The haemanthamine and montanine perchlorate were recrystallized to constant activity to give a total of 62 mg of haemanthamine, mp 199–200 °C (lit.[11] 203–203.5 °C, 3.52×10^7 dpm/mM), and 200 mg of montanine perchlorate, mp 250–251 °C (lit.[11] mp 249–250 °C, 4.21×10^6 dpm/mM).

Both alkaloids were diluted with inactive alkaloid for oxidative degradation. Haemanthamine (1.5 g, 1.46×10^5 dpm/mM) gave 50 mg of *N*-ethylhydrastimide, mp 169–170 °C (1.39×10^5 dpm/mM). Oxidation of 1.5 g of montanine (1.31×10^5 dpm/mM) gave 25 mg of imide, mp 169–170 °C (1.27×10^5 dpm/mM).

Registry No.—**1,** 510-67-8; **2a,** 25375-48-8; **2b,** 639-41-8; **2c,** 59013-69-3; **2d,** 510-69-0; **2e,** 466-75-1; **4a** perchlorate, 58944-40-4; **4a** imide, 3990-41-8; *O*-acetylvittatine, 58944-39-1; undulatine, 6882-09-3; belladine HCl, 58944-41-5; ambelline, 3660-62-6.

References and Notes

(1) Taken in part from the Ph.D. Dissertation of A.I.F., Iowa State University of Science and Technology, June 1967. This work was supported in part by funds from the National Institutes of Health (HL-7503).

(2) R. E. Lyle, E. A. Kielar, J. R. Crowder, and W. C. Wildman, *J. Am. Chem. Soc.*, **82,** 2620 (1960).
(3) H.-G. Boit and H. Ehmke, *Ber.*, **89,** 2093 (1956).
(4) H.-G. Boit and W. Döpke, *Ber.*, **90,** 1827 (1957).
(5) H.-G. Boit, *Ber.*, **89,** 1129 (1956).
(6) H.-G. Boit and W. Döpke, *Naturwissenschaften*, **45,** 315 (1958).
(7) S. Uyeo, K. Kotera, T. Okada, S. Takagi, and Y. Tsuda, *Chem. Pharm. Bull.* **14,** 793 (1966).
(8) H.-G. Boit and H. Ehmke, *Ber.*, **90,** 369 (1957).
(9) S. H. Hung and K. E. Mas, *Yao Hsueh Hsueh Pao*, **11,** 1 (1964).
(10) Y. Inubushi, H. M. Fales, E. W. Warnhoff, and W. C. Wildman, *J. Org. Chem.*, **25,** 2153 (1960).
(11) W. C. Wildman and C. J. Kaufman, *J. Am. Chem. Soc.*, **77,** 1248 (1955).
(12) W. C. Wildman, C. F. Murphy, K.-H. Michel, C. H. Brown, D. T. Bailey, N. Heimer, and R. Shaffer, *Pharmazie*, **72,** 725 (1967).
(13) W. C. Wildman and C. L. Brown, *J. Am. Chem. Soc.*, **90,** 6439 (1968).
(14) G. A. Bray, *Anal. Biochem.*, **1,** 279 (1960).
(15) H.-G. Boit, *Chem. Ber.*, **89,** 1129 (1956).
(16) E. W. Warnhoff and W. C. Wildman, *J. Am. Chem. Soc.*, **79,** 2192 (1957).
(17) L. H. Mason, E. R. Puschett, and W. C. Wildman, *J. Am. Chem. Soc.*, **77,** 1253 (1955).

Interconversions in the Pluviine–Lycorenine Series[1]

R. D. Harken, C. P. Christensen, and W. C. Wildman*

Department of Chemistry, Iowa State University of Science and Technology, Ames, Iowa 50011

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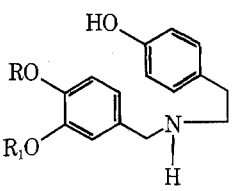
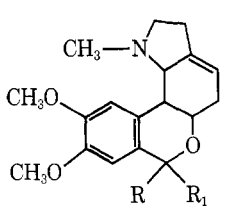
Norpluviine is converted in *Narcissus pseudonarcissus* L. (King Alfred) primarily to alkaloids of the lycorenine type while pluviine undergoes oxidation of the hydroaromatic ring to form unrearranged products in *N. poeticus*. Consistent with our findings on the mechanism of the late stage oxidation of caranine to lycorine, pluviine is converted to galanthine with inversion at C_2. A chemical conversion of lycorine to 7-hydroxylycorine (**12–13**) has been accomplished. Some spectral and chemical properties of the compound are described.

It was recognized in the original biosynthetic hypothesis of oxidative phenyl–phenyl coupling[2] that alkaloids related to lycorenine (**2b**) could not be derived from a norbelladine-type precursor (**1**) directly. The well-documented hemiami-



1

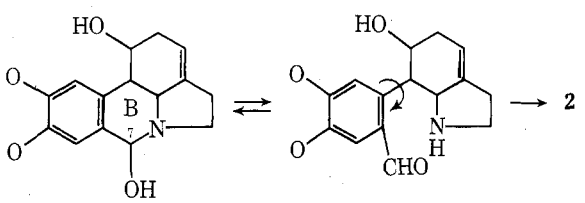
a, R = CH_3; R_1 = H (*O*-methylnorbelladine)
b, R, R_1 = H (norbelladine)



2

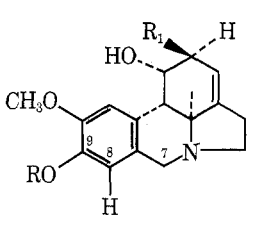
a, R, R_1 = O (homolycorine)
b, R = H, R_1 = OH (lycorenine)

nal–hemiacetal interconversion of haemanthidine and pretazettine[3] suggested that a similar process might occur in alkaloids of the lycorine type.[4] Benzylic oxidation of the CH_2 at C_7 would afford **3**; ring opening to form an amino aldehyde followed by hemiacetal formation and methylation could provide derivatives of **2.**

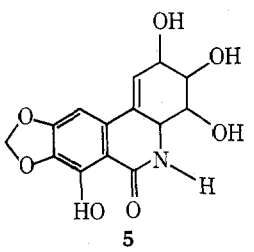


3

To test the validity of this pathway, [8-^{3}H]norpluviine (**4a**) was prepared by tritium exchange. [8-^{3}H]Pluviine (**4b**) was obtained easily by the action of diazomethane on **4a.** The



4



5

a, R = R_1 = H (norpluviine)
b, R = CH_3; R_1 = H (pluviine)
c, R = CH_3; R_1 = OH (methylpseudolycorine)
d, R = CH_3; R_1 = OCH_3 (galanthine)

distribution of the tritium label was determined for radioactive **4b** by oxidation to *m*-hemipinic acid (4,5-dimethoxyphthalic acid) which was converted to the *N*-ethyl imide. The specific molar activity of the imide was 78% of that found for **4b,** indicating that this percentage of the tritium was located in the aromatic ring. An aqueous solution (pH 6) of 5 mg of

Table I. [8-^{3}H]Norpluviine [a] Feeding to *Narcissus* "King Alfred"

Alkaloid	Activity, dpm/mM	% inc.
Lycorenine	34.9×10^5	0.11
Homolycorine	12.3×10^5	0.018
Pluviine	23.4×10^5	0.029
Methylpseudolycorine	2.46×10^5	0.0023
Galanthine	6.79×10^5	0.017
Narciclasine	0.40×10^5	0.0034

[a] The total activity of [8-^{3}H]norpluviine fed was 0.10 mCi.

Table II. [8-^{3}H]Pluviine [a] Feeding to *Narcissus poeticus*

Alkaloid	Activity, dpm/mM	% inc.
Lycorenine	0	0.00
Galanthine	1.09×10^8	3.26
Methylpseudolycorine	1.35×10^7	0.25
Narcissidine	7.50×10^5	0.021
Lycorine	1.91×10^6	0.14

[a] The total activity of [8-^{3}H]pluviine fed was 0.10 mCi.

[8-^{3}H]norpluviine was injected into the flower stalks of King Alfred daffodils. After 18 days, the plants were harvested and the alkaloids were isolated by standard methods. The major alkaloid, haemanthamine, was found to be inactive as expected. Both lycorenine (**2b**) and galanthine (**4d**) were degraded to *N*-ethylhemipinimide which had 79 and 75%, respectively, of the specific molar activity (dpm/mM) of the original [8-^{3}H]norpluviine. Table I summarizes the incorporation data. From these results it is clear that norpluviine is converted much more readily into the rearranged nucleus (**2**) than it is oxidized or simply O-methylated within its own ring system. Most surprising was the observation that the narciclasine (**5**) isolated from this feeding was radioactive. Furthermore, the percent conversion of [8-^{3}H]norpluviine to narciclasine given in Table I must be grossly low, since all tritium at C_8 in the precursor (78%) would have been lost in the transformation. Previous work has indicated that **5** is derived from vittatine[5] and that norpluviine is not converted to narciclasine.[6]

Strikingly different results were obtained when [8-^{3}H]-pluviine (**4b**) was the biosynthetic precursor. The plant host chosen for the study was *Narcissus poeticus* L. since it was readily available and contained lycorenine, pluviine, methylpseudolycorine, galanthine, and narcissidine in isolable amounts. Table II shows that pluviine is an excellent precursor for more highly oxygenated alkaloids of the same nucleus.

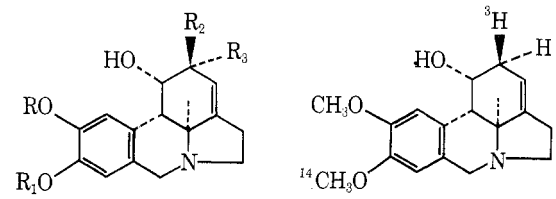
It is most interesting that the lycorenine was completely inactive. It would appear that a free phenolic group at C_9 in **4** promotes the benzylic hydroxylation necessary for rearrangement to the ring system of **2**.[7]

The in vivo conversion of *O,O*-dimethoxy alkaloids to the methylenedioxy related compounds has not been observed before. In this feeding experiment pluviine was converted to lycorine rather efficiently. The mechanism for this transformation probably does not involve a simple O-demethylation at C_9, since this intermediate would be norpluviine, a known precursor of lycorenine (vide supra) which should then be radioactive when isolated.

The stereochemistry of the oxidation process by which C_2 in **4** is converted from a methylene to either a hydroxyl or methoxyl group has been the subject of conflicting results. In the first report[8] [2β-^{3}H]caranine (**6a**) was converted to [2-α-^{3}H]lycorine (**6b**) in *Zephyranthes candida* by hydroxylation with inversion of configuration of the original 2β-^{3}H atom. A similar mechanism was reported by Bruce and Kirby[9] using both a different biosynthetic precursor and degradative route. A retention mechanism was proposed by Fuganti and Mazza[10] for the oxidation of caranine to lycorine in *Clivia miniata*. Since our initial experiments were with singly labeled material, it was considered of value to repeat the sequence with a doubly labeled precursor. [2β-^{3}H,9-*O*-methyl-^{14}C]pluviine (**7**) was selected for this purpose.

Table III. Pluviine Feeding to *Narcissus* "King Alfred"

Alkaloid	Quantity, mg	Specific activity, mCi/mg	Total activity, mCi	^{3}H/^{14}C ratio
Pluviine	9	0.77×10^{-3} ^{3}H	6.9×10^{-3} ^{3}H	
		0.578×10^{-3} ^{14}C	5.2×10^{-3} ^{14}C	1.3
Galanthine	29	0.73×10^{-5} ^{14}C	2.1×10^{-4}	
		0.75×10^{-5} ^{3}H	2.2×10^{-4}	1.0



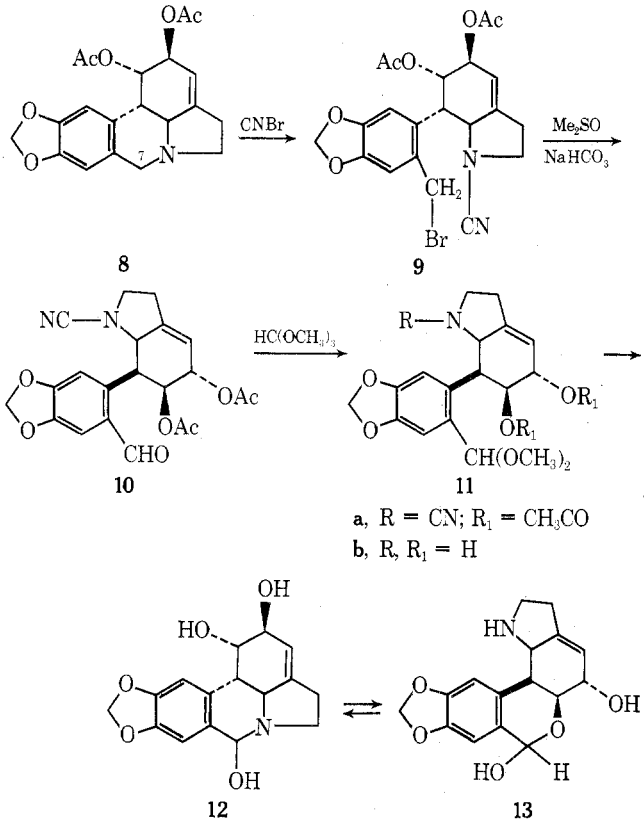
6 7

a, R, R_1 = $CH_2<$; R_2 = ^{3}H; R_3 = H
b, R, R_1 = $CH_2<$; R_2 = OH; R_3 = ^{3}H
c, R, R_1 = H; R_2 = OH; R_3 = H

Lycorine (**6b**, no label) was cleaved to **6c** with boron tribromide. Methylation with diazomethane provided methylpseudolycorine (**4c**) which was converted to [2β-^{3}H]-pluviine by the method used in our [2β-^{2}H]caranine synthesis.[8] [2β-^{2}H]Pluviine was also prepared to determine the label distribution and stereochemistry. Pyrolysis of *O*-acetyl[2β-^{2}H]pluviine provided anhydropseudolycorine with 75% of the deuterium retained. This figure was considered to represent the label at the β-C_2 position since the pyrolysis should proceed by a cyclic cis mechanism. [9-*O*-Methyl-^{14}C]pluviine (**4b**) was prepared by the methylation of **4a** with [^{14}C]diazomethane. A combination of these precursors gave doubly labeled material which was crystallized to constant activity and introduced into blooming "King Alfred" daffodils. After 2 weeks, radioactive galanthine was isolated from the plants with 4.1% incorporation into the alkaloid based on ^{14}C. The carbon–tritium ratio in the isolated galanthine showed a retention of 79% of the tritium (Table III). This result supports our previous work[8] and that of Bruce and Kirby.[9]

To complete our research on the chemistry and biosynthesis of alkaloids related to **2** and **4** it was desirable to attempt a synthesis of a compound related to **3** for chemical and spectral investigation. Although *O,O*-diacetyl-7-oxolycorine (**8**, C=O at C_7) was available by permanganate oxidation of *O,O*-diacetyllycorine (**8**), no chemical reducing agent was found to convert the lactam to the hemiaminal in reasonable yield. A successful route was found in an extension of the work of Mizukami[11] as outlined in Chart I. With cyanogen bromide in chloroform solution **8** provided **9** in greater than 80% yield. The reaction product was not isolated but oxidized with dimethyl sulfoxide–sodium bicarbonate to give **10**. Protection of the aldehyde as the acetal followed by lithium aluminum hydride reduction gave **11b**. This product was unstable and characterized only by ^{1}H NMR and ir spectra. Acid hydrolysis of **11b** provided a crystalline product which reacted with lithium aluminum hydride to form lycorine (**6b**, R_3 = ^{1}H). On this basis structure **12** should be assigned to the hydrolysis

Chart I. Interconversions of Lycorine



product of **11b** since **13** would be expected to give a dihydroxybenzyl alcohol under these conditions.

Spectral evidence, however, seemed more in accord with **13** for the structure of the hydrolysis product as shown in Table IV. The benzylic proton of **12–13** shows a chemical shift in agreement with model compounds containing the hemiacetal rather than the hemiaminal moiety. Further chemical and spectral studies on this compound are in progress.

Experimental Section

Melting points were taken on a Köfler microscope hot stage apparatus and are corrected. Infrared spectra were taken on either a Beckman Model IR-12 or IR-18A recording spectrophotometer in chloroform solution or as a potassium bromide pellet. The proton magnetic resonance spectra were obtained on a Varian A-60 or HA-100 in chloroform-d_1 unless another solvent was indicated. Mass spectra were recorded on an MS-902 mass spectrometer. This spectrometer was purchased on NSF Grant-GP 10226. The measurements of radioactivity were made with a Packard Tri-Carb liquid scintillation spectrometer (Model 3002) at ambient temperature. Solutions for counting were either toluene–PPO–POPOP [4.9 g of 2,5-diphenyloxazole (PPO) and 0.1 g of 1,4-bis-2-(5-phenyloxazole)benzene (POPOP) in 1 l. of dry toluene] or Bray's solution.[14] Efficiency of counting tritium was determined for each sample by means of an internal standard of [^{3}H]- or [^{14}C]toluene. Reproducibility of the assays was ±3%.

Preparative-scale layer chromatography used 20 × 20 cm glass plates coated with silica gel (Merck PF 254 + 366) 0.5 mm in thickness. The plates were eluted once with the solvent system specified. The alkaloids were detected by ultraviolet light. The bands of silica gel and alkaloid were removed from the plate and covered with 80–100 ml of 10% HCl for a minimum of 1 h. The acid was neutralized with 10% aqueous ammonia and the aqueous layer including the silica gel was extracted with chloroform until the aqueous layer gave a negative silico-tungstic acid test.

The alkaloids were identified by their TLC behavior, melting point, mixture melting point, and by comparison of their ir spectra with known reference spectra. The alkaloids were purified from chromatographic fractions via recrystallization from appropriate solvents to constant activity. The percent incorporation was calculated as (100 × total activity of isolated alkaloid) divided by (total activity fed). For this purpose, the final constant activity of the alkaloid per milligram was multiplied by the quantity of alkaloid isolated (mg) which was of good chemical purity as determined by TLC, melting point, and ir.

Table IV. Benzylic Proton Chemical Shifts

Compd	Chemical shift(s), δ	Assignment	Ref
12–13	6.02	Hemiacetal	*a*
Cotarnine	5.39	Carbinolamine	*a*
6-Hydroxybuphanidrine	5.31	Carbinolamine	12
6-Hydroxypowelline	5.38	Carbinolamine	12
6-Hydroxycrinamine	5.0, 5.6	Carbinolamine	13
Haemanthidine	5.0, 5.6	Carbinolamine	13
Pretazettine	6.06	Hemiacetal	3
Lycorenine	6.04	Hemiacetal	3
6-Hydroxyundulatine	5.20	Carbinolamine	12

[a] This work.

[8-^{3}H]Norpluviine (4a). The material was prepared by New England Nuclear Corp., Boston, Mass., by heating norpluviine in tritiated acetic acid in the presence of a platinum catalyst. A portion of the [8-^{3}H]norpluviine was purified by reprecipitation from aqueous acid solution using solid sodium bicarbonate, and finally by crystallization from methanol. This purification procedure gave 15 mg of [8-^{3}H]-norpluviine, mp 237–239 °C (lit.[15] mp 239–241 °C), with a specific activity of 1.22×10^{10} dpm/mM, 5.51 mCi/mM. The infrared spectrum of this material was identical with the infrared spectrum of authentic norpluviine. Dilution with pure inactive norpluviine resulted in no gain or loss of total activity.

A diluted sample of [8-^{3}H]norpluviine was purified as above to give 29 mg (5.62×10^9 dpm/mM). This material was suspended in 2 ml of methanol and treated with 10 ml of ethereal diazomethane at 25 °C for 6 h. The solvent was removed and the residue resuspended in 2 ml of methanol and treated again with 10 ml of ethereal diazomethane for 4 h. The solution was concentrated and chromatographed on a preparative-scale TLC plate, and eluted with chloroform/methanol/diethylamine (93/2/5). This afforded 20 mg (67%) of crude [8-^{3}H]pluviine which was recrystallized from benzene/hexane (mp 218–219 °C, lit.[15] mp 220–221 °C), 5.68×10^9 dpm/mM, 2.55 mCi/mM. The specific molar activity of [8-^{3}H]norpluviine relative to the specific molar activity of [8-^{3}H]pluviine was 1.01. The infrared spectrum of [8-^{3}H]pluviine was identical with the spectrum of authentic pluviine. Dilution of the sample with inactive pluviine did not alter the total activity.

Conversions to *N*-Ethylhemipinimide. The following oxidation is representative. Pluviine (200 mg, 1.33×10^7 dpm/mM) was dissolved in 30 ml of 10% hydrochloric acid. The solution was adjusted to pH 8 with solid sodium carbonate. A solution of 1.2 g of potassium permanganate was added at room temperature over 35 min. Stirring was continued for 4 h. Sulfur dioxide was passed through the reaction mixture until clear. After acidification to pH 2 with concentrated sulfuric acid and concentration to 50 ml under reduced pressure, the solution was extracted continually with ether for 36 h. Concentration of the ether gave a wet residue that was partitioned between ethyl acetate and 5% sodium bicarbonate solution. The aqueous basic layer was acidified with 50% sulfuric acid to pH 2 and extracted with eight 30-ml portions of ethyl acetate. The organic extract was dried ($MgSO_4$), filtered, and evaporated to dryness with acetic anhydride. The residue was sublimed at 120 °C (0.1 mm). The sublimate was triturated with 70% ethylamine and resublimed. The product was recrystallized from ethanol to give 11 mg of product, mp 230–231 °C (lit.[16] mp 229–230 °C), 1.04×10^7 dpm/mM. The specific molar activity of the imide relative to that of the [8-^{3}H]pluviine was 0.78.

Feeding Experiments and Isolation of Alkaloids. A typical procedure is as follows. Five milligrams of [8-^{3}H]norpluviine (2.22×10^8 dpm, 0.10 mCi) in 1.1 ml of aqueous HCl solution at pH 6 was injected with fine hypodermic syringe in equal amounts into the flower stalks of 13 blooming *Narcissus pseudonarcissus* L. var. "King Alfred" plants. The plants were allowed to grow for 18 days. The plants (1.47 kg) were processed by grinding in a Waring Blendor using 6 l. of 95% ethanol. The solid material was filtered, covered with 3 l. of ethanol, and allowed to stand overnight. This treatment was repeated twice. The combined ethanolic extracts were concentrated in vacuo to 1.5 l. The aqueous suspension was acidified to pH 4 with 10% tartaric acid. The aqueous solution was washed five times with 200-ml portions of diethyl ether. Three extractions of the aqueous layer with 200-ml portions of 1-butanol yielded, after removal of the butanol,

9.5 g of brown gum. Alkaloid fractions were obtained by extraction of the aqueous layer with six 200-ml portions of chloroform at pH 7, 9, and 12. The combined chloroform extracts yielded 2.6 g of crude alkaloid mixture which was chromatographed on preparative-scale TLC plates eluting with 70/30/1 ethyl acetate/methanol/ammonia to give four fractions (1–4). Fraction 2, R_f 0.7, gave 172 mg of haemanthamine, mp 198–200 °C (lit.[17,18] mp 200–201 °C), that was devoid of tritium activity. The mother liquors and fraction 3 were rechromatographed on plates in 90/10/0.5 chloroform/methanol/ammonia. The lycorenine containing fractions (as determined by analytical TLC) were combined and chromatographed on a column of 600 mg of alumina (Merck). Lycorenine was eluted in ethyl acetate and ethyl acetate/chloroform mixtures. Trituration with acetone yielded 12 mg of impure lycorenine. The material was diluted with 30 mg of nonradioactive lycorenine and recrystallized several times from ethyl acetate to yield 23 mg, mp 197–199 °C (lit.[19] mp 198–200 °C). Constant activity was attained at 1.11×10^4 dpm/mg. The total activity was 0.11 μCi.

Fractions containing homolycorine from the thick layer plate and column chromatography were combined and purified further on preparative-scale TLC plates using chloroform/methanol/acetone (60/20/20) as the solvent system (R_f 0.8). The homolycorine was obtained in trace quantities and was diluted with 40 mg of nonradioactive homolycorine. Recrystallization from ethanol afforded 10 mg of homolycorine, mp 174–176 °C (lit.[20] mp 175 °C) with constant activity of 3.92×10^3 dpm/mg and a total activity of 0.018 μCi.

Column and TLC fractions containing pluviine were combined and further purified via preparative-scale TLC eluting with 60/20/20 chloroform/methanol/acetone (R_f 0.7). Several recrystallizations from acetone gave 8 mg of pluviine, mp 217–219 °C (lit.[21] mp 225 °C). The activity was constant at 8.16×10^3 dpm/mg with a total activity of 0.029 μCi.

By analytical TLC, the mother liquor of pluviine crystallization contained methylpseudolycorine. Dilution with 10 mg of inactive methylpseudolycorine and recrystallization initially from ethanol and then from acetone yielded 7 mg, mp 234–237 °C (lit.[22] mp 234–242 °C). Constant activity was attained at 7.11×10^2 dpm/mg with a total activity of 2.3×10^{-3} μCi.

Fractions rich in galanthine were rechromatographed on preparative-scale TLC plates using 60/20/20 chloroform/acetone/methanol (R_f 0.6). Recrystallization from ethyl acetate/hexane afforded 18 mg of galanthine, mp 135–136 °C (lit.[22] mp 134–136 °C). Radioactivity was constant at 2.14×10^3 dpm/mg. The total activity was 0.017 μCi.

The gum isolated by extraction with butanol was dissolved in 200 ml of chloroform and 50 ml of ethanol and extracted with three 50-ml portions of 15% sodium hydroxide. The combined basic layers were washed with three 50-ml portions of chloroform. These chloroform extracts were added to the initial chloroform/ethanol solution, evaporated to dryness, and dissolved in 10% HCl (150 ml). The acidic solution was extracted with ether, basified to pH 7, and extracted with chloroform. The chloroform extracts were added to the alkaloid fractions. The initial aqueous basic layers were acidified with 20% HCl and extracted four times with 100-ml portions of ethyl acetate. Concentration of the extract gave 0.70 g of material. This material, in ethyl acetate, was chromatographed on a 20-g column of silica gel, packed in ethyl acetate. Narciclasine was eluted with ethyl acetate and recrystallized from acetic acid to give 59 mg of product, dec above 220 °C (lit.[23] mp 232–234 °C). Its activity was constant at 1.29×10^2 dpm/mg. Its total activity was 3.4×10^{-3} μCi.

Methylpseudolycorine (4c). One gram of lycorine was added to a solution of 2 ml of boron tribromide in 25 ml of methylene chloride at −80 °C. After 2 h, the solution was allowed to warm to ambient temperature and the reaction solution was stirred overnight. The solution was evaporated to dryness under reduced pressure and the residue was dissolved in methanol and treated twice with 10 mmol of diazomethane. The crude product was dissolved in chloroform and extracted with 10% sodium hydroxide solution. After the solvent was removed, 4c was recrystallized from ethanol to give 300 mg (28%), mp 233–235 °C (lit.[22] 234–242 °C dec).

[9-Methoxy-^{14}C]pluviine (4b). Diazomethane (0.6 mmol) was generated from [*N*-methyl-^{14}C]-*N*-nitroso-*p*-toluenesulfonamide (1.0 mmol) by adding an ethereal solution of the precursor into a solution of ethanolic potassium hydroxide at 60 °C. The labeled diazomethane was distilled into a cold trap at 0 °C containing 100 mg of norpluviine in methanol. The reaction mixture was stirred overnight and evaporated to dryness. The residue was dissolved in chloroform and extracted with 1% sodium hydroxide and the resulting chloroform solution was dried and evaporated to dryness. The labeled pluviine was recrystallized from benzene–hexane, yield 18 mg (17%), mp 225–226 °C.

Methylpseudolycorine α-Epoxide. The synthesis was patterned after the approach of Wildman and Heimer[8] to lycorine α-epoxide. To a mixture of 200 mg of sodium chloride and 150 mg of methylpseudolycorine, 1 ml of phosphorus oxychloride was added. The slurry was heated to 35 °C with stirring; after 5 min, 2 drops of 6 N hydrochloric acid was added, and the reaction mixture was stirred for another 30 min. The reaction mixture was hydrolyzed in ice water and neutralized with sodium hydroxide. The aqueous solution was extracted with ether and evaporated to yield methylpseudolycorine α-epoxide (50 mg) which was contaminated with anhydromethylpseudolycorine; exact mass determination of $[M - 1]^+$ 284.1282 (calcd for $C_{17}H_{19}NO_3$, 284.1287).

The ^{1}H NMR spectrum of methylpseudolycorine α-epoxide was in good agreement with that found for lycorine α-epoxide when peaks due to anhydromethylpseudolycorine were subtracted.

Anhydromethylpseudolycorine. Galanthine (100 mg) was dissolved in 6 N hydrochloric acid and refluxed for 4 h. The reaction mixture was neutralized and extracted with chloroform to yield anhydromethylpseudolycorine which was recrystallized from ethanol, 75 mg, mp 170–173 °C, recrystallized 176 °C, dec above 230 °C; exact mass determination for $[M - 1]^+$ 266.1178 (calcd for $C_{17}H_{19}NO_2$, 266.1181).

[2β-^{2}H]Pluviine (4a). Methylpseudolycorine α-epoxide (50 mg) was dissolved in 10 ml of dry ether and added to 25 mg of lithium aluminum hydride-^2H in dry ether. The reaction mixture was refluxed for 1 h and the excess lithium aluminum hydride was hydrolyzed with wet ether. Saturated sodium potassium tartrate was added and the [2β-^{2}H]pluviine was extracted with chloroform and purified by TLC (R_f 0.6; 6/2/2 chloroform/acetone/methanol), 25 mg (50%); mp 222–225 °C; exact mass determination 288.1594 (calcd for $C_{17}H_{20}{}^2HNO_3$, 288.1584).

[2β-^{2}H]-*O*-Acetylpluviine. [2β-^{2}H]Pluviine was dissolved in acetic anhydride–pyridine. After 24 h, the reaction mixture was evaporated to dryness under reduced pressure and the product was sublimed at 160 °C (0.001 Torr), exact mass determination 330.1668 (calcd for $C_{19}H_{22}{}^2HNO_4$, 330.1690).

Pyrolysis of [2β-^{2}H]-*O*-Acetylpluviine. The sample was sealed in a Pyrex tube at 0.001 Torr and heated in a 240 °C oven for 45 min. The volatile fraction was shown by MS to contain acetic acid and the nonvolatile fraction contained [2-^{2}H]anhydromethylpseudolycorine.

Feeding of the Doubly Labeled Pluviine. The labeled pluviine (0.77 mCi/mg ^{3}H, 0.58 mCi/mg ^{14}C, 9 mg) was dissolved in dilute hydrochloric acid and injected into the flower stalks of nine blooming *Narcissus* "King Alfred" plants, which were harvested after 2 weeks. The bulbs and the flower stalks (514 g) were processed. The pH 8 and 10 chloroform extracts were combined and chromatographed on silica gel with 60/20/20 chloroform/acetone/methanol to yield galanthine (29 mg). The identity of the galanthine was confirmed by comparison with an authentic sample. Tracer results are given in Table III.

Addition of Cyanogen Bromide to *O,O*-Diacetyllycorine. A mixture of 1 g of *O,O*-diacetyllycorine[24] and 1 g of cyanogen bromide was dissolved in 50 ml of chloroform. After 500 mg of potassium carbonate was added, the solution was refluxed for 6 h. The potassium bromide was removed by filtration and the filtrate was evaporated to dryness. The product (**9**) was contaminated with the isomer in which the five-membered ring was opened. They were not separated but used for the next reaction, yield 1.2 g, mp 186–187 °C, exact mass determination 476.0602 (calcd for $C_{21}H_{21}N_2O_6Br$, 476.0583).

Me_2SO Oxidation of 9 to 10. To 5 ml of Me_2SO and 500 mg of sodium bicarbonate, 500 mg of **9** was added and the reaction mixture was heated at 120 °C for 1 h. The Me_2SO was removed under reduced pressure and the residue was dissolved in a mixture of chloroform and water. The chloroform layer was removed, dried, and chromatographed on a silica gel column. The undesired isomer of **9** was eluted with chloroform/benzene (3:1) and **10** was eluted with chloroform, giving 420 mg (80%), mp 186–187 °C, exact mass determination 412.1266 (calcd for $C_{21}H_{20}N_2O_7$, 412.1270).

Conversion of the Aldehyde 10 to the Acetal 11a. To a solution of 200 mg of the aldehyde **10** in 1 ml of acidic methanol, 3 ml of methyl orthoformate was added. The solution was refluxed for 3 h. The excess methyl orthoformate and methanol were removed in vacuo and the residue was recrystallized from acetone, 220 mg (95%), mp 81–83 °C, exact mass determination 458.1710 (calcd for $C_{23}H_{26}N_2O_8$, 458.1688).

Acetal 11b. A solution of **11a** (100 mg) in dry tetrahydrofuran was added to lithium aluminum hydride (200 mg) and refluxed for 3 h. The excess lithium aluminum hydride was destroyed with saturated sodium potassium tartrate and the organic layer was separated. The aqueous layer was extracted twice with tetrahydrofuran, the organic layers were combined and dried, and the solvent was removed under

reduced pressure to yield 50 mg of amorphous **11b**. High-resolution mass spectra were unobtainable. The infrared spectrum showed the loss of both the acetate carbonyl and the *N*-cyano bands: NMR ($CDCl_3$) δ 7.3 (1 H, s), 7.1 (1 H, s), 5.95 (2 H, s), 5.6 (1 H, s), 5.5 (1 H, s), 3.3 (3 H, s).

Hydrolysis of 11b. A sample (25 mg) of **11b** was dissolved in tetrahydrofuran/water (1:1) and a drop of 6 N hydrochloric acid was added. The solution was warmed on a steam bath and allowed to stand overnight. On evaporation at room temperature the product appeared as a crystalline solid, 10 mg, mp 205–210 °C dec, NMR [CH_3CN/D_2O (1/1)] δ 7.2 (1 H, s), 7.4 (1 H, s), 6.2 (2 H, s), 6.0 (1 H, s), 5.8 (1 H, s).

Reduction of 10 mg of the solid with 50 mg of lithium aluminum hydride in tetrahydrofuran for 1 h followed by a standard workup gave lycorine (**6b**, $R_3 = {}^1H$) identified by comparison of its mass spectrum and infrared spectrum with those of authentic lycorine.

Registry No.—**2a**, 477-20-3; **2b**, 477-19-0; **4a**, 517-99-7; **4b**, 548-11-8; **4c**, 476-29-9; **4d**, 517-78-2; **5**, 29477-83-6; **8**, 2492-05-9; **9**, 58958-43-3; **10**, 58944-33-5; **11a**, 58944-34-6; **11b**, 58944-35-7; **12**, 58958-42-2; *N*-ethyl hemipinimide, 27002-36-4; haemanthamine, 466-75-1; lycorine, 476-28-8; methylpseudolycorine α-epoxide, 58944-36-8; anhydromethylpseudolycorine, 58944-37-9; *o*-acetylpluviine, 58944-38-0; cyanogen bromide, 506-68-3.

References and Notes

(1) Abstracted in part from the Ph.D. Dissertations of R.E.H., June 1970, and C.P.C., August 1974. This work was supported in part by funds from the National Institutes of Health (HL-7503).
(2) D. H. R. Barton and T. L. Cohen, "Some Aspects of Phenol Oxidation", in "Festschrift Arthur Stoll", Birkhauser, Basel, Switzerland, 1957, pp 117–143.
(3) W. C. Wildman and D. T. Bailey, *J. Am. Chem. Soc.*, **91**, 150 (1969).
(4) A conversion of norpluviine to galanthine has been reported earlier: G. W. Kirby and H. P. Tiwari, *J. Chem. Soc. C*, 676 (1966).
(5) C. Fuganti and M. Mazza, *J. Chem. Soc., Chem. Commun.*, 239 (1972).
(6) C. Fuganti, J. Staunton, and A. R. Battersby, *Chem. Commun.*, 1154 (1971).
(7) A nonphenolic aromatic ring, however, does not preclude benzylic oxidation and rearrangement to **2**. Caranine is converted to hippeastrine by *Zephyranthes candida* 0.005% incorporation: N. E. Heimer, Ph.D. Dissertation, Iowa State University, Ames, Iowa, 1968.
(8) N. E. Heimer and W. C. Wildman, *J. Am. Chem. Soc.*, **89**, 5265 (1967).
(9) I. T. Bruce and G. W. Kirby, *Chem. Commun.*, 207 (1968); *Chimia*, **22**, 314 (1968).
(10) C. Fuganti and M. Mazza, *J. Chem. Soc., Chem. Commun.*, 936 (1972).
(11) S. Mizukami, *Tetrahedron*, **11**, 89 (1960).
(12) M. R. Slabaugh and W. C. Wildman, *J. Org. Chem.*, **36**, 3202 (1971).
(13) R. W. King, C. F. Murphy, and W. C. Wildman, *J. Am. Chem. Soc.*, **87**, 4912 (1965).
(14) G. A. Bray, *Anal. Biochem.*, **1**, 279 (1960).
(15) G. W. Kirby and H. P. Tiwari, *Chem. Commun.*, 676 (1966).
(16) "Dictionary of Organic Compounds", Vol. 4, Oxford University Press, London, 1965, p 2096.
(17) W. C. Wildman and C. J. Kaufman, *J. Am. Chem. Soc.*, **77**, 1248 (1955).
(18) H. M. Fales and W. C. Wildman, *Chem. Ind. (London)*, 561 (1958).
(19) H.-G. Boit, *Chem. Ber.*, **87**, 681 (1954).
(20) T. Kitagawa, W. I. Taylor, S. Uyeo, and H. Yajima, *J. Chem. Soc.*, 1066 (1955).
(21) H.-G. Boit and H. Ehmke, *Chem. Ber.*, **89**, 163 (1956).
(22) H. M. Fales, L. D. Gluffrida, and W. C. Wildman, *J. Am. Chem. Soc.*, **78**, 4145 (1956).
(23) F. Piozzi, C. Fuganti, R. Mondelli, and G. Ceriotti, *Tetrahedron*, **24**, 1119 (1968).
(24) A. Hunger and T. Reichstein, *Helv. Chim. Acta*, **36**, 824 (1953).

Crystal Structure and Absolute Configuration of Astrocasine Methobromide

W. M. Bright[1]

Chemistry Department, Georgetown University, Washington, D.C. 20007

H. A. Lloyd and J. V. Silverton*

Laboratory of Chemistry, NHLI, National Institutes of Health, Bethesda, Maryland 20014

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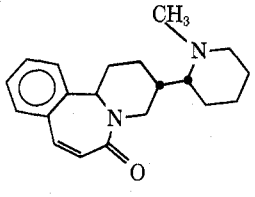
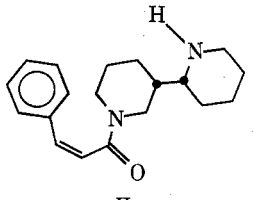
The determination of the crystal structure of the methanol-solvated methobromide of astrocasine, $(C_{21}H_{29}N_2O)^+Br^-\cdot CH_3OH$, confirms the molecular structure proposed by one of us and the absolute configuration was found to be the same as that of the biogenetically related alkaloid, astrophylline. Crystal data: orthorhombic, $P2_12_12_1$, $a = 7.654$ (1), $b = 8.678$ (1), $c = 32.589$ (3) Å, $Z = 4$, $d_x = 1.342$ g/cm^3, $d_m = 1.34$ (1) g/cm^3, x-ray intensity data were collected with an automatic diffractometer out to $\sin\theta/\lambda = 0.624$ Å^{-1} (3451 observed and 552 "unobserved" reflections.) The structure was solved using the heavy atom and "phase correction" methods. A final *R* factor of 3.1% (based on observed reflections) resulted.

Several new alkaloids[2-4] were isolated a few years ago from *Astrocasia phyllanthoides Robinson and Millspaugh*, a shrub belonging to the Euphorbiaceae family and growing in Central America.

A structure (I) was advanced[2] for the predominant alkaloid,



I



II

astrocasine ($C_{20}H_{26}N_2O$, mp 171–172 °C) based mostly on spectral data (ir, uv, NMR, MS) and on a partial degradation. Later[3] the isolation of astrophylline ($C_{19}H_{26}N_2O$) from the same plant and its characterization as *N-cis*-cinnamoyl-3(*S*)-[2′(*R*)-piperidyl]piperidine (II) provided strong support for structure I. Astrophylline was the first *cis*-cinnamoyl alkaloid reported in nature and astrocasine is simply its cyclic analogue.

More recently[4] *N*-methylastrophylline, $C_{20}H_{28}N_2O$, and astrocasidine, $C_{20}H_{24}N_2O$, related to astrocasine but possessing an additional double bond conjugated with the aromatic ring, were also found in *A. phyllanthoides*.

Two alkaloids related to astrophylline, orensine and isoorensine, had been identified earlier in several *Adenocarpus* species (Leguminosae) by Ribas et al.[5] and both had been assigned *N-trans*-cinnamoyl tetrahydroanabasine skeletons; however, a later revision[6] indicated that isoorensine was the *cis*-cinnamoyl isomer of orensine.

A possible biogenetic pathway for demethylastrocasine and isoorensine,[4] postulating tautomerism of a charged intermediate, has been proposed as a variant of the scheme of Schütte et al.[7] for orensine.

Since the proposed structure for astrocasine contained a new and unique heterocyclic ring system, it seemed worth-