μM; in each case dose-response effects were observed. However, comparison of the inhibitory activity was carried out at a standard concentration of 20 μM. The results are summarized in Table 1. Only compounds **15** and **18** showed stimulation of ascorbic acid biosynthesis. It was apparent that the differences in activity were not random, but were rather determined systematically by the nature and combination of the structural modifications.

Protonation of the nitrogen atom (lycorine HCl, [6]) did not modify the inhibitory activity of lycorine. It was therefore possible for us to bioassay the alkaloids as bases or as salts. Cleavage of the acetal bonds and the consequent opening of the dioxole ring (galanthine **13**, [2] and methylpseudolycorine **14**, [2]) caused a slight decrease in activity. Therefore the total loss of the inhibitory activity showed by norpluviine (**15**, [2]) was due to the absence of the 2-hydroxyl group in this compound. This result was unaffected by the different nature of the substituent group on C-9 (cf. activity of **15** with those of **13** and **14**). Substitution on C-8 of the aromatic ring with a methoxy group gave derivatives which were devoid of activity. In fact, ambelline (**20**, [2]), an alkaloid of the crinine-type, had no biological effect, whereas buphanisine (**19**, [2]), an alkaloid belonging to the same group, was almost as active as lycorine. The different nature of the substituent group on C-11 (D ring), present in these

*This work was supported by Italian National Research Council (CNR).

Table 1. Inhibition of ascorbic acid biosynthesis in the presence of lycorine derivatives and naturally occurring Amaryllidaceae alkaloids

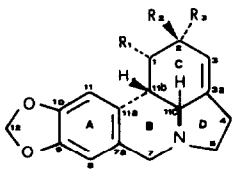
Alkaloid	% Inhibition of ascorbic acid biosynthesis				
	5 μM	10 μM	15 μM	20 μM	40 μM
1	18	45	55	88	—
1 HCl	8	24	55	83	—
2	0	5	11	22	—
3	0	0	19	26	—
4	—	—	—	12	14
5	13	13	—	16	—
6	0	0	—	12	—
7	10	33	68	100	—
8	8	40	—	95	—
9	0	14	—	17	—
13	45	59	—	63	—
14	48	75	—	70	—
15	+7	+7	—	2	—
16 HCl	10	26	36	38	—
17 HCl	0	6	15	22	—
18 HBr	+16	+21	—	+38	—
19	50	62	—	80	—
20	0	0	—	0	—
21	6	16	—	32	—
22	17	20	—	23	—
23	0	12	16	30	—
24	0	0	0	10	—
25 HBr	—	—	—	6	23

The relative inhibition given from each compound, under standard conditions, is reported. Stimulation was observed in the cases of compounds **15** and **18**, the values represent the relative increase of ascorbic acid biosynthesis. The data are the mean of three experiments run in triplicate at least.

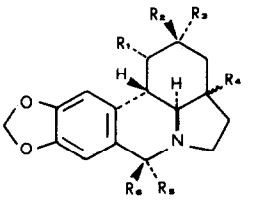
latter two compounds, as negligible. The result demonstrated that modification of the C/D ring junction combined with the D ring β configuration did not cause variation in activity. Therefore the substantial decrease in activity measured in the undulatine (**21**, [2]) bioassay was probably due to 8-methoxylation and was independent from the presence of the 1,2-epoxy group.

Modification of the C/D ring junction did not affect the biological properties of these compounds, therefore, the great decrease of inhibitory activity found in testing haemanthamine (**22**, [2]) and the consistent decrease observed in the haemanthidine (**23**, [2]) bioassay were ascribed to the α configuration of the D ring. The presence of either hydrogen or a hydroxyl group on C-6 (D ring) of these two alkaloids was of secondary importance. Furthermore, inspection of Dreiding models showed that lycorine and buphanisine possessed a plane molecule with low conformational freedom. Moreover, they had the same structure and configuration, relative to the α side of the molecule, except for the D ring. This latter result suggested that the D nucleus was of little importance with regard to inhibitory activity and that substitution of the 1,2-glycol system (**1**) with a 1,2-double bond (**19**) did not affect the activity.

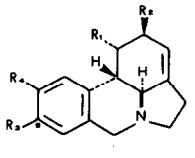
The 1,2-diol system of the C ring was essential for the inhibitory activity of **1**. Modifications such as 1- and/or 2-acetylation (**3**, [7]; **2**, [7]; **4**, [1]), 2-oxidation (**5**) and 2-epimerization (**6**, [7]) caused the loss of the activity. In contrast, hydrogenation of the 3,3a-double bond yielded, by two different routes, the α (**7**, [7]) and β (**8**) dihydro derivative of **1** both of which were more active than lycorine. These results were attributed to a larger conformational freedom of **7** and **8** as compared to **1**. Therefore, in this study of ascorbic acid biosynthesis inhibition, the **7** and **8** derivatives of lycorine were the best inhibitors. The substantial decrease in activity observed with the α-dihydro-lycorine-lactam (**9**, [8]) as compared to **7** dem-



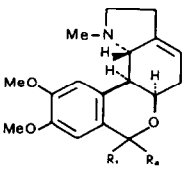
1 R_1= R_2= OH, R_3= H
2 R_1= R_2= OAc, R_3= H
3 R_1= OAc, R_2= OH, R_3= H
4 R_1= OH, R_2= OAc, R_3= H
5 R_1= OH, R_2+ R_3= O
6 R_1= R_3= OH, R_2= H



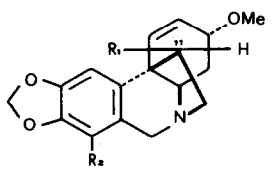
7 R_1= R_2= OH, R_3= R_5= R_6= H, R_4= ---H
8 R_1= R_2= OH, R_3= R_5= R_6= H, R_4= ◄H
9 R_1= R_2= OH, R_3= H, R_4= ---H, R_5+ R_6= O
10 R_1= R_2= OAc, R_3= R_5= R_6= H, R_4= ---H
11 R_1= R_2= OAc, R_3= R_5= R_6= H, R_4= ◄H
12 R_1= R_2= OAc, R_3= H, R_4= ---H, R_5+ R_6= O



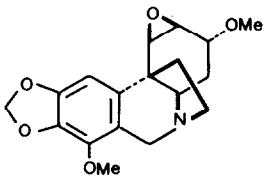
13 R_1= OH, R_2= R_3= R_4= OMe
14 R_1= R_2= OH, R_3= R_4= OMe
15 R_1= R_3= OH, R_2= H, R_4= OMe



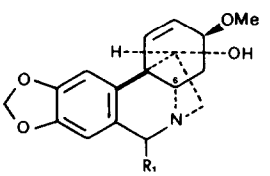
16 R_1+ R_2= O
17 R_1= OH, R_2= H



19 $R_1 = R_2 = H$
20 $R_1 = OH$, $R_2 = OMe$



21

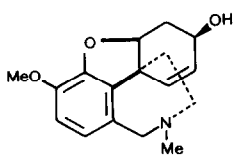


22 $R_1 = H$
23 $R_1 = OH$

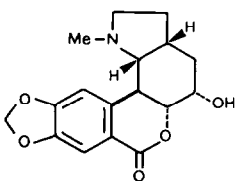
onstrated that modifications on the B ring, combined in this case to the drastic decrease of the basic properties of the nitrogen atom, produced the loss of the activity.

The radical modifications of the lycorine structure found in the Amaryllidaceae alkaloids of the lycorenine-type (homolycorine [2] HCl, **16** HCl; lycorenine [2] HCl, **17** HCl and clivonine **24**, [2]), galanthamine-type (galanthamine [2] HBr, **18** HBr) and lycoramine-type (lycoramine [9] HBr, **25** HBr) produced, as expected, great decreases or total loss of the activity; although **18** showed stimulatory activity.

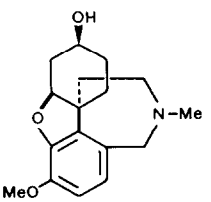
The data presented in this study show that the lycorine structural features needed to preserve the inhibitory activity of ascorbic acid biosynthesis were an integral A and B ring system, β configuration of the D ring when the C/D ring junction is changed and the presence of an electrophilic site located between C-1 and C-2 of the C ring.



18



24



25

EXPERIMENTAL

General methods. Optical rotations were measured in aq. 1% H_2SO_4. Mps are uncorr. TLC was carried out on silica gel (prepared plates): the spots were visualized by exposure to I_2 vapour or UV radiation. Solvent systems: (A) EtOAc–*iso*-PrOH (49:1); (B) $CHCl_3$–*iso*-PrOH (9:1); (C) $CHCl_3$–Me_2CO–diethylamine (5:4:1).

Inhibition in vivo *of ascorbic acid biosynthesis.* To determine the effect of lycorine derivatives and naturally occurring alkaloids, an ascorbic acid biosynthesis, cylinders of potato tuber (*Solanum tuberosum* L.) tissue, 0.9 cm in diameter, were removed with a cork borer and sliced on a sliding microtome. The slices, 1 mm thick, were washed repeatedly with tap H_2O and used either immediately or after incubation at 20° for 13 hr in H_2O with or without lycorine derivatives and other alkaloids at the concs indicated. The medium was aerated by continuous shaking. Ascorbic acid was determined after the slices were washed with tap H_2O. Tuber slices (2 g) were homogenized in a soln of 5% H_3PO_4 and centrifuged at 25000 *g* for 15 min. 0.3 ml of this extract was added to 2.7 ml 1.5 M citrate–Na-Pi buffer, pH 6.2, and $A_{280\,nm}$ measured. The ascorbic acid was oxidized by adding a small quantity of purified ascorbic acid oxidase and the decrease in *A* determined. The conc of ascorbic acid (μg/100 mg fr. wt) was then obtained from a standard curve (10–100 μg of pure ascorbic acid).

Chemicals. Lycorine (**1**) was obtained from dried bulbs of *Sternbergia lutea* Ker-Gawl by extraction with 1% H_2SO_4 soln [10]. 1,2-*O*,*O*-Diacetyl-lycorine **2** [7], 1-*O*-acetyl-lycorine **3** [7] and 2-*O*-acetyl-lycorine **4** [1] were prepared by known procedures.

Lycorine-2-one (**5**). To a soln of **1** (574 mg) in dry DMSO (3 ml) were added crystalline H_3PO_4 (300 mg) and DCC (1.23 g). After stirring for 17 hr at room temp., the mixture was diluted with 30% HOAc soln (100 ml) filtered through a glass funnel which was then washed with the same soln (4 × 50 ml). The combined filtrates and washings were neutralized with 1 M NaOH and, successively, extracted with $CHCl_3$ (5 × 150 ml); the combined extracts were dried (Na_2SO_4). Evaporation of the solvent gave an oily residue which was purified by CC (solvent A). The residue of **5** crystallized from *iso*-PrOH (98 mg, 20%): mp 143–145°; $[\alpha]_D^{25} = -99.7°$ ($c = 2.5$); UV $\lambda_{max}^{CHCl_3}$: 292 and 245 nm ($\varepsilon = 2920$ and 3120 respectively); IR ν_{max}^{nujol} cm^{-1}: 3150 (OH), 1655 (C=O); ^1H NMR 270 MHz ($CDCl_3$–CD_3COOD, 3:1, using $CDCl_3$, δ7.26, as internal standard) δ3.17 (2H, *m*, H-4), 3.49 (1H, *dd*, $J_{AM} = 10.7$ Hz, $J_{MX} = 2.6$ Hz, H-11b), 3.56 (1H, *m*, $J_{AB} = 10.0$ Hz, H-5), 3.74 (1H, *m*, $J_{AB} = 10.0$ Hz, H-5), 4.29 (1H, *d*, $J_{AM} = 10.7$ Hz, H-11c; 1H, *d*, $J_{AB} = 14.7$ Hz, H-7; by ^1H NMDR), 4.36 (1H, *d*, $J_{AB} = 14.7$ Hz, H-7), 4.73 (1H, *d*, $J_{MX} = 2.6$ Hz, H-1), 5.92 (2H, *s*, H-12), 6.05 (1H, *s*, H-3), 6.65 (1H, *s*, H-8) and 6.82 (1H, *s*, H-11); MS 70 eV, *m/z* (rel. int.): 285 $[M]^+$ (30), 284 (27), 267 (100) and 226 (17).

β-Dihydro-lycorine (**8**). To soln of **1** (2 g) in HOAc (distilled, 57 ml) was added Ni-Raney/Merck catalyst (17.8 g). Hydrogenation was carried out overnight in an H_2 atmosphere at room temp. under stirring. After filtration of the catalyst the solvent was evaporated to leave a residue which on TLC analysis (solvent C) was found to contain α-dihydro-lycorine (**7**), unreacted **1** and a minor product (R_f 0.35). The mixture was acetylated under usual conditions with Ac_2O (10 ml) and C_5H_5N (10 ml). Purification of the acetyl-derivatives by CC (silica gel, solvent B) gave diacetyl-derivatives of **1** (**2**, 100 mg) and **7** (**10**, [8], 1.6 g) and 76 mg of 1,2-*O*,*O*-diacetyl-β-dihydro-lycorine (**11**). **11** was dissolved in MeOH (10.7 ml) and hydrolysed for 5 hr with 37% HCl (2.1 ml) on a water-bath (100°). The mixture was concd under red. pres. and

then percolated through a column of Dowex-50W × 2, 100–200 mesh (20 ml in the NH_4^+ form) which was washed with H_2O (100 ml) and successively eluted with 1 M NH_4OH (100 ml). The latter effluent was lyophilized to give **8** as an amorphous solid (54 mg, 2.7%): mp 120–123°; $[\alpha]_D^{25} = -85.0°$ ($c = 0.5$); UV $\lambda_{max}^{1\%\ H_2SO_4}$ nm: 289 and 236 ($\varepsilon = 2341$ and 1936 respectively); IR ν_{max}^{nujol} cm^{-1}: 3400 (OH); ^1H NMR 270 MHz (CD_3OD–CD_3COOD, 3:1, using CD_3OD, δ3.31, as internal standard): δ1.81 (1H, *dd*, $J_{AB} = 13.7$ Hz, $J = 2.3$ Hz, H-3; 1H, *m*, H-4; by ^1H NMDR), 2.11 (1H, *ddd*, $J_{AB} = 13.7$ Hz, $J = 3.8$ Hz, H-3), 2.23 (1H, *m*, H-4), 2.36 (1H, *m*, H-3a), 3.14 (1H, *t*, $J_{AB} = 12.1$ Hz, $J = 12.1$ Hz, H-11c), 3.31 (1H, *dd*, $J_{AB} = 12.1$ Hz, $J_{AX} = 1.9$ Hz, H-11b), 3.45 (1H, *m*, H-5), 3.82 (1H, *m*, H-5), 4.09 (1H, *m*, $J = 1.9$ Hz, H-2), 4.27 (1H, *d*, $J_{AB} = 14.0$ Hz, H-7), 4.52 (1H, *d*, $J_{AB} = 14.0$ Hz, H-7; 1H, *t*, $J_{AX} = 1.9$ Hz, $J = 1.9$ Hz, H-1; by ^1H NMDR), 5.96 (2H, *s*, H-12), 6.70 (1H, *s*, H-8) and 6.91 (1H, *s*, H-11); MS 70 eV, *m/z* (rel. int.): 289 $[M]^+$ (11), 288 (41) and 270 (6).

α-Dihydro-lycorine (**7**). **1** (1 g) in HOAc (distilled, 7 ml) was stirred with 5% Pt–C (1.2 g). Hydrogenation was carried out overnight in an H_2 atmosphere (2 kg/cm^2) at room temp. After removal of the catalyst, the mixture was evaporated *in vacuo*. The solid residue, dissolved in 1 M HCl (2 ml), was purified on Dowex-50W × 2, 100–200 mesh (20 ml in the NH_4^+ form) as reported for **8**. **7** (800 mg, 80%) crystallized from the basic eluent had mp, $[\alpha]_D^{25}$ and spectral values very close to those reported in the literature [7, 11] for **7** prepared by hydrogenation followed by hydrolysis of the 1-*O*-acetyl-derivative of **5**.

2-epi-*Lycorine* (**6**). **5** (238 mg) and $NaBH_4$ (200 mg) in EtOH (73.4 ml) was kept at room temp. under stirring. After 12 hr the excess of the reagent was destroyed with 0.1 M HCl and the mixture was diluted with H_2O and extracted with $CHCl_3$ (3 × 60 ml). The combined extracts were washed with H_2O and dried (Na_2SO_4); evaporation of the solvent gave an oily residue (220 mg). Purification of the reduced mixture was performed according to the procedure used for **11**. It yielded an amorphous solid of **6** (60 mg, 40%) which was identical to 2-*epi*-lycorine prepared by another route [7].

α-Dihydro-lycorine-lactam (**9**). **10** (96 mg) was transformed into 1,2-*O*-*O*-diacetyl-α-dihydro-lycorine-lactam (**12**) according to the reported procedure [8]. **12** was hydrolysed (4% NaOH in EtOH) to **9**; crystallization from $CHCl_3$ gave white needles (27 mg, 45%) similar in all respects to α-dihydro-lycorine-lactam prepared by an other route [8].

Acknowledgements—We thank Professor H. M. Fales, Department of Health, Education and Welfare, Bethesda, MD 20014, U.S.A., for generously providing samples of galanthamine, undulatine, buphanisine, haemanthamine, ambelline and methylpseudolycorine; Professor C. Fuganti, Istituto di Chimica, Politecnico di Milano, Italy, for kindly supplying samples of norpluviine and clivonine; Dr. Xu Ren-sheng, Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China, for generously giving samples of homolycorine HCl, lycorenine HCl, lycoramine HBr and galanthamine HBr; and Professor F. Sandberg, Biomedicinska Centrum, Institution for Farmakognosi Uppsala Universittets, Sweden, for a sample of haemanthidine.

REFERENCES

1. Nakagawa, Y., Uyeo, S. and Yajima, H. (1956) *Chem. Ind. (London)* 1238.
2. Wildman, W. C. (1968) in *The Alkaloids* (Manske, R. H. F., ed.) Vol. XI, p. 307. Academic Press, New York.
3. De Leo, P., Dalessandro, G., De Santis, A. and Arrigoni, O. (1973) *Plant. Cell. Physiol.* **14**, 481.
4. Arrigoni, O., Arrigoni Liso, R. and Calabrese, G. (1975) *Nature (London)* **256**, 513.
5. Arrigoni, O., Arrigoni Liso, R. and Calabrese, G. (1976) *Science* **194**, 332.
6. Kondo, H. and Tomimura, K. (1928) *J. Pharm. Soc. Japan* **48**, 223.
7. Nakagawa, Y. and Uyeo, S. (1959) *J. Chem. Soc.* 3736.
8. Yoshisuke, T., Takchiro, S., Jun'ichi, T., Kimiaki, I., Jun, T., Shuzo, T., Masae, Y., Mayumi, M., Hiroshi, I. and Hirokazu, T. (1978) *J. Chem. Soc.* 1358.
9. Kobayashi, S., Yuasa, K., Imakura, Y., Kihara, M. and Shingu, T. (1980) *Chem. Pharm. Bull.* **28**, 3433.
10. Arrigoni, O., Arrigoni Liso, R., Calabrese, G., Cicala, M. R., Evidente, A., Randazzo, G. and Riccio, R. (in preparation).
11. Evidente, A., Cicala, M. R., Giudicianni, I., Randazzo, G. and Riccio, R. (1983) *Phytochemistry* **22**, 581.