acidic solutions, the observation of stable bridged phenylethyl cation (phenonium ions) in the present work completes the study of all three possible ion types in these systems.

Experimental Section

β-*p*-Anisylethyl alcohol was prepared by $LiAlH_4$ reduction of *p*-methoxyphenylacetic acid (Aldrich), bp 122–125° (5 mm) [lit.[29] 102–105° (0.3 mm).

β-*p*-Anisylethyl chloride was formed from the alcohol using thionyl chloride and pyridine in ether solution at 0°. After work-up, the chloride was collected by fractional distillation, bp 90–92° (2 mm).

α-*p*-Anisylethyl Alcohol. *p*-Methoxyacetophenone (Eastman) was reduced with $LiAlH_4$ in ether solution, bp 84° (3 mm) [lit.[30] 104° (3 mm)].

β-Mesitylethyl Chloride. 2-Mesitylethyl alcohol[31] was converted to the chloride with thionyl chloride and pyridine in ether solution at 0°, bp 128–132° (12 mm).

α-Mesitylethyl Alcohol. Mesityllithium (from butyllithium and 2-bromomesitylene) (Eastman) was treated with acetaldehyde in ether solution at −10°. After work-up, the product was isolated by distilling off volatile impurities under vacuum and recrystallizing from pentane and then acetone, mp 72–74°.

1-*p*-Anisyl-2-propanol. The Grignard reagent from *p*-bromoanisole (Eastman) was prepared in the usual manner from 200 g (1.07 moles) of the halide in 400 ml of ether. After addition of 800 ml of benzene and cooling to −20°, a cold (−40°) solution of 80 g (1.4 moles) of propylene oxide in 100 ml of benzene was added over a period of 10 min. After standing overnight at room temperature and refluxing for 2 hr, the reaction mixture was cooled to −20 and hydrolyzed with 200 ml of H_2O. Work-up yielded 100 g (58%) of the title compound, bp 75° (0.007 mm).

1-*p*-Anisyl-2-chloropropane. 1-*p*-Anisyl-2-propanol (40 g, 0.24 mole) was cooled to −60° and slowly dissolved in 200 ml of cold (−40°) $SOCl_2$. The reaction mixture was stirred and allowed to warm to room temperature. After stirring for 2 hr at room temperature, the excess $SOCl_2$ was removed on a rotary evaporator. Distillation yielded 38 g (87%) of the title compound, bp 125° (10 mm).

β-(Pentamethylphenyl)ethyl Alcohol. Using the above procedure for 1-*p*-anisyl-2-propanol 50 g (52%) of the title compound was prepared from 114 g of bromopentamethylbenzene and 40 g of ethylene oxide, mp 108–109.5° (from hexane).

β-(Pentamethylphenyl)ethyl Chloride. β-(Pentamethylphenyl)-ethyl alcohol (5 g) was dissolved in 50 ml of cold (−40°) $SOCl_2$. After warming to 35° and stirring for 2 hr, excess $SOCl_2$ was removed on a rotary evaporator, mp 64–65° (from ether), yield 4.9 g (86%).

1-Mesityl-2-propanol. Using the above procedure for 1-*p*-anisyl-2-propanol the title compound was analogously prepared, bp 138–142° (0.5 mm).

1-Mesityl-2-chloropropane was prepared from the corresponding alcohol with thionyl chloride and pyridine in ether at 0°, bp 124–126° (15 mm).

1-*p*-Anisyl-1-methoxyethane. A solution of 16 g (0.097 mole) of α-*p*-anisylethyl alcohol in 60 ml of dry DMSO was added dropwise with stirring to a suspension of 6 g (0.167 mole) of NaH (in mineral oil) in 100 ml of dry DMSO. After stirring for 0.5 hr 50 g of CH_3I was added over a period of 60 min. After hydrolysis and work-up, the product was isolated by distillation, bp 72° (10 mm). Similarly prepared were 1-*p*-anisyl-2-methoxyethane, bp 80° (1.0 mm); 1-mesityl-1-methoxyethane, bp 60° (10 mm); 2-mesityl-2-methoxyethane (solid melting point not determined); and 2-pentamethylphenyl-2-methoxyethane, mp 59–60.5° (from ether).

Preparation of Carbonium Ions. A saturated solution of antimony pentafluoride (or SbF_5–FSO_3H, 1:1) in sulfur dioxide was prepared (at −10°). Portions (2 ml) of this solution were cooled to −78°, causing some acid to crystallize from solution. To this suspension was slowly added with rapid stirring approximately 0.3–0.4 g of halide (alcohol, hydrocarbon) in SO_2. Slight warming was generally required to complete the ionization, whereupon a homogeneous solution resulted with only slight traces of color. Ion concentrations were approximately 10%. Nmr spectra were recorded on a Varian Associates Model A56-60A spectrometer with external capillary TMS as reference.

Methanolysis of the carbonium ions was accomplished by adding slowly the solution of the carbonium ion in sulfur dioxide to cold (−98°) rapidly stirred methanol slush. The reaction mixture was then poured on ether. After washing and drying, the ethereal layer was concentrated and analyzed by gas chromatography (capillary technique using a Perkin-Elmer Model 226 gas chromatograph). Identification of products was made by comparison of retention times and peak enhancement with authentic samples and also by nmr and infrared spectroscopy of isolated products.

Acknowledgment. Generous support of this work by grants of the National Science Foundation and the National Institutes of Health is gratefully acknowledged.

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Alkaloid Biosynthesis and Interconversions. The Conversion of Caranine to Lycorine[1]

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Abstract: A synthesis of [2β-^{3}H]caranine from lycorine has been accomplished. When introduced into *Zephyranthes candida*, this radioactive precursor was transformed into lycorine in good yield. This interconversion indicates that the oxidation at C_2 may occur relatively late in the biosynthetic process by an inversion mechanism. The relative configurations at C_1 and C_2 in several lycorine derivatives were determined by hydrogen-bonding studies in the infrared.

Radioactive tracer studies have shown that both phenylalanine and tyrosine are utilized in the biosynthesis of lycorine (**1**).[3,4] The former amino acid is utilized specifically for the construction of the aromatic ring and C_7 (C_6–C_1 unit). Tyrosine provides the hydroaromatic ring and the aliphatic carbon atoms at

(1) This research was supported in part by Grant HE 7503 from the National Institutes of Health.
(2) NASA Fellow, 1966.
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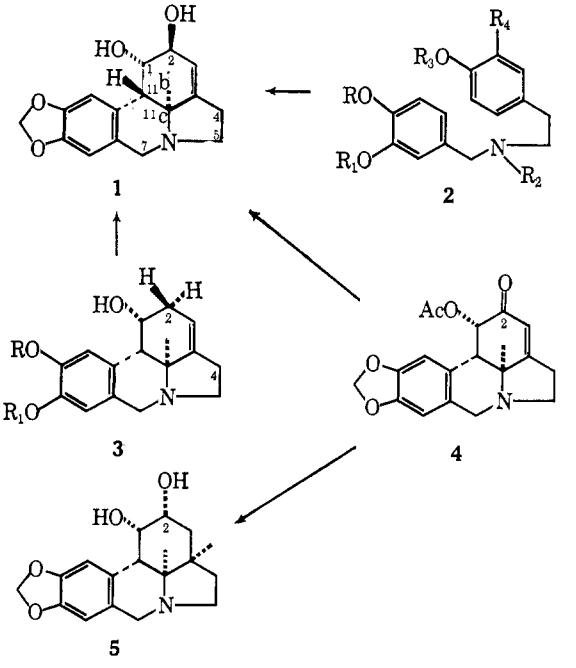
positions 4 and 5 (C_6–C_2 unit). Phenylalanine is not incorporated into the C_6–C_2 unit, and tyrosine is not a precursor of the C_6–C_1 fragment. It is particularly striking that the C_6–C_1 unit of these alkaloids may contain as many as three aromatic, oxygenated substituents, while the C_6–C_2 unit may contain no more oxy substituents than its precursor, tyrosine. Evidence for the discrete stages of hydroxylation in the C_6–C_1 unit of the *Amaryllidaceae* alkaloids have been reported.[5-11] The observations that norbelladine (**2**; R, R_1, R_2, R_3, R_4 = H) and O-methylnorbelladine (**2**; R_1, R_2, R_3, R_4 = H; R = CH_3) were incorporated well into the alkaloids, while hydroxynorbelladine (**2**, R, R_1, R_2, R_3 = H; R_4 = OH) was an ineffective precursor, suggested that additional hydroxylation of the C_6–C_2 moiety might occur at a relatively late stage in the biosynthetic scheme. Additional support for this hypothesis was found in the conversion of radioactive norpluviine (**3**; R = CH_3; R_1 = H) to lycorine in both the "Twink" and "Deanna Durbin" daffodils.[3] *o*-O-Methylphenols are known precursors of the methylenedioxy group,[12] and it seemed likely that caranine (**3**; R, R_1 = CH_2) might also serve as a precursor of lycorine. Preliminary feeding experiments using randomly labeled [^{3}H]caranine afforded radioactive lycorine.[13] This paper confirms the *in vivo* conversion of caranine to lycorine and defines more clearly the stereochemical process of oxidation.

At present, 23 alkaloids containing the lycorine-type nucleus have been isolated from various *Amaryllidaceae*. A majority of these have an oxygenated substituent at C_2 possessing the β configuration (steroid convention). Jonquilline (**4**)[14] and zephyranthine (**5**)[15] are notable exceptions to this generalization. The introduction of an oxygenated substituent at C_2 in a caranine-like precursor might occur *via* one of three general mechanisms. Jonquilline might serve as the important intermediate. Reduction to the alcohol at C_2 and appropriate hydrolysis and/or reduction of the double bond could lead to either lycorine (**1**) or zephyranthine (**5**). It is also possible that caranine can be converted to lycorine and zephyranthine without a 2-oxo intermediate. Direct insertion of oxygen between carbon and a given hydrogen at C_2 would lead to a product in which the remaining C–H bond at C_2 possesses the same configuration as in the starting material (retention). In an alternative process, oxygen could be introduced in such a manner that the remaining C–H bond has an inverted configuration. In order to decide between these alternative pathways, it was necessary to devise a synthesis of caranine labeled in an unequivocal manner at C_2.

Synthesis. Several papers have described various transformations of the functional groups at C_1 and C_2 in lycorine (**1**) and dihydrolycorine.[16-18] It seemed attractive to prepare the known lycorine chlorohydrin (**6**) and examine its reduction to a derivative tritiated at C_2. The chlorohydrin **6** in our hands was unstable and was contaminated with anhydrolycorine (**7**). That **6** was a *cis*-chlorohydrin was shown by the strong intramolecular hydrogen bond between the two groups (3585 cm^{-1}).[19-21] This frequency is in good agreement with 3578 cm^{-1} found for the bonded hydroxyl of 2-epilycorine (**1**; OH at C_2 in α configuration). The chlorohydrin formed a 2-methyl ether (**8**; hippamine) with methanolic potassium hydroxide. The *trans* configuration of **8** was evident from the lack of strong hydrogen bonding. Weak hydrogen bonding of the C_1-hydroxyl to the aryl π system was observed at 3602 cm^{-1}. This compares favorably with 3602, 3595, and 3598 cm^{-1} found for galanthine (**8**; dimethoxy instead of methylenedioxy), caranine (**3**; R, R_1 = CH_2), and α-dihydrocaranine (**5**; no OH at C_2).



In an attempt to separate **6** from **7**, the crude material was chromatographed on Florisil. The early eluates contained a crystalline, chlorine-free base which was assigned structure **9** from the following evidence. The ultraviolet absorption spectrum showed a normal methylenedioxyaryl system. The infrared spectrum showed no absorption which might be attributed to hydroxyl or carbonyl groups. The mass spectrum of **9** showed a parent ion at *m/e* 269 which confirms the molecular formula $C_{16}H_{15}NO_3$. The base peak in the spectrum occurs at *m/e* 268 corresponding to the loss of one hydrogen atom. Other major peaks occur at *m/e* 250 (M − 19) and *m/e* 240 (M − 29). The nmr spectrum showed two aromatic protons, one methylenedioxy group, and one olefinic proton. The remainder of the spectrum was complicated by superposition of coupling patterns but was simplified by spin decoupling techniques to show the expected AB pattern for the

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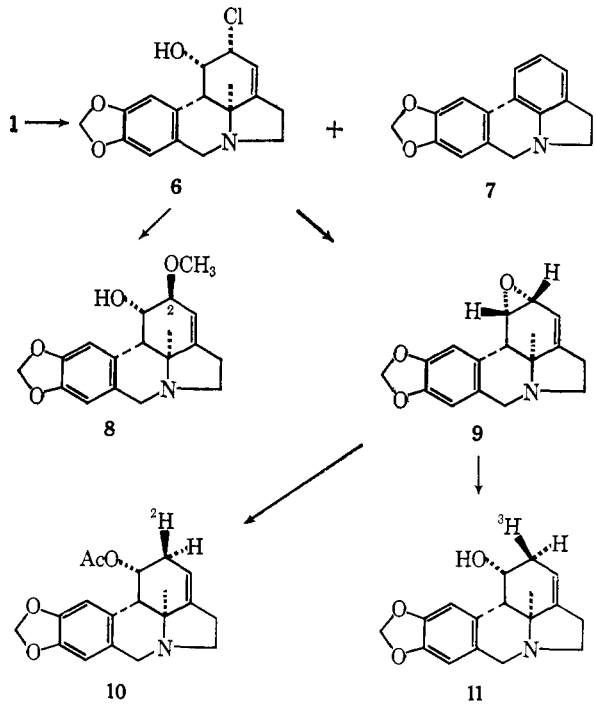
C_7 protons and a broad doublet for the proton at C_1. The C_2 proton was initially observed as a triplet which collapsed to a singlet upon irradiation of the olefinic and C_1 protons. The remainder of the spectrum strongly resembles that of O,O-diacetyllycorine.[22]

Hydrolysis of **9** by dilute sulfuric acid afforded lycorine as the sole product. Lithium aluminum hydride reduction of **9** gave a good yield of caranine. Reduction of **9** with lithium aluminum deuteride (followed by acetylation) gave deuterated acetylcaranine (**10**) which should bear the deuterium atom in the 2β position, since nucleophilic attack by hydride ion should occur from the least hindered side of the molecule. The location of the deuterium atom was verified by nmr and mass spectroscopy. In the mass spectrum of **10**, the parent ion occurs at *m/e* 314, and the fragment corresponding to loss of atoms C_1 and C_2 and their substituents as vinyl acetate occurs at the same mass-to-charge ratio as the corresponding loss in natural acetylcaranine (mol wt 313).[23] This indicates that the deuterium



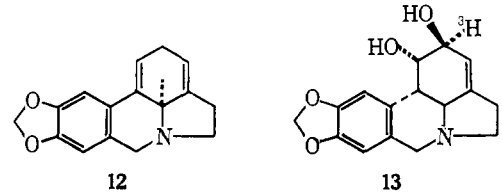
atom must have been at C_2. Comparison of the nmr spectra of natural and [2β-^{2}H]acetylcaranine in a benzene–chloroform solvent showed little difference except for a one-proton decrease in the integral of the multiplet located at δ 2.32 and a marked decrease in the half band width of both the C_3 proton at δ 5.23 and the C_1 proton at δ 5.90. It might be possible to assign the configuration of the C_2 deuterium atom by nmr, but the conformational mobility of the hydroaromatic ring of **10** and the unavailability of the C_2 epimer of **10** complicate an assignment by this method.

If the evidence cited above limits the deuterium atom to C_2, it would be desirable to have other data (other than chemical precedent) to establish the configuration of the deuterium atom as assigned in **10**. The mass spectrum of **10** shows the loss of acetic acid (*m/e* 254) which is accompanied by a metastable ion at 205.5 (calcd 205.5). The same fragmentation in natural acetylcaranine (mol wt, 313) leads to an ion at *m/e* 253 which is accompanied by a metastable ion at 204.5 (calcd 204.5). If the deuterium atom were in the α configuration, a significant loss of acetic acid should occur by analogy with the loss of acetamide in acetylated 3-amino steroids[24] and acetic acid in peracetylated sugars.[25] This would result in a significant increase in the intensity of the M − 61 ion at *m/e* 253 in the spectrum of **10** relative to the same loss in undeuterated acetylcaranine. Since the intensities are nearly the same in both spectra (76% base peak in **10** and 72% base peak in natural acetylcaranine), the deuterium atom must occupy the β position at C_2.

Radioactive caranine (**11**) was prepared by the reduction of **9** with 5 mc of lithium aluminum [^{3}H]hydride. The radioactive caranine (1.35×10^6 dpm/mg) was shown to be pure by crystallization to constant activity and by chromatographic criteria.

Incorporation Data. A solution of 19.5 mg of **11** in 1 ml of an aqueous solution buffered to pH 6 was injected into 35 bulbs of *Zephyranthes candida*. The plants were allowed to grow for 3 weeks and processed in a standard manner.[26] The lycorine isolated was radioactive (7.05% incorporation). All attempts to isolate radioactive zephyranthine by isotopic dilution failed. Thus our *Z. candida* either contained no zephyranthine or the zephyranthine present was not radioactive.

Degradation of Radioactive Lycorine. The lycorine isolated from the feeding experiments with *Z. candida* was converted to **6** and reduced directly to caranine with lithium aluminum hydride in order to conserve labeled material. Radioactive **7** was isolated as a by-product of the sequence. Treatment of the radioactive caranine with phosphorus oxychloride and pyridine afforded anhydrocaranine (**12**). The specific activities of the anhydrocaranine and anhydrolycorine were the



same (within experimental error) as that of the original radioactive lycorine, and the tritium label cannot reside at either C_{11b} or C_{11c}. The method of synthesis for **11** makes it highly improbable that the tritium could be at either C_1 or C_3. The tritium was located at C_2 by a degradation of the radioactive lycorine[13] to jonquilline (**4**) which proved to be virtually devoid of radioactivity. This conversion utilized the well-known pathways of acetylation of **1** and partial hydrolysis to 1-O-acetyllycorine, followed by manganese dioxide oxidation to **4**. Specific and relative activities for the compounds in the degradation scheme are given in Table I.

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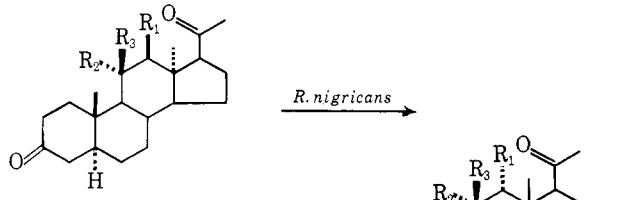
Table I. Relative and Specific Activities of Lycorine and Its Degradation Products

Compound	Act. $\times 10^{-4}$, dpm/mmole	Rel act.
Lycorine (**1**)	5.37	1.00
Caranine (**3**)	5.36	1.00
Anhydrolycorine (**7**)	5.00	0.93
Anhydrocaranine (**12**)	5.36	1.00
O,O-Diacetylycorine	5.37	1.00
Jonquilline (**4**)	0.21	0.04

Discussion

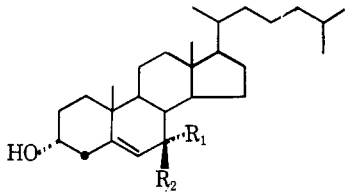
The conversion of C–H to C–OH by microbiological techniques is a well-known process.[27] Although this hydroxylation process has been examined primarily with substrates containing the steroid nucleus, the method has been applied to agroclavine[28] and yohimbine and its derivatives.[29,30] The configuration of the newly introduced hydroxyl group was established, but appropriately labeled precursors, which would permit evaluation of the over-all stereochemical process, were not employed. Microbiological hydroxylations in the steroid group have provided some information in this regard. It has been shown that the introduced oxygen atom is derived from molecular oxygen.[31] Stereospecifically labeled steroids undergo selective hydroxylation through a process which can be visualized as the insertion of an oxygen atom into a carbon–hydrogen bond. Upon incubation of *Rhizopus nigricans*, with [11α,12β-^{3}H]pregnane-3,20-dione (**14a**), the tritium atom at C_{11} is lost from the resulting 11α-hydroxyl derivative, **15a**.[32] In a similar experiment it was shown



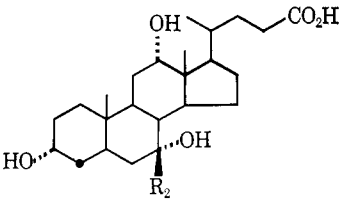
14a, $R_1 = R_2 = {}^3H$; $R_3 = H$
b, $R_1 = R_2 = H$; $R_3 = {}^2H$

15a, $R_1 = {}^3H$; $R_2 = OH$; $R_3 = H$
b, $R_1 = H$; $R_2 = OH$; $R_3 = {}^2H$
c, $R_1 = R_2 = {}^3H$; $R_3 = OH$

that incubation of *R. nigricans* with [11β-^{2}H]pregnane-3,20-dione (**14b**) gave the 11-hydroxyl derivative **15b** in which the deuterium atom was retained.[33] Administration of **14a** to surviving bovine adrenals afforded **15c** in which the tritium atoms at both C_{11} and C_{12} were retained.[28] In another experiment doubly labeled materials, [4-^{14}C,7α-^{3}H]cholesterol (**16a**) and the 7β-^{3}H epimer, **16b**, were administered to living rats and cholic acid (**17**) was isolated.[34,35] In this case the hydroxylation at C_7 has proceeded with retention of configuration as well. From these results it was suggested that the hydroxylation process involved attack of an



16a, $R_1 = {}^3H$; $R_2 = H$
b, $R_1 = H$; $R_2 = {}^3H$



17a, $R_2 = H$
b, $R_2 = {}^3H$

electrophilic agent on the carbon–hydrogen bond.[33,34]

Our experimental results eliminate the possibility of either a 2-oxo intermediate or direct insertion of oxygen into the 2β-CH bond. Direct nucleophilic displacement of the α-C_2 hydrogen atom by a hydroxyl anion is not an attractive alternative. Our current working hypothesis involves electrophilic attack at C_2 in a manner similar to that proposed in the steroid hydroxylation process. An allylic carbonium ion develops at C_2 which can either react with water from the least hindered side of the molecule and form lycorine directly or form the α-epoxide **9** which is hydrolyzed to lycorine. The experimental evidence which we have at present does not indicate whether the loss of hydrogen at C_2 in the hydroxylation process is stereospecific. It is possible that some tritium is lost in the interconversion as well. Further research dealing with this aspect is in progress. The biogenesis of zephyranthine may occur by reduction of caranine to α-dihydrocaranine followed by oxygenation at C_2 as in the steroidal series.

Experimental Section[36]

Lycorine α-Epoxide (9). To a mixture of 1 ml of phosphorus oxychloride and 200 mg of powdered sodium chloride was added 330 mg of lycorine. The mixture was heated to 35° in a water bath. After 5 min, two drops of 6 *N* hydrochloric acid was added. The temperature was raised to 40°, and the reaction mixture was

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(36) Melting points were taken on a Kofler hot-stage apparatus and are not corrected. Infrared spectra were obtained with a Perkin-Elmer Model 21 spectrophotometer. Hydrogen bonding studies were carried out with a Beckman Model IR-12 spectrophotometer using 2.0-cm silica cells and dry carbon tetrachloride as the solvent. Proton nuclear magnetic resonance spectra were obtained in either deuteriochloroform or a 4:1 mixture of benzene and chloroform at 60.0 MHz on a Varian HR-60 spectrometer. Mass spectra were determined with an Atlas CH-4 mass spectrometer operating at 70-ev electron energy. Vapor phase chromatographic analyses were obtained with a Chromolab A-210 chromatograph containing a 12-ft glass column packed with 1% SE-30 on Gaschrom Q. Radioactive assays were determined in Bray's solution with a Packard Tricarb Model 314X liquid scintillation spectrometer.

maintained at this temperature for 35 min. The reaction mixture was hydrolyzed with ice–water, made basic (pH 8) with sodium hydroxide and sodium carbonate, and extracted with ether. The ether extract was dried with a saturated sodium chloride solution and evaporated under reduced pressure to give 300 mg of noncrystalline residue which was pure by thin-layer chromatographic criteria. The residue was dissolved in a minimum amount of anhydrous ether and passed rapidly through a Florisil column (15 g) which had been packed in ether. Elution with 200 ml of ether provided 80 mg (26%) of crystalline epoxide, mp 145–153°. Recrystallization from methanol gave colorless prisms, mp 148–150°; $[\alpha]^{20}D$ −192° (*c* 0.76, $CHCl_3$); ultraviolet maxima (95% ethanol) at 290 mμ (ε 5500) and 235 (sh) mμ.

Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.35; H, 5.44; N, 5.20; mol wt, 269. Found: C, 71.09; H, 5.46; N, 5.15 (mass spectrum *m/e* 269 (M^+)).

Conversion of Lycorine α-Epoxide to Caranine. To a solution of 100 mg of lithium aluminum hydride in dry ether was added 50 mg of lycorine α-epoxide. The mixture was refluxed for 3 hr. The excess lithium aluminum hydride was destroyed with wet ether. The ether layer was separated, washed with a dilute solution of sodium hydroxide, and evaporated to dryness to provide 33 mg of crude caranine. Recrystallization from ethyl acetate gave colorless prisms, mp 174–177° (lit.[37] mp 176–177°). The infrared spectra (KBr and $CHCl_3$) were identical with those of authentic caranine in these phases.

Hydrolysis of Lycorine α-Epoxide. A solution of 20 mg of lycorine α-epoxide in 3.0 ml of water and six drops of 6 *N* sulfuric acid was heated at 95° for 0.5 hr. The reaction mixture was cooled and made basic. The precipitated lycorine (14 mg) was removed by filtration, mp 225–235° dec. The infrared spectrum (KBr) was identical with that of authentic lycorine.

[2β-^{3}H]Caranine. To a solution of 5 mc of lithium aluminum [^{3}H]hydride (5 mg) and 6 mg of lithium aluminum hydride in dry ether was added 150 mg of lycorine α-epoxide. After the mixture had refluxed for 2 hr, an additional 25 mg of lithium aluminum hydride was added. The mixture was heated under reflux for an additional 3 hr. Excess lithium aluminum hydride was destroyed with wet ether and water was added, and the mixture was extracted with ether and then chloroform. The combined residues from the extractions were chromatographed on 10 g of Merck alumina. Elution with benzene and chloroform gave 67 mg of caranine (1.345×10^6 dpm/mg). Analysis of the product by vapor phase chromatography showed no chemical impurities. Dilution of a small sample of the [2β-^{3}H]caranine with nonradioactive caranine, followed by repeated recrystallization, gave no change from the initial total radioactivity.

Feeding and Isolation. An aqueous solution (2 ml, pH 6) of 19.5 mg of caranine (1.345×10^6 dpm/mg) was introduced into 35 *Zephyranthes candida* bulbs by syringe injection. After 2 weeks of active growth, the bulbs (347 g) were processed according to the usual procedure[26] to give 113 mg of lycorine (1.470×10^4 dpm/mg; 7.05% incorporation). To the basic chloroform extract, after removal of lycorine, was added 30 mg of zephyranthine prepared by the procedure of Nakagawa and Uyeo.[38] This material was recrytallized five times from acetone. The radioactivity fell drastically with each recrystallization. Only 2.6% remained after five recrystallizations, and the activity was still decreasing rapidly.

(37) K. Takeda, K. Kotera, S. Mizukami, *J. Am. Chem. Soc.*, **80**, 2562 (1958).

(38) Y. Nakagawa and S. Uyeo, *J. Chem. Soc.*, 3736 (1959).

[^{3}H]Lycorine Dilution. To 3.950 g of nonradioactive lycorine was added 53 mg of [^{3}H]lycorine (1.470×10^4 dpm/mg) which had been obtained from the feeding experiment. The total mixture was recrystallized from methanol to give 3.50 g of [^{3}H]lycorine (5.37×10^4 dpm/mmole).

O,O-Diacetyllycorine. To a mixture of 5 ml of dry pyridine and 3.5 ml of acetic anhydride was added 1.213 g of lycorine (5.37×10^4 dpm/mmole). The mixture was allowed to stand in the dark for 1 week. The solution was poured into water, neutralized with ammonium hydroxide, and extracted with ether. The ether was evaporated to dryness, and the residue was chromatographed on 20 g of alumina in chloroform to provide 1.480 g of O,O-diacetyllycorine, mp 210–212° (lit.[38] mp 215–216°); 5.37×10^4 dpm/mmole.

1-O-Acetyllycorine. To a solution of 1.45 g of O,O-diacetyllycorine (5.37×10^4 dpm/mmole) in 63 ml of methanol was added 13 ml of 35% hydrochloric acid. The mixture was heated on a steam bath for 4 min. The solution was cooled as rapidly as possible in an ice bath, and 13 ml of concentrated ammonium hydroxide was added. The resulting mixture was poured into 300 ml of cold water and extracted six times with 50-ml portions of chloroform. Evaporation to dryness under reduced pressure gave 1.2 g of material which was purified by chromatography on alumina. Elution with chloroform and 10% methanol in chloroform gave 0.254 g of O,O-diacetyllycorine and 0.850 g of 1-O-acetyllycorine, mp 212–213° (lit.[38] mp 215–216°).

Oxidation of 1-O-Acetyllycorine. To a solution of 0.805 g of 1-O-acetyllycorine in 85 ml of chloroform was added 8.0 g of manganese dioxide. The reaction mixture was stirred for 19 hr at 13°. The manganese dioxide was removed by filtration, and the chloroform solution was evaporated to dryness under reduced pressure to give 520 mg of crude reaction product which was chromatographed on 52 g of alumina. Elution with chloroform provided 300 mg of 1-O-acetyllycorin-2-one, mp 188–192° dec (lit.[38] mp 191° dec), 0.21×10^4 dpm/mmole.

Caranine and Anhydrolycorine from Lycorine. To a mixture of 3.0 ml of phosphorus oxychloride and 1.0 g of powdered sodium chloride was added 0.977 g of lycorine (5.37×10^4 dpm/mmole). After 10 min at 37°, one drop of 35% hydrochloric acid was added. An additional drop was added after 10 min. After 45 min, the mixture was cooled to room temperature and added dropwise to 250 ml of a mixture of water and ice. The solution was made slightly basic with sodium carbonate, allowed to stand 1 hr, and then extracted four times with ether. The ether extract was washed with a saturated solution of sodium chloride and evaporated to dryness under reduced pressure. The residue (600 mg) was dissolved in 20 ml of dry ether and added slowly to 125 ml of a refluxing ethereal solution containing 600 mg of lithium aluminum hydride. After 3 hr, the excess lithium aluminum hydride was destroyed with ethyl acetate and the reaction mixture was treated with 10% sodium hydroxide. The aqueous solution was extracted several times with benzene–ethyl acetate (1:1). The combined organic extracts were evaporated to dryness under reduced pressure, and the residue (500 mg) was chromatographed on alumina. Elution with benzene and ethyl acetate afforded 30 mg of anhydrolycorine (4.99×10^4 dpm/mmole), mp 110–112° (lit.[33] mp 110–111°), and 160 mg of caranine, mp 176–177° (5.37×10^4 dpm/mmole).

Anhydrocaranine from Caranine. Caranine (5.37×10^4 dpm/mmole) was converted, by the published procedure,[33] to anhydrocaranine, mp 150–151° (lit.[33] mp 153–154°); activity 5.36×10^4 dpm/mmole.