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Alkaloids of the Amaryllidaceae. VII. Alkaloids Containing the Hemiacetal or Lactone Group[1]

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Five new alkaloids of the Amaryllidaceae have been isolated and named albomaculine, clivonine, krigeine, neronine and nivaline. The molecular formulas of these alkaloids have been determined and tentative structures have been assigned.

This paper describes the isolation and characterization of five new alkaloids of the Amaryllidaceae, four of which are lactones and thus related to homolycorine.[2] The fifth, krigeine, is a hemiacetal which is readily oxidized to a lactone. Without exception, the alkaloids are either moderately abundant in plant materials not readily available to us, or they are present in trace amounts (*ca.* 0.0007%) in the more accessible plants. Since it is our intention to continue investigation of these alkaloids only if the supplies become more bountiful, it seems justifiable to record our observations and postulate structures based on the existing evidence.

The new alkaloids were found in four genera which have been studied to a considerable extent in this Laboratory as well as in others throughout the world.[3] Isolation techniques were similar to those of our previous papers. A summary of the isolations is given in Table I.

The physical properties and expanded molecular formulas of the new alkaloids are shown in Table II.

TABLE I

ALKALOIDAL CONTENT OF AMARYLLIS SPECIES[a]

Alkaloid	*Clivia miniata*	*Galanthus nivalis*	*Haemanthus albomaculatus*	*Hymenocallis occidentalis*	*Nerine krigeii*
Albomaculine	...	...	0.004	...	...
Clivonine	0.07[b]	...	....	...	...
Coccinine	...	...	.004	...	...
Krigeine	...	...	....	...	0.058
Lycorenine	...	...	.009	...	...
Lycorine	.53[b]	0.018	....	0.007	.021
Neronine	...	...	....	...	.088
Nivaline	...	.0007	....	.0002	...
Tazettine	...	.04	Ref. 17	.03	...

[a] Percentage yield based on wet bulb weight unless otherwise indicated. [b] Percentage yield based on dried weight of rhizomes.

Facile identification of the new alkaloids (except krigeine) as lactones was accomplished by spectrographic and chemical means. The presence of a carbonyl group conjugated with the aromatic ring causes strong absorption near 225 mμ as well as infrared absorption near 5.85 μ.[4]

The structures that we suggest for the new alkaloids are based on Ia, the formula assigned to homolycorine.[2] The presence of a lactone rather than an ester function in the relatively abundant albomaculine, clivonine and neronine was demonstrated by their ready solution in warm alkali; upon acidification, facile relactonization occurred and the hydrochlorides of the bases were extracted into chloroform.[5] Neutralization of the acid solutions with sodium bicarbonate followed by chloroform extraction afforded the free bases. The quantity of nivaline at our disposal precluded examination by this technique.

Perhaps the most rewarding study was that of the alkaloid neronine. The presence of one methoxyl and one N-methyl function was established by standard analytical procedures. In addition to intense carbonyl absorption at 5.86 μ, the infrared spectrum of neronine showed hydroxyl absorption at 2.72 and 3.05 μ. The alkaloid formed a basic monoacetate with ease; the carbonyl band of the acetate function was found at 5.75 μ, and no absorption then was present in the 3 μ region. These data are consistent with the presence of one primary or secondary aliphatic alcohol group. The methylenedioxy group was identified by absorption at 3.60 and 10.65 μ.[6] The base gave a positive Labat test. The ultraviolet spectrum provided considerable information concerning the placement of these functional groups. Neronine showed maxima at 228 mμ (log ε 4.41) and 285 mμ (log ε 3.85) and a shoulder at 310 mμ (log ε 3.55). The first two bands are good evidence for the lactone structure proposed in Ie. The absence of a dis-

(1) Previous paper, H. M. Fales, E. W. Warnhoff and W. C. Wildman, THIS JOURNAL, **77**, 5885 (1955).

(2) T. Kitigawa, W. I. Taylor, S. Uyeo and H. Yajima, *J. Chem. Soc.*, 1066 (1955), and references cited therein.

(3) The isolation of alkaloids from genera of the Amaryllidaceae up to 1950 is reviewed by J. W. Cook and J. D. Loudon in R. H. F. Manske, "The Alkaloids," Vol. II, Academic Press, Inc., New York, N. Y., 1952, p. 331. For more recent isolations: (a) *Clivia miniata*, H.-G. Boit, *Chem. Ber.*, **87**, 1704 (1954); (b) *Galanthus* spp., G. R. Clemo and D. G. I. Felton, *Chemistry & Industry*, 807 (1952); H.-G. Boit, *Chem. Ber.*, **87**, 724 (1954); **88**, 1590 (1955); N. F. Proskurnina and L. Ya Areshkina, *J. Gen. Chem. (U.S.S.R.)*, **17**, 1216 (1947); N. F. Proskurnina and A. P. Yakovleva, *ibid.*, **22**, 1899 (1952); (c), *Haemanthus* spp. W. C. Wildman and C. J. Kaufman, THIS JOURNAL, **77**, 1248 (1955); H.-G. Boit, *Chem. Ber.*, **87**, 1339, 1448 (1954); (d) *Hymenocallis* spp., W. C. Wildman and Carol J. Kaufman, THIS JOURNAL, **76**, 5815 (1954); (e) *Nerine* spp., W. C. Wildman and Carol J. Kaufman, *ibid.*, **77**, 4807 (1955); H.-G. Boit, *Chem. Ber.*, **87**, 1704 (1954).

(4) The latter wave length lends support to the δ-lactone rather than a γ-lactone structure. The γ-lactone structure for homolycorine, postulated by H.-G. Boit, L. Paul and W. Stender, *Chem. Ber.*, **88**, 133 (1955), has been refuted in spectral grounds by reference 2 as well as by: (a) R. J. Highet and W. C. Wildman, THIS JOURNAL, **77**, 4899 (1955), and (b) G. R. Clemo and R. Robinson, *Chemistry & Industry*, 1086 (1955).

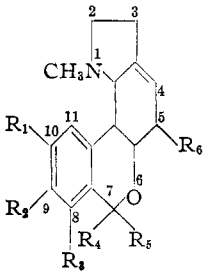
(5) Similar solubility of homolycorine hydrochloride in chloroform was reported in reference 2.

(6) A detailed report of the infrared absorption of methoxyl and methylenedioxy groups will be published in another journal. In previous articles, we have assigned bands near 9.7 and 10.7 μ to the methylenedioxy group. The results of more recent investigations show that the most reliable bands for the identification of this group are near 3.6 and 10.7 μ

TABLE II

Alkaloid	Formula	M.p., °C.	$[\alpha]_D$
Albomaculine	$C_{14}H_{11}(OCH_3)_3(NCH_3)(COO)$	180–181	+ 71.1°
Clivonine	$C_{14}H_{13}(O_2CH_2)(NCH_3)(OH)(COO)$	199–200	+ 41.2
Krigeine	$C_{15}H_{11}(O)(O_2CH_2)(OCH_3)(NCH_3)(OH)_2$	209.5–210 d.	+232.2
Neronine	$C_{14}H_{10}(O_2CH_2)(OCH_3)(NCH_3)(OH)(COO)$	196–197	+161.6
Nivaline	$C_{14}H_{11}(O_2CH_2)(OCH_3)(NCH_3)(COO)$	131.5–132.5	+268

tinct maximum near 305 mμ and the shifted wave length of the 285 mμ band differentiate neronine from the possible spectral model II.[7] II shows three distinct maxima at 225 mμ (log ε 4.39), 263 mμ (log ε 3.72) and 307 mμ (log ε 3.73). These studies together with the spectral analysis of tetrahydroneronine (*vide infra*) allow us to assign the methoxyl group to position 8 of the aromatic ring.[8]



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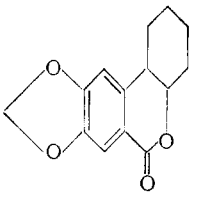
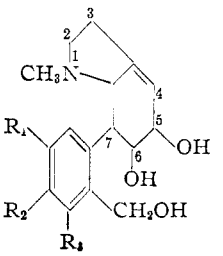


II

Ia, R_1, R_2 = OCH_3; R_3 = H; R_4, R_5 = O; R_6 = H (homolycorine)
b, R_1, R_2, R_3 = OCH_3; R_4, R_5 = O; R_6 = H (albomaculine)
c, R_1, R_2 = O_2CH_2; R_3 = H; R_4, R_5 = O; R_6 = OH no double bond at 3a-4 (clivonine)
d, R_1, R_2 = O_2CH_2; R_3 = OCH_3; R_4 = H; R_5, R_6 = OH (krigeine)
e, R_1, R_2 = O_2CH_2; R_3 = OCH_3; R_4, R_5 = O; R_6 = OH (neronine)
f, R_1, R_2 = O_2CH_2; R_3 = H; R_4, R_5 = O; R_6 = OCH_3 (nivaline)

If neronine has the homolycorine ring system, only the double bond and the hydroxyl group remain to be located. Chemical and spectral properties of neronine and its derivatives do not permit its formulation as either an aldehyde-ammonia or an enol. Reduction of neronine with lithium aluminum hydride afforded a crystalline tetrahydroneronine, m.p. 178–179°. It showed no carbonyl absorption in the infrared. The ultraviolet spectrum was similar to that of falcatine, ambelline and hydrocotarnine. This spectral relationship, in conjunction with the presence of strong absorption at 6.20 μ, affords more evidence that the aromatic ring is substituted with both the methylenedioxy and methoxyl groups.[9] Tetrahydroneronine gave a positive qualitative test with periodic acid, and the vicinal glycol function was confirmed by the quantitative method of Siggia.[10] Tetrahydroneronine then may be represented by IIIa. An alternative 6,7-dihydroxy structure is excluded because of the facile acetylation of the parent alkaloid and our subsequent oxidation experiments.



IIIa, R_1, R_2 = O_2CH_2; R_3 = OCH_3
b, R_1, R_2 = O_2CH_2; R_3 = H; no double bond at 3a–4

If the double bond of neronine is in the 3a,4-position postulated for homolycorine, lycorenine and the other alkaloids of this paper, its hydroxyl group must be allylic and should be susceptible to oxidation with manganese dioxide.[11] A chloroform solution of neronine was stirred with manganese dioxide at room temperature for three hours. Portions of the reaction mixture were removed at timed intervals and centrifuged; the solutions were examined spectrally. Gradual loss of the hydroxyl band was observed and, concurrently, absorption at 5.95 μ appeared. No appreciable change in the spectrum occurred after a reaction period of two hours. The crude reaction product gave a crystalline picrate, m.p. 175–178°, which analyzed correctly for the picrate of a base $C_{18}H_{17}NO_6$. The free base was regenerated from the picrate and crystallized from ether, m.p. 140–150°. The melting range appears to be due to hydration, since hydroxyl absorption at 3.0 μ is present in addition to the expected bands at 5.82 and 5.95 μ. Further evidence for an α,β-unsaturated ketone function in oxoneronine is found in its ultraviolet spectrum. When the extinction coefficients of neronine are subtracted from those of oxoneronine, an apparent maximum of 10,800 is found at 245 mμ. These data can place the hydroxyl only in the 5-position and the double bond at 3a–4. This represents the first assignment by chemical means of the position of unsaturation in alkaloids of this family.

The second new alkaloid found in *Nerine krigeii* was named krigeine. We decided to separate krigeine from traces of neronine by chromatography on alumina. This was an unfortunate choice since only a small portion of the krigeine was recovered. In turn, the eluates from the column gave more neronine than we had anticipated from

(7) This compound was prepared by Dr. H. M. Fales of this Laboratory, and experimental details of the synthesis will be published at a later date.

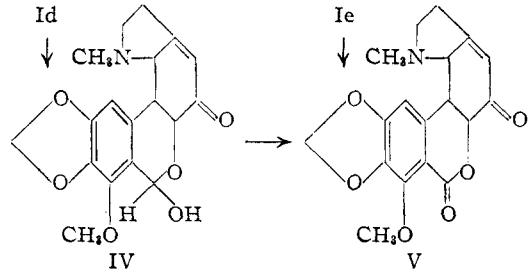
(8) An alternative aromatic substitution (R_1 = OCH_3; R_2, R_3 = O_2CH_2) has been suggested by L. G. Humber and W. I. Taylor, *Can. J. Chemistry*, **33**, 1268 (1955), for the alkaloid, m.p. 189°, from *Boophone disticha*, on spectral grounds. It is our opinion that a clear preference for one arrangement of these groups in the *Boophone* alkaloid, as well as in ambelline, krigeine and neronine, is not possibe from the present spectral or chemical information or from biogenetic considerations.

(9) For a discussion of absorption in the 6 μ region and its relationship to aromatic substitution, see W. C. Wildman and Carol J. Kaufman, THIS JOURNAL, **77**, 4807 (1955).

(10) S. Siggia, "Quantitative Organic Analysis *via* Functional Groups," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 8.

(11) B. C. L. Weedon, *Ann. Repts.*, **50**, 169 (1953), and references cited therein.

an examination of the infrared spectrum of the crude material. In retrospect, the alkalinity of the column undoubtedly effected considerable disproportionation of krigeine into neronine and tetrahydroneronine. A similar conversion of lycorenine to homolycorine and tetrahydrohomolycorine with alkali has been reported.[2] Analytical data reveal that krigeine has the molecular formula $C_{18}H_{21}NO_6$. One methylenedioxy and one methoxyl group are present. The ultraviolet spectrum shows a broad maximum of low intensity, similar to that found for tetrahydroneronine, falcatine and ambelline. Except for strong absorption at 6.20 μ, which we believe is indicative of the coexistence of methylenedioxy and methoxyl in the same aromatic ring, no significant absorption is present from 3.7 to 6.5 μ. Hydroxyl absorption is present at 2.78 and 2.95 μ but acetylation of the alkaloid gives no crystalline derivative. The perchlorate, obtained from the determination of the neutral equivalent, is an oil. Since it seemed most probable that krigeine was a hemiacetal type of dihydroneronine, the remaining material was oxidized with manganese dioxide in the hope that oxoneronine (V) would be obtained. This reaction



would involve oxidation of both the benzylic and the allylic hydroxyl groups. After a chloroform solution of krigeine had been stirred with manganese dioxide for six hours, the infrared spectrum of the solution was identical with that of oxoneronine. Spectra observed during the course of the reaction show that the allylic alcohol is oxidized more rapidly than the hemiacetal. In pure V, the lactone band at 5.82 μ is considerably more intense than the conjugated ketone band at 5.95 μ. In the oxidation of krigeine, after two hours, the band at 5.95 μ is stronger than the one at 5.82 μ; at four hours, the two are of equal intensity. Only after six hours of reaction time is the spectrum of the reaction mixture identical with that of V obtained from neronine. The reaction product was converted to its picrate which has the same melting point as the picrate obtained by the oxidation of neronine.

The methoxyl determination for albomaculine indicated that three of these groups were present. Thus, with two oxygen atoms in the lactone unit, all oxygen atoms were accounted for. Since the alkaloid and all of its derivatives showed strong absorption at 6.28 μ, the presence of at least two methoxyl groups in the aromatic ring seemed probable.[9,12] The third methoxyl has been placed on the aromatic ring for spectral reasons. If this methoxyl were attached to any other part of the molecule than the aromatic ring, the ultraviolet spectrum of albomaculine should be very similar to that of homolycorine. Further, the spectrum of tetrahydroalbomaculine then should resemble that of lycorenine and its dihydrodesoxy derivatives. Neither of these suppositions holds true. Homolycorine showed three well-defined maxima at 228, 268 and 303 mμ. Albomaculine showed only two maxima, 224 and 266 mμ, with an indefinite shoulder about 295 mμ. The ultraviolet spectrum of tetrahydroalbomaculine (sh. 230 mμ, log ϵ 3.85, and λ_{max} 273–280 mμ, log ϵ 2.85) resembled that of mescaline and 2,3,4-trimethoxybenzsuberane-5,6-diol (sh. 228 mμ, log ϵ 3.61 and λ_{max} 273–280 mμ, log ϵ 2.86)[13] more closely than that of lycorenine (λ_{max} 233 mμ, log ϵ 3.96 and λ_{max} 283 mμ, log ϵ 3.51).[14] If the basic ring system of homolycorine is considered to be present in albomaculine also, albomaculine would be represented as Ib.[15]

Nivaline seems best represented by formula If. The methoxy group appears to be aliphatic since no band is present near 6.20 μ. In agreement with this, the ultraviolet spectrum can almost be superimposed upon those of clivonine and II. The method of isolation and chemical behavior of nivaline leave only three possible locations for this methoxyl group: 3, 5 or 11b. We favor the 5-position because of the likelihood that oxygenated functions are present at the 5-position in krigeine, neronine and clivonine. However, the other possibilities by no means are excluded.

Clivonine possesses no methoxyl group; it contains one aliphatic hydroxyl which gives a basic monoacetate, m.p. 194–196°. A positive Labat test and infrared absorption at 3.58 and 10.68 μ give positive proof of the methylenedioxy function. The methylenedioxy group is formulated in the 9,10-position since the ultraviolet spectrum of clivonine is nearly identical with that of II. In contrast to all other lactonic alkaloids of this family, no unsaturation was detected. Clivonine absorbed no hydrogen and was recovered unchanged when treated with hydrogen and either palladium or platinum catalysts at room temperature and atmospheric pressure. When reduced with lithium aluminum hydride, clivonine gave tetrahydroclivonine which we represent as IIIb.

(12) Monomethoxy alkaloids of this family, galanthamine and lycoramine, and their derivatives invariably show a doublet near 6.15 and 6.27 μ.

(13) We are indebted to Dr. G. N. Walker of this Laboratory for the ultraviolet spectrum of this compound.

(14) It is recognized that this argument for placement of the methoxyl groups does not exclude the alternative 9,10,11 arrangement. An unsymmetrical type of substitution appears less likely on biogenetic grounds. Vicinal substitution is found in many of the alkaloids of the cactus family as well as in the non-alkaloidal colchicine.

(15) It has been suggested (H.-G. Boit and H. Ehmke, *Chem. Ber.*, **88**, 1590 (1955); **87**, 1704 (1954)) that nerinine, $C_{18}H_{25}NO_5$, is a methoxylcorenine or a dimethoxygalanthamine. Nerinine has the expanded formula $C_{16}H_{12}(O)(OH)(OCH_3)_3(NCH_3)$. Although nerinine has not been characterized completely, a hint that it is a hemiacetal is found in the fact that it gives a yellow color with concentrated hydrochloric acid as do the known hemiacetals lycorenine and krigeine. It seems to us that albomaculine and nerinine are related in the same manner as homolycorine and lycorenine. An examination of the molecular rotations of the compounds involved supports this contention. Oxidation of lycorenine, $[\alpha]_D$ +180°, to its lactone, homolycorine, results in a decrease in rotation of 87°.[16] On a molecular basis this becomes a ΔM_D of −277°. Similarly, the decrease in dextrorotation going from nerinine to albomaculine is 84° with a resultant ΔM_D of −293°.

The infrared spectrum of IIIb shows no carbonyl absorption. The vicinal glycol structure of IIIb was confirmed by periodate titration. The 5,6-dihydroxy structure in IIIb is most probable by analogy with neronine and krigeine. A dihydroxy system such as represented by IIIb would relate clivonine to dihydrolycorine, for dihydrolycorine would result from a hypothetical ring closure involving the benzylic alcohol and the NH of *nor*-clivonine.

Acknowledgment.—The procurement and botanical aspects of the work were carried out by Dr. B. G. Schubert of the Section of Plant Introduction, U. S. Agricultural Research Service. The assistance of Mr. D. L. Rogerson of this Laboratory in the processing of plant materials is gratefully acknowledged.

Experimental[16]

Neronine and Krigeine.—The bulbs of *Nerine krigeii* Barker (5280 g.) were processed by a standard procedure[8d] to give 20.39 g. of a crude alkaloid fraction. A portion, 5.45 g., of this material was extracted with two 100-ml. portions of hot benzene. The benzene-insoluble residues, upon trituration with ethanol, gave 294 mg. of lycorine, m.p. 227–237° dec. The infrared spectrum of this material was identical with that of authentic lycorine. The benzene-soluble fraction was concentrated and chromatographed on 300 g. of Fisher alumina. Elution with benzene, ethyl acetate or chloroform gave only traces of non-crystalline material, and when 500 ml. of chloroform had been passed through the column with no effect, the benzene-insoluble fraction (less the lycorine) was freed of ethanol, dissolved in chloroform, and passed over the column. From the material eluted with chloroform, 1.24 g. of neronine was obtained by recrystallization from aqueous ethanol. The fractions eluted with 5% ethanol in chloroform gave 0.826 g. of krigeine, m.p. 202–206° dec., upon recrystallization from acetone.

Neronine was purified by recrystallization from ethyl acetate or benzene–cyclohexane to give colorless prisms, m.p. 196–197°, $[\alpha]^{24}D$ +161.6° (*c*, 0.57, chloroform). The alkaloid was quite hygroscopic and after standing a few minutes in the air, it melted at 130–136°, resolidified, and then remelted at 196–197°, $[\alpha]^{23}D$ +151° (*c* 1.32, chloroform).

Anal. Calcd. for $C_{18}H_{19}NO_6 \cdot H_2O$: C, 59.49; H, 5.83; N, 3.86; neut. equiv., 363.4. Found: C, 59.72; H, 5.74; N, 3.54; neut. equiv., 364.6. Calcd. for $C_{18}H_{19}NO_6$: C, 62.60; H, 5.55; N, 4.06; OCH_3, 8.98; NCH_3, 4.34. Found: C, 62.62; H, 5.51; N, 4.08; OCH_3, 8.80; NCH_3, 3.31.[17]

The ultraviolet absorption spectrum showed maxima at 228 mμ (log ε 4.41), 285 mμ (log ε 3.85) and a shoulder at 310 mμ (log ε 3.55).

Saponification of Neronine.—An ethanolic solution of 86.8 mg. of neronine was saponified by the method described for albomaculine. Neronine was recovered in 77% yield.

Neronine Methiodide.—Prepared in acetone from 152 mg. of neronine and recrystallized from water, 100 mg. of neronine methiodide was obtained as colorless prisms, m.p. 260–265° dec. The odor of a low molecular weight amine was observed during the melting point determination.

Anal. Calcd. for $C_{18}H_{19}NO_6 \cdot CH_3I$: C, 46.83; H, 4.55; N, 2.88. Found: C, 47.04; H, 4.70; N, 2.87.

Neronine Picrate.—Prepared in aqueous ethanol from 50 mg. of neronine, the picrate was recrystallized from aqueous ethanol as yellow prisms, 37 mg., m.p. 205–209° dec.

Anal. Calcd. for $C_{18}H_{19}NO_6 \cdot C_6H_3N_3O_7$: C, 50.18; H, 3.86; N, 9.76. Found: C, 50.12; H, 3.77; N, 9.84.

O-Acetylneronine.—A solution of 100 mg. of neronine in 2 ml. of dry pyridine and 1 ml. of acetic anhydride was allowed to stand two days at room temperature. The solvents were removed, and a chloroform solution of the residue was washed with dilute sodium carbonate and water. The chloroform was removed by evaporation, and the residue was crystallized from ethanol, 80 mg., m.p. 201–202°. The material was basic.

Anal. Calcd. for $C_{20}H_{21}NO_7$: C, 62.01; H, 5.46; N, 3.62. Found: C, 62.02; H, 5.35; N, 3.61.

Dihydroneronine.—A solution of 300 mg. of neronine in 15 ml. of ethanol was hydrogenated at room temperature and atmospheric pressure with 10% palladium-on-charcoal catalyst. The dihydroneronine crystallized from aqueous acetone as a hydrate which melted at 80–100°, resolidified, and remelted at 155–157°. The anhydrous base was obtained by recrystallization from benzene–cyclohexane, m.p. 157–158°.

Anal. Calcd. for $C_{18}H_{21}NO_6$: C, 62.24; H, 6.10. Found: C, 62.12; H, 6.11.

Dihydroneronine Picrate.—Prepared in aqueous ethanol in quantitative yield, the picrate was recrystallized from ethanol to give hygroscopic yellow prisms that were trimorphic, m.p. 115–120°, 158–160° and finally 211–213° dec.

Anal. Calcd. for $C_{18}H_{21}NO_6 \cdot C_6H_3N_3O_7 \cdot H_2O$: C, 48.49; H, 4.41; N, 9.42. Found: C, 48.36; H, 4.44; N, 9.29.

Tetrahydroneronine.—A solution of 50 mg. of neronine in ether was refluxed with an excess of lithium aluminum hydride for 16 hours. The mixture was hydrolyzed with alkali, and the ethereal solution was separated. The aqueous solution was extracted several times with chloroform. The chloroform extracts were combined with the ethereal solution and concentrated to 66 mg. of yellow oil that crystallized upon trituration with ethyl acetate; 22 mg. of pure tetrahydroneronine was obtained by recrystallization from aqueous ethyl acetate, m.p. 178–179°. The sample was dried four hours at 130° (loss in weight, 4.65%).

Anal. Calcd. for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64. Found: C, 62.02; H, 6.81.

The ultraviolet absorption spectrum showed a shoulder at 240 mμ (log ε 3.72) and a broad maximum at 281–287 mμ (log ε 3.12). The infrared spectrum showed no carbonyl absorption. A vicinal glycol determination by the method of Siggia[10] gave a value of 110.2%.

Manganese Dioxide Oxidation of Neronine.—A solution of 87.1 mg. of neronine in 20 ml. of chloroform was stirred at room temperature with 300 mg. of manganese dioxide[18] for three hours. The solution was filtered to remove the oxide, and a portion of the filtrate was examined spectroscopically. Hydroxyl absorption at 2.72 and 3.0 μ, present in neronine, had vanished, and new, strong absorption was found at 5.95 μ. The chloroform solution was concentrated to a yellow oil which was dissolved in ethanol and treated with ethanolic picric acid. The precipitated picrate was recrystallized twice from 95% ethanol to give 30 mg. of oxoneronine picrate, m.p. 175–178° dec.

Anal. Calcd. for $C_{18}H_{17}NO_6 \cdot C_6H_3N_3O_7$: C, 50.35; H, 3.52; N, 9.79. Found: C, 50.53; H, 3.64; N, 9.59.

Oxoneronine was regenerated from its picrate by passing a chloroform solution of the picrate over 1 g. of alumina. The free base, 5 mg., was crystallized from ether, m.p. 140–150°. The infrared spectrum (KBr disc) showed absorption at 2.90 (perhaps due to water of solvation), 5.81 and 5.96 μ. The ultraviolet absorption spectrum showed maxima at 229 mμ (log ε 4.52) and 284 mμ (log ε 3.79).

Krigeine was purified by recrystallization from aqueous acetone, m.p. 209.5–210° dec., $[\alpha]^{25}_{589}$ +234°, $[\alpha]^{25}_{436}$ +515° (*c* 0.15, chloroform).

(16) All melting points were observed on a Kofler microscope hot-stage equipped with polarizer and are corrected. Spectral measurements and optical rotations were performed by Mr. H. F. Byers and Miss Catherine Monaghan. The ultraviolet spectra were run in Pharmco absolute ethanol. Analyses were performed by Dr. W. C. Alford and his staff, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md., and the Clark Microanalytical Laboratory, Urbana, Ill.

(17) Although the analytical results for the N-methyl group were disappointing (varying from 50–80% of theory), we believe that the values are sufficiently good to assign the presence of an N-methyl group to each alkaloid. The alkaloids of this family known not to contain the N-methyl group have shown N-methyl values not to exceed 0.2%. Since the new alkaloids have values of 2.26% and greater, we feel that the assignment of the group is warranted. Tazettine, lycoramine and galanthamine, all of which have been shown to contain the N-methyl group, have given similar low values in the hands of our analyst.

(18) J. Attenburrow, *et al.*, *J. Chem. Soc.*, 1094 (1952).

Anal. Calcd. for $C_{18}H_{21}NO_6$: C, 62.24; H, 6.10; N, 4.03; OCH_3, 8.93; NCH_3, 4.33; neut. equiv., 347.4. Found: C, 62.16; H, 6.09; N, 4.08; OCH_3, 8.45; NCH_3, 2.11; neut. equiv., 345.

The ultraviolet spectrum showed maxima at 279 mμ (log ε 3.06) and 287 mμ (log ε 3.03).

Manganese Dioxide Oxidation of Krigeine.—A solution of 51 mg. of krigeine in 20 ml. of chloroform was stirred at room temperature with 300 mg. of manganese dioxide for two hours. The oxide was removed by filtration, and 2 ml. of the filtrate was examined spectroscopically. Strong absorption was present at 5.97 μ, along with a band of lesser intensity at 5.81 μ. Fresh oxide (300 mg.) was added to the solution, and the mixture was stirred for an additional two hours. At the end of this time, the absorption at 5.81 and 5.97 μ was of equal intensity. After an additional two hours of reaction time with fresh oxide, the infrared spectrum of the solution was identical with that of oxoneronine. The solution was filtered and concentrated to a yellow-brown oil that was dissolved in ethanol and converted to the picrate, 11 mg., m.p. 175–178°.

Albomaculine.—The bulbs of *H. albomaculatus* Baker[19] were obtained from the moist, forested areas near Durban, Natal, South Africa, in October, 1954. After a qualitative test showed the presence of alkaloids, all remaining material, 3575 g., was processed by a standard procedure[3d] to give 3.71 g. (0.10%) of an alkaloid mixture. Albomaculine hydrochloride is chloroform soluble. Thus, when the acidified aqueous solution of the alkaloid mixture was washed with chloroform, albomaculine hydrochloride dissolved in the organic layer. Albomaculine was isolated from the chloroform layer on basification. Recrystallization from ethyl acetate gave 134 mg. (0.004%) of pure alkaloid, m.p. 180–181°. The 3.71 g. of crude alkaloids was dissolved in benzene and chromatographed on 300 g. of alumina (Woelm, "almost neutral"). Elution with ethyl acetate–chloroform (4:1) and recrystallization of the resulting solid from acetone gave 317 mg. (0.009%) of lycorenine, m.p. 195–199°. Elution with chloroform gave 140 mg. (0.004%) of coccinine, m.p. 161–163°.[20] Lycorenine and coccinine were identified by melting points and spectral comparisons with the authentic alkaloids. When crystallized from ethyl acetate, albomaculine formed a solvate, m.p. 180–181°, which lost solvent when dried at 110° under 0.1 mm. pressure for five hours, m.p. 180–181°, $[\alpha]^{25}D$ +71.1° (*c* 0.52, chloroform).

Anal. Calcd. for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06; 3 OCH_3, 26.95; NCH_3, 4.35; neut. equiv., 345.4. Found: C, 66.11; H, 6.56; N, 4.03; OCH_3, 26.29; NCH_3, 2.96; neut. equiv., 347.3.

The ultraviolet absorption spectrum of albomaculine showed maxima at 224 mμ (log ε 4.44), 266 mμ (log ε 4.03) and a shoulder at 295 mμ (log ε 3.41).

Saponification of Albomaculine.—A solution of 68.8 mg. of albomaculine in 5 ml. of ethanol and 5 ml. of 0.1 *N* sodium hydroxide was boiled under reflux for 30 minutes. The solution was poured into water, acidified with dilute hydrochloric acid, and allowed to stand for one hour. The solution was made basic and extracted with chloroform–ethanol (4:1). The organic layer was concentrated to constant weight and recrystallized from ethyl acetate to give 45 mg. of albomaculine, m.p. 180–181°. A second crop, 17 mg., m.p. 175–179°, was obtained from the filtrate. The infrared spectrum of each in chloroform was identical with that of pure albomaculine.

Albomaculine perchlorate was recovered from the neutral equivalent determination and recrystallized from methanol to give colorless prisms, m.p. 285–289° dec. The melting point was lower when the sample was heated more slowly.

Anal. Calcd. for $C_{19}H_{23}NO_5 \cdot HClO_4$: C, 51.18; H, 5.43; N, 3.14; 3 OCH_3, 20.88; NCH_3, 3.37. Found: C, 51.38; H, 5.48; N, 3.34; OCH_3, 20.62; NCH_3, 1.48.

The ultraviolet absorption spectrum was identical with that of the free base.

Albomaculine Picrate.—An aqueous ethanolic solution of 0.119 g. of albomaculine was treated with aqueous picric acid. The picrate precipitated upon scratching and after two hours was separated by filtration, 0.140 g., m.p. 189–198°. Four recrystallizations from ethanol and one from methanol did not improve the melting point.

Anal. Calcd. for $C_{19}H_{23}NO_5 \cdot C_6H_3N_3O_7$: C, 52.26; H, 4.56; N, 9.75. Found: C, 52.24; H, 4.66; N, 9.48.

Albomaculine Methopicrate.—The non-crystalline methiodide was prepared in acetone, and the solvent was removed. The residue was dissolved in water, and aqueous picric acid was added until no further precipitation occurred. The solid was recrystallized from aqueous ethanol to give yellow prisms, m.p. 244–246° dec.

Anal. Calcd. for $C_{19}H_{23}NO_5 \cdot CH_3 \cdot C_6H_2N_3O_7$: C, 53.06; H, 4.80; N, 9.52. Found: C, 53.06; H, 4.92; N, 9.59.

Dihydroalbomaculine.—Albomaculine absorbed one mole of hydrogen at atmospheric pressure and room temperature when reduced in ethanol with 10% palladium-on-charcoal catalyst. The resulting material was an oil, probably consisting of two isomers since no constant-melting derivative was obtained.

Tetrahydroalbomaculine.—To a suspension of 50 mg. of lithium aluminum hydride in 20 ml. of ether was added an ethereal solution of 56 mg. of albomaculine. The mixture was heated under reflux for two days, then hydrolyzed with alkali in the usual manner. The product was an oil which could not be crystallized. The infrared spectrum showed the presence of a hydroxyl group and the absence of any unreacted lactone. The ultraviolet absorption spectrum of the oil showed a shoulder at 230 mμ (log ε 3.85) and a broad maximum at 273–280 mμ (log ε 2.85).

Clivonine.—The dried rhizomes[19] (2774 g.) were ground in a Ball and Jewell grinder. The material was divided into two equal portions and each was extracted first with 10 l. of 1% ethanolic tartaric acid for two hours at 60°. The solutions were decanted, and each residue was reextracted with 6 l. of the same solvent. The combined extracts were concentrated to 3 l. and treated with 2 l. of water and 500 ml. of 2 *N* hydrochloric acid. The solution was filtered through SuperCel to remove traces of suspended material, and the filter cake was washed with 250 ml. of 2 *N* acid. The aqueous solution was divided in half, and each half was washed five times with 200-ml. portions of chloroform. This chloroform wash contained alkaloidal material, probably clivonine. It was extracted eighteen times with 200-ml. portions of 2 *N* hydrochloric acid; the aqueous solution was basified with solid sodium carbonate and extracted exhaustively with chloroform–ethanol (4:1). Concentration of this solution gave 6.0 g. of alkaloidal material. The washed aqueous solution was made basic with sodium carbonate and extracted with chloroform until no alkaloid was present in the aqueous solution. The chloroform was removed under reduced pressure. During this concentration, a solid precipitated. When one liter of solution remained, the solid was removed by filtration. This solid, 14.6 g., was identified as lycorine by melting point and infrared spectrum. Concentration of the filtrate gave 35.0 g. of a crude alkaloid mixture. This was combined with the 6.0 g. obtained from the acid wash.

The crude alkaloids were treated with 100 ml. of ethanol, and the lycorine which precipitated on standing overnight was removed by filtration. It was purified and isolated as its hydrochloride, 3.32 g., m.p. 211–218° dec. The ethanolic filtrate was concentrated to constant weight under reduced pressure, and several attempts were made to isolate pure alkaloids by chromatographing this mixture. Traces of a lactonic material, m.p. 273–275°, were obtained, but no satisfactory analysis of this compound or of its picrate, m.p. 205°, could be obtained. The pure alkaloid clivonine, was isolated in the following manner. A portion of the remaining crude alkaloid fraction was treated with 3 *N* hydrochloric acid, and to the resulting suspension was added a generous portion of Darco. The insoluble material and charcoal were removed by filtration, and the nearly colorless filtrate was made basic with 6 *N* sodium hydroxide. The basic solution was digested for one hour on a steam-bath, cooled and extracted with chloroform. Chromatography of a concentrate of this chloroform extract gave no crystalline material. The aqueous basic solution was acidified with hydrochloric acid, treated again with Darco and filtered. The colorless filtrate was concentrated under reduced pres-

(19) The assistance of Professor F. L. Warren of the University of Natal, Pietermaritzburg, South Africa, in the procurement of this material is gratefully acknowledged.

(20) The alkaloids of *H. albomaculatus* appear to vary with the time of collection since a small collection of these bulbs gathered in December a year earlier gave tazettine in addition to the alkaloids mentioned above. Tazettine could not be detected in the current study.

sure to a solid mass. Fractional crystallization from dry methanol separated clivonine hydrochloride, m.p. 263–275° dec., from the sodium chloride. The last traces of clivonine were obtained by dissolving all residues containing alkaloidal material in water, neutralizing the solution with sodium bicarbonate solution and extracting the alkaloid with chloroform–ethanol (4:1). Concentration of this extract and chromatography on alumina gave crude clivonine, m.p. 190–195°. By this procedure, a total of 2.00 g. of clivonine was obtained. A pure sample of clivonine was obtained by recrystallization from ethyl acetate, m.p. 199–200°, $[\alpha]^{23}_{589}$ +41.24°, $[\alpha]^{23}_{436}$ +103.44° (*c* 1.11, chloroform).

Anal. Calcd. for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41; NCH_3, 4.73. Found: C, 64.39; H, 5.95; N, 4.41; NCH_3, 2.26. No methoxyl was present.

The ultraviolet absorption spectrum showed maxima at 226 mμ (log ε 4.33), 268 mμ (log ε 3.77) and 308 mμ (log ε 3.76).

Clivonine Hydrochloride.—The crude hydrochloride was recrystallized from methanol–ether (which contained a drop of water) as colorless prisms, m.p. 282–287° dec.

Anal. Calcd. for $C_{17}H_{19}NO_5 \cdot HCl$: C, 57.81; H, 5.70; N, 3.96. Found: C, 57.67; H, 5.74; N, 3.91.

The ultraviolet absorption spectrum showed maxima at 226 mμ (log ε 4.35), 268 mμ (log ε 3.67) and 308 mμ (log ε 3.75).

Clivonine Picrate.—An ethanolic solution of 75 mg. of clivonine was treated with saturated aqueous picric acid until the precipitation of picrate ceased. The resulting solid was recrystallized from aqueous ethanol to give a quantitative yield of derivative, m.p. 250–254° dec.

Anal. Calcd. for $C_{17}H_{19}NO_5 \cdot C_6H_3N_3O_7$: C, 50.55; H, 4.06; N, 10.25. Found: C, 50.55; H, 4.07; N, 10.19.

Clivonine Methopicrate.—A methanolic solution of 129 mg. of clivonine was refluxed overnight with excess methyl iodide. The neutral gum obtained on concentration was triturated with ethanol–ether to give a very hygroscopic solid. The material was converted to the methopicrate in the usual manner and recrystallized from aqueous ethanol, 111 mg., m.p. 285° dec.

Anal. Calcd. for $C_{17}H_{19}NO_5 \cdot CH_3 \cdot C_6H_2N_3O_7$: C, 51.43; H, 4.32; N, 10.00. Found: C, 51.66; H, 4.88; N, 9.89.

Attempted Hydrogenation of Clivonine.—Clivonine absorbed no hydrogen and was recovered unchanged from atmospheric hydrogenation conditions employing either palladium-on-charcoal (10%) or platinum oxide catalysts.

O-Acetylclivonine.—A solution of 110 mg. of clivonine in 2 ml. of acetic anhydride containing twenty drops of pyridine was allowed to stand one day at room temperature and then concentrated under reduced pressure to a colorless gum that was dissolved in chloroform. The solution was washed with dilute sodium bicarbonate solution and water. Concentration of the chloroform solution gave an oil that crystallized on trituration with ethyl acetate. Recrystallization from ethyl acetate gave 87 mg. of colorless needles, m.p. 194–196°. The acetate showed basic properties.

Anal. Calcd. for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.74; H, 5.80; N, 3.81.

Tetrahydroclivonine.—Reduction of 313 mg. of clivonine in tetrahydrofuran with an excess of lithium aluminum hydride gave 261 mg. of a colorless oil that was chromatographed on 30 g. of aluminum oxide (Merck). Elution with chloroform removed 60 mg. of unreacted starting material. Elution with 5% ethanolic chloroform gave 200 mg. of colorless oil which could not be crystallized. The ultraviolet spectrum of this oil showed maxima at 238 mμ (log ε 3.58) and 292 mμ (log ε 3.51). A vicinal glycol determination[10] on 68.4 mg. of this oil indicated that 81.6% of the material had a vicinal glycol structure.

Nivaline.—The bulbs of *Galanthus nivalis* L. were purchased from Kipiteijn and Sons, Hillegon, The Netherlands. By the usual extraction method, 9800 g. of fresh bulbs gave 25.6 g. (0.26%) of crude alkaloids. Ethanolic trituration of this material gave 1.76 g. (0.018%) of lycorine, m.p. 250–255° dec. The filtrate was concentrated under reduced pressure to remove the ethanol. The benzene-soluble portion of the residue was chromatographed on aluminum oxide (Merck) to give 4.01 g. (0.04%) of tazettine, m.p. 200–205°. The oils (0.776 g.) eluted prior to the tazettine were rechromatographed. Elution with 5% ethyl acetate in benzene gave 92 mg. of crude nivaline, m.p. 121–125°, which was recrystallized from ethanol to give 73 mg. (0.0007%) of pure nivaline, m.p. 131.5–132.5°, $[\alpha]^{24}D$ +268° (*c* 1.0, ethanol).

Anal. Calcd. for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.82; N, 4.25; OCH_3, 9.42; NCH_3, 4.56. Found: C, 65.88; H, 5.84; N, 4.36; OCH_3, 9.34; NCH_3, 3.30.

The ultraviolet absorption spectrum showed maxima at 230 mμ (log 4.43), 269 mμ (log ε 3.75) and 308 mμ (log ε 3.77).

A similar processing of 26,900 g. of *Hymenocallis occidentalis* (le Conte) Kunth gave lycorine (0.007%), tazettine (0.03%) and nivaline (0.0002%).

Dihydronivaline.—A solution of 48 mg. of nivaline in 20 ml. of ethanol absorbed 3.05 ml. of hydrogen when reduced at atmospheric pressure and room temperature with 40 mg. of 10% palladium-on-charcoal catalyst. The dihydro derivative was an oil.

BETHESDA, MARYLAND

COMMUNICATIONS TO THE EDITOR

REDUCED TRIPHOSPHOPYRIDINENUCLEOTIDE REQUIREMENT FOR THE ENZYMATIC FORMATION OF 3-HYDROXYKYNURENINE FROM L-KYNURENINE

Sir:

Two recent reviews on the metabolism of tryptophan[1,2] have stressed the lack of information concerning the formation of 3-hydroxykynurenine from kynurenine. In an effort to account for the absence of the usual tryptophan metabolites from cat urine[3] certain *in vitro* studies were carried out. This letter describes a cell-free system occurring in liver mitochondria of cats and rats which forms 3-hydroxykynurenine from L-kynurenine.

The disappearance of substrates and the appearance of the metabolites were followed by ascending paper chromatography on Whatman No. 1 filter paper. The spots were identified by comparison with authentic compounds according to the criteria in Table I.

Washed mitochondria equivalent to 200 to 400 mg. wet weight of liver was incubated aerobically at 37° for two hours in 0.05 *M* phosphate buffer at *p*H 7.4 containing 5.0 μmoles of citrate, 0.67 μmole of triphosphopyridinenucleotide (TPN), 33.0 μmoles of nicotinamide and 2.5 μmoles of L-kynurenine. The final volume was 1.5 ml. It was not necessary to deproteinize before spotting the

(1) C. E. Dalgliesh in Advances in Protein Chemistry, **10**, 86 (1955).

(2) A. H. Mehler in "Amino Acid Metabolism," edited by W. D. McElroy and H. B. Glass, The Johns Hopkins Press, Baltimore, Md., 1955, p. 898.

(3) R. R. Brown and J. M. Price, *J. Biol. Chem.*, **219**, 985 (1956).