6-Hydroxybuphanidrine and 6-Hydroxypowelline[1]

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6-Hydroxybuphanidrine and 6-hydroxypowelline have been isolated from *Nerine bowdenii* W. Wats. and characterized. Evidence is presented to show that the alkaloids are represented by structures **1** and **9**, respectively. The reactions and spectroscopic properties of these alkaloids are compared with those found for 11-hydroxy analogs. Oxime derivatives of several α-carbinolamines have been prepared and a mechanism is proposed to account for their formation.

The bulbs of *Nerine bowdenii* W. Wats. contain a large number of *Amaryllidaceae* alkaloids. At present, 19 alkaloids have been reported to be present in the plant.[3,4] Sixteen alkaloids were isolated from this plant source in 1960,[4] but it was reported that 66% of the total alkaloid fraction remained noncrystalline and uncharacterized. In view of the recent advances in preparative thin layer chromatography, an investigation of these amorphous residues was undertaken. Two alkaloids containing the 5,10b-ethanophenanthridine nucleus have been isolated. This paper describes spectra and reactivity of the new alkaloids and defines their structures.

Preliminary fractionation of the alkaloids by column chromatography[4] produced considerable quantities of a mixture which contained crinamine and an unknown compound. Thin layer chromatography of this mixture on a silica gel plate developed in chloroform–methanol–diethylamine (90:5:5) led to the isolation of crinamine and 6-hydroxybuphanidrine (**1**, mp 95–96°, $[\alpha]^{24}D$ −64°). The infrared spectrum of the compound indicated the presence of a hydroxyl group (3595 cm^{-1}) and an aromatic ring substituted with both methylenedioxy (945 and 1050 cm^{-1}) and methoxyl (1618 cm^{-1}) groups. The ultraviolet absorption spectrum (λ_{max} 285 nm, ϵ 1880) is in agreement with this formulation.[5] The alkaloid was stable to chemical methods of reduction but gave a single dihydro derivative upon catalytic reduction. The *O*-acetyl derivative ($C_{20}H_{23}NO_6$) was obtained by routine acetylation conditions. The nmr spectrum of **1** confirms the presence of a hydroxyl group (5.10 ppm) which disappears upon exchange with deuterium oxide. The spectrum also reveals an aliphatic methoxyl (3.33 ppm), an aromatic proton (6.60 ppm), and two olefinic protons centered at 6.00 and 6.58 ppm. Since the base contains no *N*-methyl or NH groups and yet possesses two olefinic protons, it could be tentatively assigned the 5,10b-ethanophenanthridine nucleus by a process of elimination.[6]

To verify this conclusion, it seemed desirable to convert the alkaloid to a deoxy compound which might be identical with an alkaloid of known structure. Treatment of **1** with thionyl chloride followed by lithium aluminum hydride gave a compound which, by all spectroscopic and chromatographic criteria, was identical with the alkaloid buphanidrine (**2**). This interconversion established the basic nucleus of the alkaloid, its absolute configuration, the configuration of the aliphatic methoxyl at C_3, and the location of the olefinic bond at C_1–C_2. Additional proof that the double bond is located at C_1–C_2 was obtained from the nmr spectrum. The single aromatic proton appeared as a singlet at 6.60 ppm and was shifted to 6.38 ppm in dihydro-6-hydroxybuphanidrine. This upfield shift has been observed in other alkaloids of this series and results from the loss of the deshielding of the C_{10} proton by the C_1,C_2 unsaturation.[7] The two olefinic protons appeared as an AB pattern which overlapped partially with methylenedioxy absorption. The proton at C_2 (6.00 ppm) is further split by coupling to the proton at C_3 (J = 5 cps).

With this information, only the location of the hydroxyl group remained to be determined. The chemical properties of the alkaloid precluded the presence of this functional group at C_1, C_2, or C_3. Substitution at C_4, C_{4a}, or C_{12} is unlikely since this type of substitution is unknown in the *Amaryllidaceae* alkaloids. Assignments of the hydroxyl group to C_{11} can be ruled out since the compound is not identical with either ambelline or epiambelline, both of which are known.[8] The infrared absorption of the hydroxyl group obtained at high dilution in chloroform shows a single band at 3595 cm^{-1}. This is compatible with a hydroxyl function weakly hydrogen bonded to the π electrons of the aromatic ring and suggested that the hydroxyl group might be located at C_6.[9] Comparable stretching frequencies (3593 and 3597 cm^{-1}) have been reported for the benzylic hydroxyl groups in 6-hydroxycrinamine (**5**) and haemanthidine (**6**), respectively, the only two other alkaloids known to contain a C_6 hydroxyl.[10,11] The appearance of a singlet in the nmr spectrum at 5.31 ppm was found by integration to be a single proton. It was considered to be a single benzylic proton by the absence of the characteristic AB methylene pattern near 4.0 ppm found in alkaloids of this ring system which are unsubstituted at this position (*e.g.*, **2**).

Attempted oxidation of 6-hydroxybuphanidrine with dimethyl sulfoxide–acetic anhydride formed only the *O*-acetyl derivative (**7**). Prolonged manganese dioxide oxidation of **1** permitted isolation of the lactam (**3**) in low yield. 6-Oxobuphanidrine has carbonyl absorption in the infrared at 1690 cm^{-1} and ultraviolet absorption at 231, 287, and 320 nm. The nmr spectrum confirms

(1) Support of this research by Public Health Service Research Grant HE 7503 is gratefully acknowledged.

(2) Part of this work is included in the Ph.D. thesis of M. R. Slabaugh, Iowa State University, 1970.

(3) H. G. Boit and H. Ehmke, *Chem. Ber.*, **89**, 2093 (1956).

(4) R. E. Lyle, E. A. Kielar, J. R. Crowder, H. M. Fales, and W. C. Wildman, *J. Amer. Chem. Soc.*, **82**, 2620 (1960).

(5) W. C. Wildman and C. J. Kaufman, *ibid.*, **77**, 4807 (1955).

(6) W. C. Wildman, *Alkaloids*, **11**, 352 (1968).

(7) A. L. Burlingame, H. M. Fales, and R. J. Highet, *J. Amer. Chem. Soc.*, **86**, 4976 (1964).

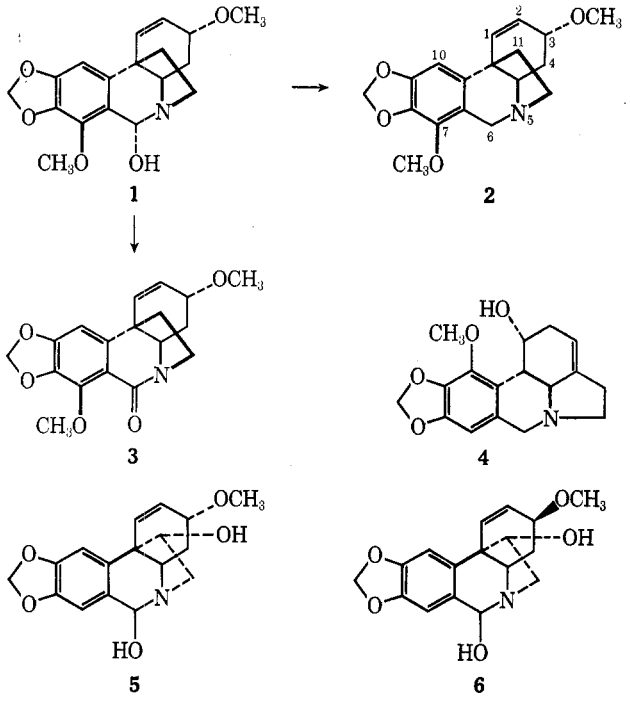
(8) P. Naegeli, E. W. Warnhoff, H. M. Fales, R. E. Lyle, and W. C. Wildman, *J. Org. Chem.*, **28**, 206 (1963).

(9) H. M. Fales and W. C. Wildman, *J. Amer. Chem. Soc.*, **85**, 784 (1963).

(10) R. W. King, C. F. Murphy, and W. C. Wildman, *ibid.*, **87**, 4912 (1965).

(11) H. A. Lloyd, E. A. Kielar, R. J. Highet, S. Uyeo, H. M. Fales, and W. C. Wildman, *J. Org. Chem.*, **27**, 373 (1963).

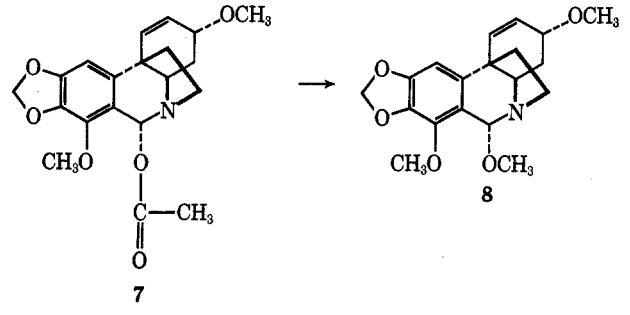
the absence of benzylic protons. This spectral evidence supports the proposed structure. The inertness to oxidation of 6-hydroxybuphanidrine contrasts with 6-hydroxycrinamine (5) and haemanthidine (6), which are readily oxidized by manganese dioxide. This difference in reactivity is attributed to the steric effect of the *peri* aromatic methoxyl group. An analogous case of steric hindrance to oxidation was observed with the base falcatine (4) which proved inert to Oppenauer conditions while caranine (4, no aromatic methoxyl) was readily oxidized.[12]



In contrast to haemanthidine (6) and 6-hydroxycrinamine (5), which exist in solution as a mixture of C_6 epimers, the nmr spectrum of 6-hydroxybuphanidrine gives no indication of an epimeric mixture. The benzylic proton appears as a singlet at 5.31 ppm which is shifted downfield (6.08 ppm) in 6-hydroxybuphanidrine hydrochloride. This observation of a singlet suggests that either one epimer is present or that two epimers exist which possess the same chemical shift. The latter possibility seems remote in view of the highly different environments present in the rigid ring system and the large difference in chemical shifts reported for the two C_6 epimers of 5. Additional evidence for a single epimer was provided by the *O*-acetyl and hydrochloride derivatives of 1, which also exhibited singlets for the benzylic proton.

Hydrolysis of 6-*O*-acetylbuphanidrine in 50% dioxane–water to 6-hydroxybuphanidrine was complete after 10 hr at room temperature. Such hydrolytic reactions ordinarily involve cleavage of the acyl–oxygen rather than the alkyl–oxygen bond. However, the reaction might also proceed by a carbonium ion intermediate which would be characterized by the occurrence of alkyl–oxygen fission. A methanolysis of 6-O-acetylbuphanidrine (7) was carried out to determine the mode of bond cleavage. The methanolysis product proved to be the methyl ether of 1. The nmr spectrum of 6-methoxybuphanidrine (8) displayed an upfield shift of the benzylic proton (4.60 ppm). Thus, alkyl–oxygen fission has occurred, indicating that the reaction proceeded by a carbonium ion mechanism.

The major factor promoting heterolysis of the alkyl–oxygen bond is conjugative electron release by the benzene ring containing both ortho and para electron-releasing substituents. Assistance by the nitrogen's lone pair of electrons would violate Bredt's rule[13] and, in view of the rigidity of the 1-azabicyclo[3,2,1]octane ring system, is highly unlikely. As a carbonium ion



develops at the benzylic position in the hydrolysis reactions, one would predict from stereoelectronic factors the formation of pseudo-axial products as depicted. Steric control would also predict the same result with attack of the nucleophile from the side opposite the ethano bridge. Thus, a consideration of both electronic and steric factors would predict the α configuration for 6-methoxybuphanidrine (8).

To demonstrate that 6-hydroxybuphanidrine also possesses the α configuration at C_6, it seemed desirable to prepare the methyl ether by a method in which the C_6–O bond was not broken and compare the derivative to the methyl ether obtained from hydrolysis of 7. When methyl *p*-toluenesulfonate was added to the potassium salt of 6-hydroxybuphanidrine, a 28% yield of the methyl ether (8) was obtained. This product proved identical with 6-methoxybuphanidrine by ir, nmr, and chromatographic data. There was no evidence for the epimeric methyl ether in the methylation reaction products. In order to rule out the possibility of epimerization of the secondary hydroxyl group under the strongly basic conditions encountered, a duplicate experiment was performed with omission of the methyl *p*-toluenesulfonate. The reaction afforded only starting material with no evidence of the epimeric alcohol. Thus, 6-hydroxybuphanidrine also contains the α-hydroxyl configuration at C_6. This fact has been verified by an X-ray crystallographic study of the methiodide salt.[14]

Separation of the alkaloid fractions from an unidentified *Crinum* species revealed the presence of buphanidrine, 6-hydroxybuphanidrine, ambelline, crinamidine, and a new alkaloid of unknown structure. Comparison by tlc of the unknown compound with the fractions from *Nerine bowdenii* also permitted its isolation from this species. The mass spectrum indicated that the alkaloid has a molecular weight of 317. The infrared spectrum revealed the presence of an aromatic ring substituted with both methylenedioxy (945 and 1050 cm^{-1}) and methoxyl (1618 cm^{-1}) groups. Of special interest in establishing the structure was the similarity

(12) H. M. Fales and W. C. Wildman, *J. Amer. Chem. Soc.*, **80**, 4395 (1958).

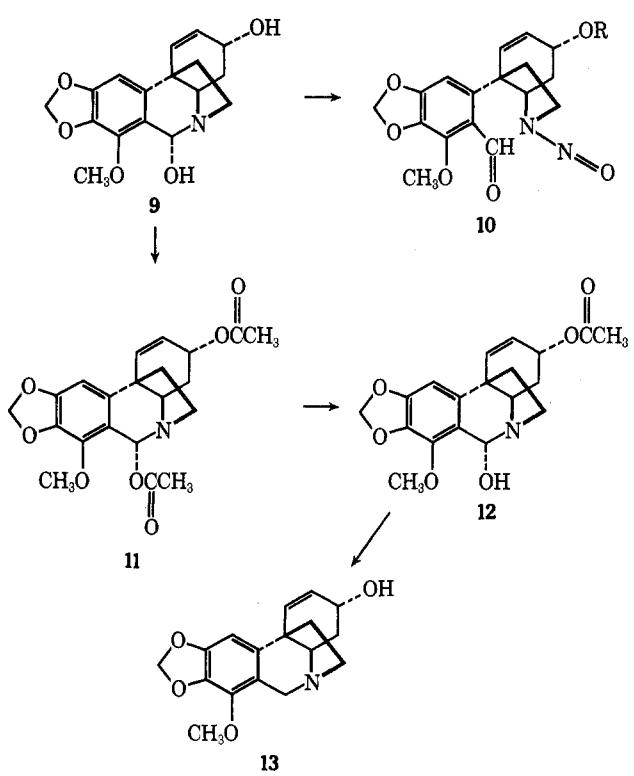
(13) F. S. Fawcett, *Chem. Rev.*, **47**, 219 (1950).

(14) J. C. Clardy, F. M. Hauser, D. Dahm, R. A. Jacobson, and W. C. Wildman, *J. Amer. Chem. Soc.*, **92**, 6337 (1970).

of the nmr spectrum to that of 6-hydroxybuphanidrine. From this comparison, the alkaloid was tentatively assigned the 5,10b-ethanophenanthridine nucleus. Particularly important in the nmr spectrum was the appearance of the singlet at 5.38 ppm which indicates the existence of a hydroxyl group at C_6. As in the spectrum of 6-hydroxybuphanidrine, there are two olefinic protons which appear as an AB pattern (6.38 and 5.85 ppm). The proton at 5.85 ppm is further split by coupling to a single proton (J = 5 cps) which must be the proton at C_3. Since the alkaloid formed an *O,O*-diacetyl derivative, the compound was tentatively assigned structure **9**.

The hydroxyl group at C_6 was assigned the α configuration (as in **9**) because the position of the benzylic proton (5.38 ppm) was comparable to the chemical shift of the corresponding proton in 6-hydroxybuphanidrine (5.31 ppm). By analogy with the spectrum of 6-hydroxycrinamine, if the β epimer were present, the benzylic proton should be approximately 0.6 ppm downfield.

To establish the configuration of the C_3 hydroxyl group, it seemed desirable to convert the alkaloid to a known 6-deoxy compound. Acetylation of **9** afforded the corresponding *O,O*-diacetyl derivative (**11**). Hydrolysis of **11** with dioxane–water (50:50) produced **12**. The position of the hydroxyl group was assigned from the nmr spectrum which showed that the benzylic proton had returned to its upfield position (5.37 ppm). Treatment of **12** with thionyl chloride followed by lithium aluminum hydride produced **13**, which was identical in all respects with the known alkaloid, powelline. This interconversion established the absolute configuration of the alkaloid and the relative configuration of the C_3 hydroxyl group.

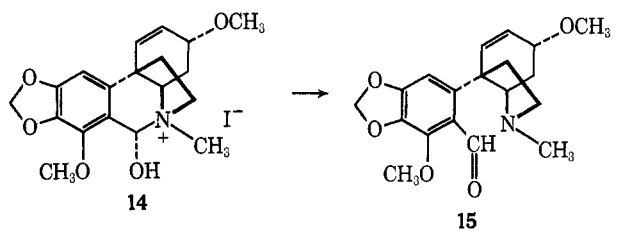


As in the case of 6-hydroxybuphanidrine, 6-hydroxypowelline showed no evidence for the presence of equilibrating epimers at C_6. Both the nmr spectra of **9** and the diacetyl derivative displayed a single peak for the benzylic proton.

6-Hydroxybuphanidrine (**1**) and 6-hydroxypowelline (**9**) are unique among the 5,10b-ethanophenanthridine alkaloids because they represent the only alkaloids containing a hydroxyl group at C_6 without a hydroxyl substituent at C_{11}. The most striking feature of the 6-hydroxy compounds is their potential to exist as the tautomeric open-chain aminoaldehyde. Infrared spectra of 6-hydroxybuphanidrine, both crystalline and in chloroform solution, indicate that the alkaloid is in the carbinolamine form. An aldehyde group is not detected by nmr and ultraviolet spectroscopy. In contrast to **5** and **6**, 6-hydroxybuphanidrine displays no tendency to epimerize at the C_6 position. Refluxing the alkaloid in either acidic or basic media fails to give epimerization.

6-Hydroxybuphanidrine and 6-hydroxypowelline, when treated with sodium nitrite in dilute acetic acid, formed *N*-nitroso aldehyde derivatives (**10**). This is a reaction characteristic of secondary amines and may indicate that the aminoaldehyde is present in the solution.

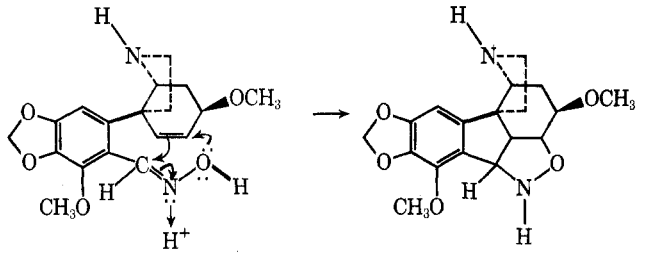
Ring opening was also observed when 6-hydroxybuphanidrine methiodide (**14**) was converted to a chloroform-soluble base ($C_{19}H_{23}NO_5$) by treatment with dilute alkali. The infrared and ultraviolet spectrum of this product [λ_{max} ($CHCl_3$) 1690 cm^{-1}, λ_{max} (95% EtOH) 320 nm (ϵ 1990)] indicated that C_6–N bond cleavage had occurred with the formation of the aminoaldehyde (**15**). The nmr spectrum showed the *N*-methyl (2.47 ppm) and the aldehydic proton (10.5 ppm) in accord with this structure.



It has been reported that haemanthidine and 6-hydroxycrinamine have proven stable to Schiff and Tollens reagents, lithium aluminum hydride, sodium borohydride, and hydroxylamine.[10] Quite surprisingly, the reaction of 6-hydroxybuphanidrine with hydroxylamine hydrochloride in refluxing 95% ethanol afforded the isoxazolidine derivative, **17**. The product lacked a C=N stretching band in the infrared and showed no absorption characteristic of benzylic conjugation. A doublet was located in the nmr spectrum at 5.15 ppm instead of much farther downfield where the benzylic proton was expected for an oxime structure. No olefinic protons were observed. The mass spectrum indicated that the compound had a molecular weight of 346, the same as that of the expected oxime.

In keeping with structure **17**, the compound formed an *N,N*-diacetyl derivative. It would appear that an oxime (**16**) was initially formed and was readily converted to **17**.[15] To confirm the intermediacy of the oxime, the reaction was carried out using 6-hydroxyundulatine, which possesses no olefinic protons. The ultraviolet and infrared spectra of the product indicated

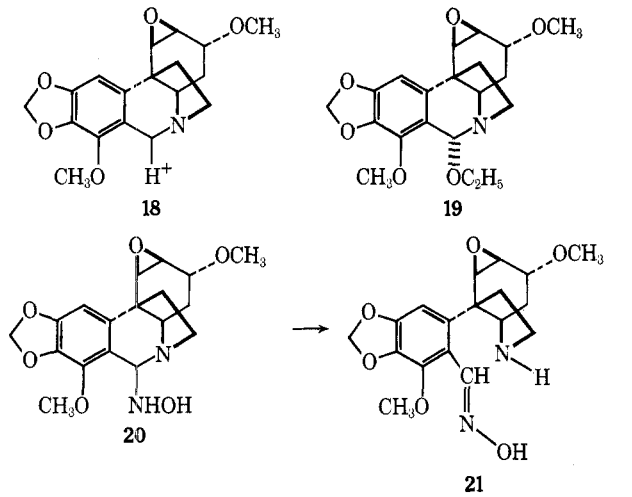
(15) The precedent for addition of oximes to double bonds is well documented; L. A. Paquette, "Principles of Modern Heterocyclic Chemistry," W. A. Benjamin, New York, N. Y., 1968, pp 183–190.



16 17

that C_6–N bond cleavage had occurred with formation of the oxime (**21**). The nmr spectrum confirmed the benzylic proton (8.1 ppm) in accord with this structure.

Although oxime formation may occur by a mechanism similar to the base-catalyzed ring opening of **14**, such a mechanism does not account for the fact that 6-hydroxybuphanidrine reacts with hydroxylamine while 6-hydroxycrinamine and haemanthidine do not. It is proposed that a small amount of the carbonium ion **18** is formed. The isolation of 6-ethoxyundulatine (**19**) from the reaction supports this possibility. The carbonium ion **18** could react with hydroxylamine to produce **20**. In the presence of acid, this hydroxylamine derivative could isomerize to the oxime **21**.



18 19

20 21

When the condensation of 6-hydroxybuphanidrine (**1**) and hydroxylamine was performed in a solution buffered with sodium acetate, no isoxazolidine was isolated. However, 6-hydroxyundulatine afforded an almost quantitative yield of the oxime when dimethyl sulfoxide was used as the solvent.

The inability of 6-hydroxycrinamine and haemanthidine to react may indicate that formation of the benzylic carbonium ion is not as favorable without the ortho electron-releasing substituent (–OCH_3). Thus, both **5** and **6** fail to form the C_6 ethers in acidic ethanol while 6-hydroxybuphanidrine and 6-hydroxypowelline react with facility. In a similar way, the 6,11-*O*,*O*-diacetates of **5** and **6** do not undergo methanolysis with cleavage of the alkyl–oxygen bond.

Experimental Section[16]

Isolation of Alkaloids.—The preliminary isolations from the bulbs of *Nerine bowdenii* W. Wats. have been described in detail previously.[4] The method of isolation involved a fractionation of the alkaloids into bases forming chloroform-soluble and chloroform-insoluble hydrochlorides. Further purification of the alkaloids was achieved by a combination of crystallization and chromatography on Florisil or alumina. Of primary concern were the chloroform-insoluble hydrochlorides which contain the more polar alkaloids. 6-Hydroxybuphanidrine was eluted from a Florisil column with 2% methanolic chloroform to give several viscous fractions which contained as much as 50% crinamine. Thin layer chromatography of these mixtures on silica gel plates (0.5 mm in thickness) developed in chloroform–methanol–diethylamine (90:5:5) separated the components into two bands: 6-hydroxybuphanidrine (R_f 0.5) and crinamine (R_f 0.4). The material was recovered by removal of the bands from the plate and elution of the alkaloids with chloroform. Most polar alkaloids were eluted with 50% methanolic chloroform to give, in addition to a large amount of amorphous material,[4] 6-hydroxypowelline, nerbowdine, *O*,*O*-deacetylbowdensine, coranicine, and powelline.

6-Hydroxybuphanidrine (**1**).—The alkaloid afforded colorless prisms from chloroform: mp 95–96°; $[\alpha]^{24}D$ –64° (*c* 0.19, MeOH); λ_{max} (95% EtOH) 215 nm (ϵ 18,250), 238 (7750), and 285 (1880); ir ($CHCl_3$) 1505, 1481, 1050, 945 (aromatic methylenedioxy), 3595 (hydroxyl), 1618 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) δ 3.33 (s, 3, aliphatic methoxyl), 4.05 (s, 3, aromatic methoxyl), 5.31 (s, 1, benzylic proton), 5.85 (s, 2, methylenedioxy), 5.10 (s, 1, hydroxyl group), 6.00 and 6.58 (2, olefinic protons), 6.60 (s, 1, aromatic proton).

Anal. Calcd for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23. Found: C, 65.08; H, 6.57; N, 4.35.

Dihydro-6-hydroxybuphanidrine.—A solution of 65 mg of **1** in 10 ml of 95% ethanol was hydrogenated at room temperature and atmospheric pressure with 30 mg of palladium on charcoal which had been equilibrated with hydrogen. The reduction stopped after the uptake of 92% of the theoretical amount of hydrogen. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure. Thin layer chromatography on a silica gel plate (0.5 mm) developed in chloroform–methanol–diethylamine (90:5:5) showed that the residue was a single component (R_f 0.7). The product (48 mg) was recovered by extraction with chloroform from a basic suspension of the silica gel. A pure sample was obtained by sublimation: mp 118–119°; $[\alpha]^{24}D$ –74° (*c* 0.21, MeOH); λ_{max} (95% EtOH) 220 nm (ϵ 19,400), 237 (8250), and 284 (2560); nmr ($CDCl_3$) δ 3.29 and 4.02 (2 s, 3, –OCH_3), 5.30 (s, 1, benzylic proton), 5.87 (s, 2, methylenedioxy), 6.38 (s, 1, –OH), 6.41 (s, 1, aromatic proton).

Anal. Calcd for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95; N, 4.20. Found: C, 65.01; H, 6.93; N, 4.20.

6-*O*-Acetylbuphanidrine (**7**).—Acetylation of a pyridine solution of 58 mg of **1** under standard reaction conditions gave 55 mg of **7**, which was purified by recrystallization from benzene: mp 165–168°; $[\alpha]^{24}D$ –42° (*c* 0.23, MeOH); λ_{max} (95% EtOH) 214 nm (ϵ 17,500), 239 (8050), and 286 (2180); ir ($CHCl_3$) 1733 (C=O), 1624 (aromatic methoxyl), 950 and 1050 cm^{-1} (aromatic methylenedioxy); nmr ($CDCl_3$) δ 2.10 (s, 3, CH_3C=O), 3.33 and 3.90 (2 s, 3, –OCH_3), 5.85 (s, 2, methylenedioxy), 6.25 (s, 1, benzylic proton), 6.58 (s, 1, aromatic proton).

Anal. Calcd for $C_{20}H_{23}NO_6$: C, 64.33; H 6.21; N, 3.75. Found: C, 64.03; H, 6.12; N, 3.83.

6-Hydroxybuphanidrine Hydrochloride.—**1** afforded an amorphous hydrochloride salt: $[\alpha]^{24}D$ –22° (*c* 0.16, MeOH); nmr ($CDCl_3$) δ 3.34 and 4.05 (2 s, 3, –OCH_3), 5.96 (s, 2, methylenedioxy), 6.06 (s, 1, benzylic proton), 6.62 (s, 1, aromatic proton), 6.88 (1, hydroxyl), 11.50 (NH).

Anal. Calcd for $C_{18}H_{21}NO_5 \cdot HCl$: C, 58.82; H, 5.99; N, 3.81. Found: C, 58.55; H, 6.05; N, 3.96.

Conversion of 6-Hydroxybuphanidrine (**1**) **to Buphanidrine** (**2**).—A solution of 75 mg of **1** in 10 ml of thionyl chloride was refluxed for 2 hr and then evaporated to dryness. The residue

(16) Melting points were taken on a Köfler microscope hot stage and are corrected. Ultraviolet spectra were determined in 95% ethanol on a Cary Model 14 spectrophotometer. Infrared spectra were obtained with a Beckman Model IR-12 spectrophotometer. Hydrogen-bonding studies were carried out in high dilution in carbon tetrachloride unless otherwise noted. Proton nuclear magnetic resonance spectra were recorded with a Varian A-60 spectrometer. Mass spectra were obtained with an Atlas CH-4 mass spectrometer operating at 70 eV. Optical rotations were determined in methanol solution with a Jasco Model 5 optical rotatory dispersion spectrometer. Thin layer chromatography was performed using silica gel PF 254 + 366 (E. Merck). Compounds were identified by exposure to ultraviolet light of the appropriate wavelength. All comparisons of alkaloids were verified by the identity of the ir spectra, melting points, mixture melting points, and chromatographic data.

was combined with 15 ml of dry tetrahydrofuran and 300 mg of lithium aluminum hydride. The solution was refluxed for 16 hr. The cooled mixture was hydrolyzed with ethyl acetate followed by water and 25% sodium hydroxide. The tetrahydrofuran solution was filtered and the filtrate was washed thoroughly with chloroform. The combined chloroform and tetrahydrofuran extracts were evaporated to dryness. Chromatography on silica gel gave 42 mg of product which was identical with buphanidrine on comparison of ir, nmr, and tlc data.

6-Oxobuphanidrine (3).—A solution of 150 mg of **1** in 15 ml of anhydrous chloroform was combined with 700 mg of activated manganese dioxide and stirred for 4 days. The manganese dioxide was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was separated by chromatography on silica gel plates developed in chloroform–methanol–diethylamine (90:5:5) to give 14 mg of 6-oxobuphanidrine (R_f 0.7) and 119 mg of 6-hydroxybuphanidrine (R_f 0.5). The product was crystallized from acetone: mp 161–163°; $[\alpha]^{24}D$ −6° (*c* 0.25, MeOH); λ_{max} (95% EtOH) 216 nm (ϵ 20,400), 238 (7200), 287 (1500), and 321 (1350); ir ($CHCl_3$) 1690 (C=O), 1616 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) δ 3.30 and 4.05 (2 s, 3, $-OCH_3$), 5.95 (s, 2, methylenedioxy), 6.65 (s, 1, aromatic proton).

Anal. Calcd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.82; N, 4.25. Found: C, 65.36; H, 5.77; N, 4.30.

6-Ethoxybuphanidrine.—A solution of 100 mg of **1** in 5 ml of 95% ethanol was made acidic (pH 2) with 0.2 ml of 20% hydrochloric acid and refluxed for 4 hr. The solution was evaporated to dryness under reduced pressure, and the residue was dissolved in 5 ml of water. The solution was made basic (pH 10) with concentrated ammonium hydroxide and extracted three times with chloroform. The chloroform extracts were evaporated under reduced pressure to give 96 mg of an oil. Separation by thin layer chromatography provided 22 mg of 6-ethoxybuphanidrine (R_f 0.8) and 68 mg of **1** (R_f 0.6). Although chromatographic data indicated that the 6-ethoxybuphanidrine was pure, it was amorphous: $[\alpha]^{24}D$ −38° (*c* 0.17, MeOH); λ_{max} (95% EtOH) 240 nm (ϵ 7940) and 287 (2130); nmr ($CDCl_3$) δ 1.20 (t, 3, CH_3CH_2O-), 3.31 and 3.95 (2 s, 3, $-OCH_3$), 4.72 (benzylic proton), 5.87 (q, 2, methylenedioxy), 6.56 (s, 1, aromatic proton).

Anal. Calcd for $C_{20}H_{25}NO_5$: C, 66.83; H, 7.01; N, 3.90. Found: C, 66.55; H, 7.07; N, 3.85.

Hydrolysis of 6-*O*-Acetylbuphanidrine.—A solution of 40 mg of 6-*O*-acetylbuphanidrine in 4 ml of water–dioxane (50:50) was allowed to stand at room temperature for 2 days. The solution was extracted three times with chloroform, and the chloroform extracts were evaporated under reduced pressure. Thin layer chromatography of the residue on a silica gel plate developed in chloroform–methanol–diethylamine (90:5:5) gave 32 mg of product (R_f 0.6), identical in all respects with **1**.

6-Methoxybuphanidrine (8). A. From 6-*O*-Acetylbuphanidrine (7).—A solution of 45 mg of 6-*O*-acetylbuphanidrine in 3 ml of absolute methanol was allowed to stand for 4 days at room temperature. Evaporation under reduced pressure and thin layer chromatography on silica gel plates revealed the presence of **7** (R_f 0.6) and a second, less polar band (R_f 0.8). Elution of this band with chloroform permitted recovery of 17 mg of 6-methoxybuphanidrine: amorphous; $[\alpha]^{24}D$ −14° (*c* 0.18, MeOH); λ_{max} (95% EtOH) 238 nm (ϵ 7300) and 285 (2200); nmr ($CDCl_3$) δ 3.33, 3.56, and 3.97 (3 s, 3, $-OCH_3$), 4.61 (s, 1, benzylic proton), 5.86 (q, 2, methylenedioxy), 6.55 (s, 1, aromatic proton).

Anal. Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 65.86; H, 6.74; N, 4.19.

B. From 6-Hydroxybuphanidrine (1).—O-Methylation of 100 mg of **1** gave 63 mg of reaction product which was purified by preparative thin layer chromatography (chloroform–methanol–diethylamine, 90:5:5). The major band (R_f 0.8, 28 mg) was recovered as an amorphous material identical in all respects with 6-methoxybuphanidrine.

Conversion to the *N*-Nitroso Derivative (10, R = CH_3).—To a solution of 65 mg of **1** in 10 ml of 1.5% aqueous acetic acid was added 65 mg of sodium nitrite. The reaction mixture was allowed to stand for 8 hr at room temperature. The aqueous solution was extracted four times with chloroform. The chloroform solution was washed with 5% sodium bicarbonate and evaporated under reduced pressure. The residue was chromatographed on silica gel to give 17 mg of the starting material and 34 mg of the amorphous product (**10**, R = CH_3): $[\alpha]^{24}D$ −7° (*c* 0.26, MeOH); λ_{max} (95% EtOH) 240 nm (ϵ 8020), 288 (2070), and 315 (1670); ir ($CHCl_3$) 1685 cm^{-1} (C=O); nmr ($CDCl_3$) δ 3.37 and 4.12 (2 s, 3, $-OCH_3$), 6.05 (s, 2, methylenedioxy), 6.90 (s, 1, aromatic proton), 10.40 (s, 1, CHO).

Anal. Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 60.00; H, 5.64; N, 7.73.

6-Hydroxybuphanidrine Methiodide (14).—Methylation of 55 mg of **1** in methanol–acetone (1:4) by methyl iodide gave 56 mg of fine needles: mp 240–242°; λ_{max} 281 nm (ϵ 1402); nmr (DMSO-d_6) δ 3.12 (s, 3, NCH_3), 3.29 and 3.97 (2 s, 3, $-OCH_3$), 6.09 (s, 2, methylenedioxy), 6.98 (s, 1, aromatic proton).

Anal. Calcd for $C_{19}H_{24}NO_5I$: C, 48.41; H, 5.10; N, 2.97. Found: C, 48.54; H, 5.22; N, 3.02.

Conversion of 14 to 15.—A solution of 48 mg of 6-hydroxybuphanidrine methiodide (**14**) in 4 ml of water was made basic (pH 10) with ammonium hydroxide, allowed to stand at room temperature for 3 hr, and then extracted four times with chloroform. The chloroform extracts were evaporated under reduced pressure to give 39 mg of an oil. Thin layer chromatography gave 28 mg of **15** (R_f 0.7): amorphous; $[\alpha]^{24}D$ −14° (*c* 0.21, MeOH); λ_{max} (95% EtOH) 240 nm (ϵ 8040), 285 (2010), and 320 (1990); ir ($CHCl_3$) 1696 cm^{-1} (C=O); nmr ($CDCl_3$) δ 2.42 (s, 3, NCH_3), 3.38 and 4.00 (2 s, 3, $-OCH_3$), 5.83 (s, 2, methylenedioxy), 5.97 (s, 2, olefinic protons), 6.76 (s, 1, aromatic proton), 10.3 (s, 1, -CHO).

Anal. Calcd for $C_{19}H_{23}NO_6$: C, 66.07; H, 6.71; N, 4.06. Found: C, 65.86; H, 6.74; N, 4.19.

Hydroxylamine Adduct (17).—A solution of 100 mg of **1** in 8 ml of 95% ethanol was combined with 40 mg of hydroxylamine hydrochloride. The solution was refluxed for 4 hr, evaporated under reduced pressure, diluted with 15 ml of water, made basic with ammonium hydroxide, and extracted three times with chloroform. Evaporation of the chloroform extracts gave 93 mg of residue which was chromatographed on silica gel plates (chloroform–methanol–diethylamine, 90:5:5). Removal of the three bands present provided 26 mg of 6-ethoxybuphanidrine (R_f 0.8), 38 mg of starting material (R_f 0.6), and 22 mg of the hydroxylamine adduct (R_f 0.5). All attempts to crystallize the adduct were unsuccessful: amorphous; $[\alpha]^{24}D$ +11° (*c* 0.18, MeOH); λ_{max} (95% EtOH) 238 nm (ϵ 8400) and 285 (2300); nmr ($CDCl_3$) δ 3.41 and 4.05 (2 s, 3, $-OCH_3$), 5.15 (d, 1, benzylic proton), 5.90 (s, 2, methylenedioxy), 6.30 (s, 1, aromatic proton).

Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.41; H, 6.40; N, 8.09. Found: C, 62.16; H, 6.13; H, 8.00.

The hydroxylamine adduct (84 mg) was converted to 74 mg of the *N*,*N*-diacetyl derivative, which was crystallized from ether–acetone: mp 133–135°; $[\alpha]^{24}D$ +18° (*c* 0.23, MeOH); λ_{max} (95% EtOH) 238 nm (ϵ 7600) and 286 (2400); nmr ($CDCl_3$) δ 2.10 and 2.19 (2 s, 3, $CH_3C=O$), 3.40 and 3.98 (2 s, 3, $-OCH_3$), 5.92 (s, 2, methylenedioxy), 6.20 (d, 1, benzylic proton), 6.30 (s, 1, aromatic proton).

6-Hydroxyundulatine.—To a solution of 259 mg of undulatine in 15 ml of benzene was added 134 mg of *N*-bromosuccinimide and 30 mg of benzoyl peroxide. The solution was maintained under a nitrogen atmosphere for 2 hr and then refluxed on a steam bath for 4 hr. The solution was evaporated to dryness under reduced pressure. The residue was dissolved in methanol and treated with 10% sodium hydroxide. The basic solution was allowed to stand at room temperature for 2 hr, diluted with water, and extracted three times with chloroform. Evaporation of the chloroform extracts under reduced pressure gave 214 mg of residue. Thin layer chromatography of the mixture gave 93 mg of undulatine (R_f 0.7) and 84 mg of 6-hydroxyundulatine (R_f 0.6). The product crystallized from acetone–ether: mp 211–213°; $[\alpha]^{24}D$ −43° (*c* 0.25, MeOH); λ_{max} (95% EtOH) 237 nm (ϵ 13,900) and 292 (2410); nmr ($CDCl_3$) δ 3.40 and 4.00 (2 s, 3, $-OCH_3$), 5.20 (s, 1, benzylic proton), 5.90 (s, 2, methylenedioxy), 6.63 (s, 1, aromatic proton).

Anal. Calcd for $C_{18}H_{21}NO_6$: C, 62.24; H, 6.10; N, 4.03. Found: C, 61.96; H, 6.05; N, 4.12.

6-Hydroxyundulatine Oxime (21). A. With 95% Ethanol as the Solvent.—By the same procedure cited for the preparation of **17**, 100 mg of 6-hydroxyundulatine was converted to 24 mg of 6-hydroxyundulatine oxime: amorphous; $[\alpha]^{24}D$ −18° (*c* 0.18, MeOH); λ_{max} (95% EtOH) 239 nm (ϵ 5490), 288 (ϵ 1380), and 335 nm (ϵ 1050); nmr ($CDCl_3$) δ 3.48 and 4.06 ppm (2 s, 3, $-OCH_3$), δ 5.97 ppm (s, 2, methylenedioxy), δ 6.48 ppm (s, 1, aromatic proton), δ 8.07 ppm (s, 1, benzylic proton).

Anal. Calcd for $C_{18}H_{22}N_2O_6$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.75; H, 5.98; N, 7.77.

B. With Dimethyl Sulfoxide as the Solvent.—To a solution of 100 mg of 6-hydroxyundulatine in 6 ml of dimethyl sulfoxide was added 50 mg of hydroxylamine hydrochloride. The solution was heated on a steam bath for 6 hr. The cooled solution was diluted with water, made basic with ammonium hydroxide, and extracted with chloroform. The chloroform extracts were evaporated under reduced pressure. The residual dimethyl sulfoxide was removed by chromatography on silica gel plates. Removal of the major band (R_f 0.5) allowed the recovery of 78 mg of 6-hydroxyundulatine oxime. Although the material remained amorphous, it was pure by tlc criteria and proved identical with **21** prepared above.

6-Hydroxypowelline (9).—The pure alkaloid afforded colorless prisms from acetone: mp 233–235°; $[\alpha]^{24}D$ −36° (*c* 0.19, MeOH); λ_{max} (95% EtOH) 218 nm (ϵ 17,500), 235 (6800), and 286 (2150); ir ($CHCl_3$) 1510, 1490, 1055, 950 (aromatic methylenedioxy), 3609 (hydroxyl group), 1623 (aromatic methoxyl); nmr ($CDCl_3$) δ 4.02 (s, 3, aromatic methoxyl), 5.38 (s, 1, benzylic proton), 5.88 (2, methylenedioxy), 5.85 and 6.38 (2, olefinic protons), 6.55 (s, 1, aromatic proton).

Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41. Found: C, 64.17; H, 5.85; N, 4.31.

3,6-*O*,*O*-Diacetylpowelline (11).—By the procedure cited for the preparation of **7**, 100 mg of **9** was converted to 88 mg of crude diacetate. The product was purified by thin layer chromatography and gave 72 mg of 3,6-*O*,*O*-diacetylpowelline after crystallization from acetone: mp 114–117°; $[\alpha]^{24}D$ −26° (*c* 0.20, MeOH); λ_{max} (95% EtOH) 241 nm (ϵ 9020) and 285 (1900); ir ($CHCl_3$) 1738 (C=O), 1625 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) δ 2.00 and 2.10 (2 s, 3, CH_3C=O), 3.97 (s, 1, aromatic methoxyl), 5.35 (C_3 methine), 5.90 (q, 2, methylenedioxy), 6.27 (s, 1, benzylic proton), 6.60 (s, 1, aromatic proton).

Anal. Calcd for $C_{21}H_{23}NO_7$: C, 62.83; H, 5.78; N, 3.49. Found: C, 63.02; H, 5.75; N, 3.36.

3-*O*-Acetyl-6-hydroxypowelline (12).—A solution of 50 mg of **11** in 5 ml of dioxane–water (50:50) was allowed to stand at room temperature for 24 hr. The solution was evaporated under reduced pressure, and the crude product was chromatographed on a silica gel plate developed in chloroform–methanol–diethylamine (90:5:5). The major band (R_f 0.6) was recovered as an amorphous material (38 mg): $[\alpha]^{24}D$ −18° (*c* 0.25, MeOH); λ_{max} (95% EtOH) 237 nm (7050) and 287 (1400); ir ($CHCl_3$) 1730 cm^{-1} (C=O); nmr ($CDCl_3$) δ 2.03 (s, 3, CH_3C=O), 4.04 (s, 3, $-OCH_3$), 5.28 (s, 1, benzylic proton), 5.30 (C_3 methine), 5.90 (2, methylenedioxy), 6.59 (s, 1, aromatic proton).

Anal. Calcd for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.66; H, 6.01; N, 4.02.

Powelline (13).—A solution of 35 mg of 3-*O*-acetyl-6-hydroxypowelline in 5 ml of thionyl chloride was refluxed for 2 hr on a steam bath and then evaporated to dryness. The residue was combined with 8 ml of dry tetrahydrofuran and 85 mg of lithium aluminum hydride. The solution was refluxed for 12 hr. The cooled solution was hydrolyzed with ethyl acetate followed by water and 25% sodium hydroxide. The tetrahydrofuran solution was filtered, and the filter cake was washed repeatedly with chloroform. The combined chloroform and tetrahydrofuran extracts were evaporated to dryness. The residue was chromatographed on silica gel plates. Removal of the major band (R_f 0.5) gave 22 mg of material which crystallized from acetone, mp 199–201° (lit.[11] mp 200–201°). The compound showed ir and nmr spectra as well as chromatographic characteristics identical with those of powelline.

***N*-Nitroso-6-hydroxypowelline (10, R = H).**—To a solution of 47 mg of **9** in 6 ml of 1.5% aqueous acetic acid was added 50 mg of sodium nitrite. The reaction mixture was allowed to stand at room temperature for 10 hr. *N*-Nitroso-6-hydroxypowelline crystallized from the solution as fine needles. The product (28 mg) was filtered from the solution and recrystallized from acetone–ether: mp 156–159°; $[\alpha]^{24}D$ −14° (*c* 0.17, MeOH); λ_{max} (95% EtOH) 238 nm (ϵ 6040), 289 (1680), and 321 (1720); ir ($CHCl_3$) 1690 (C=O), 1615 cm^{-1} (aromatic methoxyl); nmr ($CDCl_3$) δ 4.12 (s, 3, $-OCH_3$), 6.08 (s, 2, methylenedioxy), 6.07 (s, 2, olefinic protons), 6.85 (s, 1, aromatic proton), 10.4 (s, 1, CHO).

Anal. Calcd for $C_{17}H_{18}N_2O_6$: C, 58.95; H, 5.24; H, 8.09. Found: C, 59.12; H, 5.08; N, 7.92.

Registry No.—**1**, 31128-91-3; **1** (dihydro derivative), 31128-92-4; **1** HCl, 31128-93-5; **2** (6-ethoxy), 31128-94-6; **3**, 31128-95-7; **7**, 31128-96-8; **8**, 31128-97-9; **9**, 31128-98-0; **10** (R = H), 31128-99-1; **10**, (R = Me), 31129-00-7; **11**, 31129-01-8; **12**, 31129-02-9; **14**, 31129-03-0; **15**, 31129-04-1; **17**, 31081-91-1; **17** (*N*,*N*-diacetyl), 31081-92-2; **19** (6-hydroxy), 31129-05-2; **21**, 31129-06-3.

Bufadienolides. 13. Conversion of 3β-Hydroxy-17-oxoandrost-5-ene to 3β-Acetoxy-5β,14α-bufa-20,22-dienolide[1]

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A synthetic method was developed for converting the readily available dehydroepiandosterone (**1a**) to 3β-acetoxy-5β,14α-bufa-20,22-dienolide (**11**). Important steps in the transformation included condensation of ketone **1b** with diethyl cyanomethylphosphonate to afford olefin **2**. Selective reduction of olefin **2** provided formate **4** which was successively oxidized (**5**) and reduced to ketone **6b**. Application of the Henbest reagent (trimethyl phosphite–chloroiridic acid) to reduction of ketone **6b** provided a convenient pathway to 3β alcohol **7b**. The remaining steps to bufadienolide **11** proceeded *via* intermediates **8**, **9**, and **10**.

For the dual purposes of making 3β-acetoxy-5β,14α-bufa-20,22-dienolide (**11**) readily available for subsequent conversion to naturally occurring bufadienolides[3] and for comparison purposes a practical total synthesis was required. To meet these objectives the readily available dehydroepiandosterone (**1a**) was selected as starting material. Treatment of alcohol **1a** with acetic–formic anhydride provided formate **1b** in essentially quantitative yield. The formate derivative was employed as protecting group in order to eliminate a saponification step prior to an Oppenauer oxidation[4] envisaged for a later stage in the synthesis. Ketone **1b** was condensed with the carbanion derived from diethyl cyanomethylphosphonate to afford (80%) olefin **2**.

(1) For part 12, see G. R. Pettit, P. Brown, F. Bruschweiler, and L. Houghton, *Chem. Commun.*, 1566 (1971). The present study corresponds to Steroids and Related Natural Products 67; for part 66, refer to G. R. Pettit and J. R. Dias, *J. Org. Chem.*, in press. The investigation described herein was supported by Public Health Service Research Grants CA-10115-04 and CA-11451-02 from the National Cancer Institute. The mass spectrometer was obtained using National Science Foundation Grant GB-4939.

(2) Based in part on a dissertation submitted by J. R. Dias to the Graduate School, Arizona State University, Feb 1970. NIH Predoctoral Fellow, 1968–1970.

(3) Appropriate application of microbiological and synthetic manipulations to bufadienolide **11** might be expected to result easily in routes to, for example, bufalin and resibufogenin; *cf.* G. R. Pettit, L. E. Houghton, J. C. Knight, and F. Bruschweiler, *J. Org. Chem.*, **35**, 2895 (1970).

(4) H. Ringold, B. Loken, G. Rosenkranz, and F. Sondheimer, *J. Amer. Chem. Soc.*, **78**, 816 (1956).