CLIVONIDINE, A NEW ALKALOID FROM *CLIVIA MINIATA*

A. A. ALI, S. A. ROSS, A. M. EL-MOGHAZY, and S. A. EL-MOGHAZY

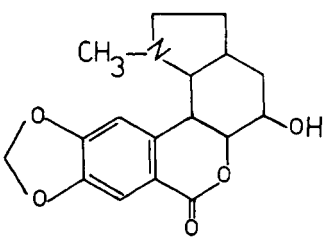
Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

ABSTRACT.—A novel alkaloid, clivonidine (**4**), was isolated from the total herb of *C. miniata* Regel cultivated in Egypt, and its structure was proved. Alkaloids lycorine (**1**), clivonine (**2**), and clivatine (**3**) were also isolated from this plant. The clivonidine alkaloid has an interesting structure from a biogenetic view-point.

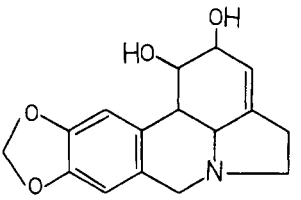
Clivia miniata Regel (Amaryllidaceae) is a handsome, ornamental perennial herb usually cultivated for its beautiful flowers. The plant is used to counteract snake-bite, to treat febrile conditions, and to facilitate delivery at childbirth (1). Leven, *et al.* (2-4) stated that the folkloric use of *C. miniata* Regel is due to its antiviral activity. They reported that alkaloid lycorine decreases the growth of polio in VERO cells through its inhibitory action on viral protein synthesis (2-4). Nine alkaloids were isolated from *C. miniata* Regel (5-15).

RESULTS AND DISCUSSION

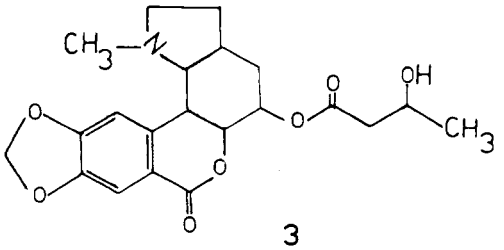
The basic chloroform fraction of the ethanolic extract of *C. miniata* Regel was chromatographed over basic alumina and silica gel columns. Four pure crystalline alkaloids were isolated. Three of them were identified by mp, chemical evidence, and spectroscopic analysis as: lycorine (**1**), clivonine (**2**) and clivatine (**3**).



2



1

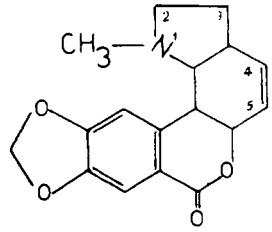


3

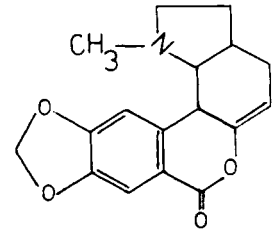
The fourth alkaloid (clivonidine) has mp 238-239°, λ max (MeOH) 258, 290 nm, and M^+ 299. Accurate mass measurements showed the composition of this peak to be $C_{17}H_{17}NO_4$ and the base peak at *m/e* 83 (C_5H_9N), indicating that this alkaloid is of the clivonine type (15). A band was revealed by ir at 1730 cm^{-1} (C=O) and two bands at 1480 and 940 cm^{-1}, assignable to methylenedioxy benzyl system and confirmed by ^{1}H-nmr, which showed a signal for 2H at 6.08 ppm. ^{1}H-nmr showed two singlets for

two aromatic protons at 7.8 and 7.5 ppm, attributed to C-11-H and C-8-H; a singlet for 3H at 2.08 ppm, assignable for N-CH_3. The molecular formula of clivonine ($C_{17}H_{19}NO_5$) and its spectral data suggested that it differs from clivonidine ($C_{17}H_{17}NO_4$) by extra H and OH functions. Because the ir and ^{1}H-nmr spectra of clivonidine show no evidence for the presence of OH group, alkaloid clivonidine is the dehydrated form of clivonine and has two possible structures (**4** or **5**). ^{1}H-nmr spectrum showed two doublets of doublets (1H each) at 5.8 and 4.2 ppm, which correspond to C-5-H and C-4-H, respectively. Therefore, the double bond in clivonidine is between C-4 and C-5, and its structure is tentatively assigned by formula **4**.

The novel alkaloid clivonidine has an interesting biogenetic structure; it is a possible biosynthetic precursor of clivonine and clivatine.



4



5

EXPERIMENTAL

PLANT MATERIAL.—The material used in this study was collected from the Experimental Station of the Faculty of Pharmacy, Assiut University, Assiut, Egypt.

EXTRACTION AND ISOLATION.—Air-dried, powdered herb (leaves, corms, and roots) (3 kg) was extracted with 95% alcohol. The extract was concentrated, digested with 300 ml dilute HCl, and filtered, and the filtrate was then washed with chloroform. The acidic solution was rendered alkaline with NH_4OH and extracted with chloroform. The chloroformic extract was concentrated (30 ml) and treated with 200 ml methanol, at which time a creamy powder was precipitated, leaving a brownish mother liquor. The precipitate was washed with methanol to give lycorine. The combined mother liquor and filtrates were fractionated over basic alumina column (800 g). Elution was carried out with ethyl acetate-methanol mixtures in a manner of increasing polarity till pure methanol was achieved. Fractions (250 ml each) were analyzed for the alkaloidal content by use of system I: chloroform-methanol (9:1).

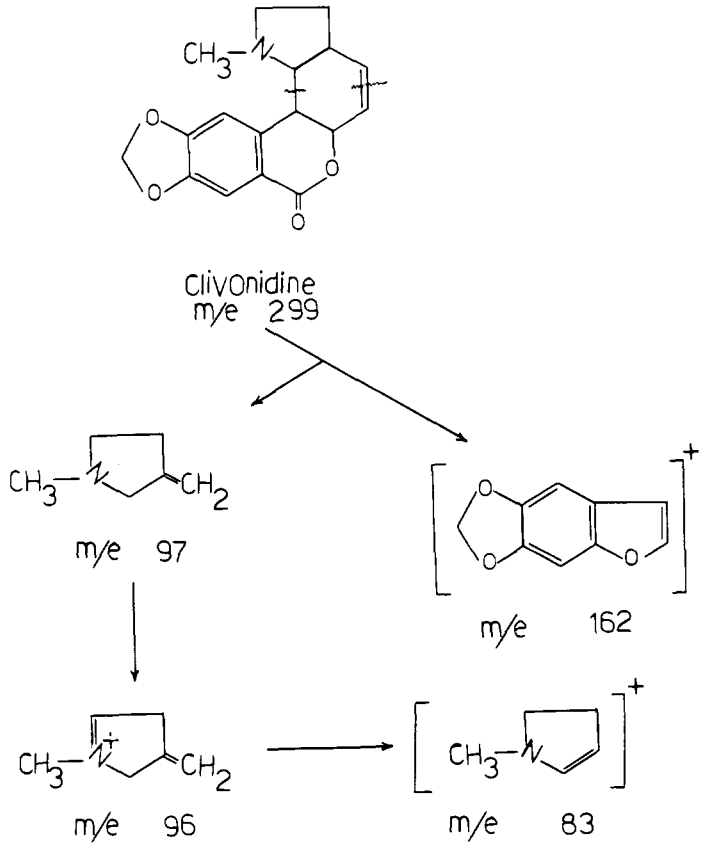
Fractions from no. 37-45 (eluent: 5% methanol in ethyl acetate) and from no. 46-58 (eluent: 10% methanol in ethyl acetate) showed, by tlc, four spots. They were mixed and chromatographed over a silica gel column (300 g). We succeeded in isolating three pure alkaloids: clivonine, clivatine, and clivonidine.

Lycorine. Prisms from ethanol; mp 268-270° [Lit. (6) 270°]; mmp showed no depression; tlc on silica gel G, system I, Rf=0.36.

Clivonine. Shiny prisms from ethanol; mp 198-200° [Lit. (6) 199-200°]; tlc, system I, Rf=0.85; ir (cm^{-1}): 3450 (OH); 1720, 1700 (C=0); 1480, 940 (-O-CH_2-O-); ms: molecular ion peak at *m/e* 317 (3) and other significant peaks at *m/e* 299 (5), 190 (1), 162 (3), 96 (24), 83 (100), 82 (25).

Clivatine. Colorless prisms from CH_3OH; mp 166-168° (Lit. (7) 166-169°]; tlc system I, Rf=0.61; uv λ max (MeOH) 203, 223, 265, 306 nm; ir (cm^{-1}): 3550 (OH); 1740, 1700 (C=0); 1480, 940 (-O-CH_2-O-). High resolution ms showed M^+ at *m/e* 403 (10.3) equivalent to molecular formula $C_{21}H_{25}NO_7$ and peaks at *m/e* 359 (3), 318 (2), 317 (6), 299 (3), 126 (2), 96 (65), 83 (100), 82 (18). ^{1}H-nmr (DMSO) δ: 7.75 (1H, s, C-11 H); 7.45 (1H, s, C-8 H); 6.15 (2H, s. -O-CH_2-O-); 5.14 (H, q, C-5 H); 2.03 (3H, s, N-CH_3); 1.2 (3H, d, J=9 Hz, secondary Me).

Clivonidine. Shiny bitter platelets from ethanol; mp 238-239°; tlc, system 1, Rf=0.75; uv λ max (MeOH) 258, 290 nm; ir (cm^{-1}): 1730 (C-0); 1480, 940 (-O-CH_2-O-). High resolution ms (scheme 1) showed M^+ at *m/e* 299 (6) equivalent to a molecular formula $C_{17}H_{17}NO_4$ and other peaks at *m/e* 162 (4), 127 (1.5), 126 (4), 97 (13), 96 (7), 83 (100). ^{1}H-nmr δ: 7.8 (1H, s, C-11 H); 7.5 (1H, s, C-8 H); 6.08 (2H, s, -O-CH_2-O-); 2.1 (3H, s, N-CH_3) and two doublets of doublets (1H each) at 5.8 and 4.2 ppm, corresponding to C-5 H and C-4 H.



SCHEME 1. Fragmentation pattern of clivonidine alkaloid.

Received 19 July 1982

LITERATURE CITED

1. V. Täckholm and M. Drar, *Bull. Fac. Sci.*, **3**(30), 344 (1954).
2. M. Leven, J. Totte, D. Van Den Berghe, and A. J. Vlietinck, *Planta Med.* **33**, 284 (1978).
3. D. Van Den Berghe, M. Leven, F. Martens, and A. J. Vlietinck, *Lloydia*, **41**, 463 (1978).
4. M. Leven, D. Van Den Berghe, and A. J. Vlietinck, *Planta Med.*, **38**, 254 (1979).
5. H. G. Boit, *Chem. Ber.* **87**, 1707 (1954).
6. C. K. Briggs, P. F. Highet, R. J. Highet, and W. C. Wildman, *J. Am. Chem. Soc.*, **78**, 2899 (1956).
7. H. G. Boit and B. Mehlis, *Naturwissenschaften*, **48**, 603 (1961).
8. B. Mehlis, *Naturwissenschaften*, **52**, 33 (1956).
9. P. W. Jeffs, W. A. Hawksworth, B. K. Tidd, and T. P. Toube, *J. Chem. Soc.*, 1491 (1956).
10. W. Dopke, M. Bienert, A. L. Burlingame, H. K. Schnoes, P. W. Jeffs, and D. S. Farrier, *Tetrahedron Lett.* **5**, 451 (1967).
11. H. K. Schnoes, D. M. Smith, A. L. Burlingame, P. W. Jeffs, and W. Dopke, *Tetrahedron*, **24**, 2824 (1968).
12. W. Dopke and M. Bienert, *Pharmasia*, **25**(11), 700 (1970).
13. W. Dopke and M. Bienert, *Tetrahedron Lett.*, **10**, 745 (1970).
14. W. Dopke and M. Bienert, *Tetrahedron Lett.* **11**, 3245 (1970).
15. S. Kobayashi, H. Ishikawa, E. Sasokawa, M. Kihara, T. Shingu, and A. Kato, *Chem. Pharm. Bull. Jap.*, **28**(16), 1827 (1980).