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NUPHACRISTINE—AN ALKALOID FROM NUPHAR LUTEUM

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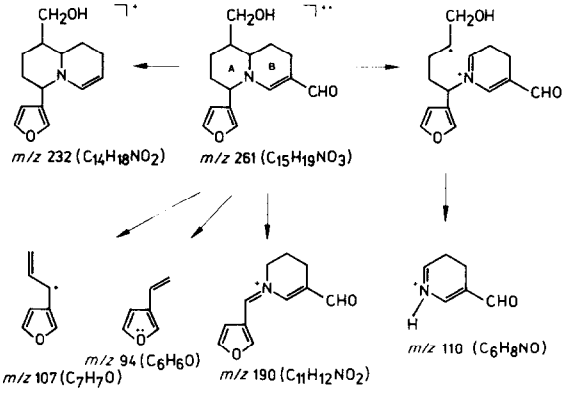
Key Word Index—*Nuphar luteum*; Nymphaeaceae; C_{15} alkaloid; nuphacristine.

Abstract—From the rhizomes of *Nuphar luteum* a new C_{15} alkaloid, nuphacristine, has been isolated. The structure and stereochemistry of nuphacristine have been established on the basis of spectral analysis and chemical transformations.

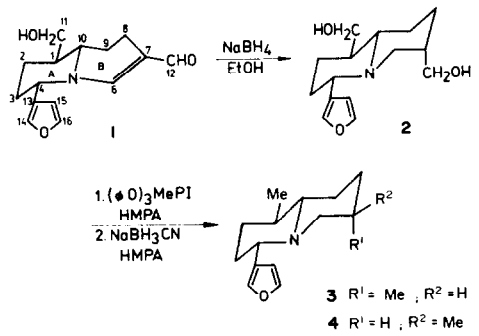
INTRODUCTION

From the rhizomes of *Nuphar luteum* a number of monomeric ($C_{15}N$) and dimeric ($C_{30}N_2S$) alkaloids have been isolated and characterized [1]. The quinolizidine ring system is present in many of the monomeric alkaloids and in all of the dimeric, sulphur-containing alkaloids. Some alkaloids occur as *N*-oxides, and others in the dimeric series, as *S*-oxides; a hemiaminal system is also present in some alkaloids [1]. We present here the structure and stereochemistry of a newly isolated $C_{15}N$ alkaloid, nuphacristine **1**, which has a novel array of functional groups. It became evident from the composition of **1** ($C_{15}H_{19}NO_3$) and from the nature of its functional groups that it belonged to the $C_{15}N$ group of *Nuphar* alkaloids containing a quinolizidine ring system (Fig. 1).



RESULTS AND DISCUSSION

Nuphacristine **1** was isolated in the following manner. The crude alkaloids of *N. luteum* were first chromatographed on alumina affording three fractions of varying polarity. The chloroform–methanol fraction, the most polar fraction, was then rechromatographed twice on silica gel. In this way nuphacristine, which gave a positive Dragendorff test, was obtained. The structure of **1** was deduced from an examination of its ^1H and ^{13}C NMR spectra and its mass spectrum, and confirmed by chemical transformation to *Nuphar* alkaloids of established structure and stereochemistry.



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In the ^{1}H NMR spectrum of **1** a signal of area one was observed at δ 8.71 indicative of the presence of an aldehyde group. A one-proton singlet at δ 6.77 indicated the presence of an olefinic proton and a signal attributed to a hydroxymethyl group was observed at δ 3.69 as an apparent doublet. There were no signals that could be assigned to methyl groups which are normally observed in $C_{15}N$ *Nuphar* alkaloids of established structure [2]. Apart from the three signals in the aromatic region attributed to a β-substituted furan and signals attributed to the protons adjacent to the nitrogen atom the remainder of the spectrum could not be assigned.

The ^{13}C NMR spectrum was more revealing and more amenable to interpretation. Signals at δ 57.4 and 59.2 were assigned to tertiary carbon atoms adjacent to nitrogen at C-4 and C-10, respectively. In the downfield region signals for two olefinic carbon atoms were observed, one a methine at δ 157.9 and the other a quaternary carbon atom at δ 122.5; these data point to the presence of a double bond possibly in an enamine between C-6 and C-7 of the quinolizidine system [3]. The fact that C-7 is quaternary indicates that it must be substituted by the aldehyde or the hydroxymethyl group. Since the signal of the aldehyde carbon atom is shielded in comparison with aldehyde carbon atoms attached to saturated carbon centres, one may infer that the aldehyde function is located at C-7 [3]. Provided that the substitution pattern of **1** is the same as that in other $C_{15}N$ quinolizidine alkaloids of the *Nuphar* group, the hydroxymethyl group must be at C-1. Its chemical shift at δ 63.0, in comparison with literature data for related lupinane derivatives [4], implies that it is equatorial. Other ^{13}C NMR signals listed in the Experimental are in harmony with structure **1**.

The composition of **1** and several of its fragment ions was established by high resolution mass spectrometry as shown in Scheme 1. The ions in the low resolution spectrum of **1** at *m/z* 107 and 94 (Scheme 1) are also found in the mass spectra of deoxynupharidine and castoramine [5,6,7] indicating that this part of the quinolizidine is the same in all three compounds. The ions at *m/z* 190 and 110 in **1** have their counterparts in the spectrum of deoxynupharidine **3** (Fig. 1) at *m/z* 178 and 98 and in the spectrum of castoramine (structure **3**, $R^1 = CH_2OH$, $R^2 = H$) [5–7] at *m/z* 194 and 114, respectively. The presence of OH, CH_2OH, and CH=O groups in **1** is reflected in the loss of 17 (*m/z* 244), 31 (*m/z* 230) and 29 (*m/z* 232) mass units, respectively, from the molecular ion (only the latter is shown in Scheme 1). Thus the mass spectrum of **1** supports the placement of the CH_2OH group in ring A and the CH=O group in ring B and otherwise is fully in accord with the structure proposed in Fig. 1.

The structure of **1** has been confirmed by its chemical transformation into a mixture of deoxynupharidine **3** and 7-epideoxynupharidine **4** by the route shown in Fig. 1. In the first step the treatment of **1** with sodium borohydride in ethanol afforded diol **2**, isolated as an oil. The spectroscopic properties of **2** agreed with the assigned structure. The ^{1}H NMR spectrum of **2**, in comparison with that of **1**, did not exhibit signals characteristic of an alkene or an aldehyde. Instead a new signal of area **2** at δ 3.80 was present which is characteristic of an axially oriented CH_2OH group. The ^{13}C NMR spectrum of **3** was, like the proton spectrum, devoid of signals for olefinic or carbonyl carbon atoms. Instead, new signals were present, attributed to C-6 and C-7 at δ 56.5 and 34.9, respectively. The signal at δ 34.9 is considerably shielded relative to that at C-1 (δ 44.3), which also bears a CH_2OH group, suggesting that the new CH_2OH group is in an axial orientation [4]. The chemical shift of C-6, in analogy with data in the literature [8], points to a *trans*-quinolizidine ring juncture. The data also favour an equatorial orientation of the furan substituent at C-4. The composition of **2** ($C_{15}H_{23}NO_3$) was confirmed by high resolution mass spectrometry, as were the compositions of a number of fragment ions. Of particular interest are ions at *m/z* 248 $(M-OH)^+$, *m/z* 234 $(M-CH_2OH)^+$ and an ion at *m/z* 114 ($C_6H_{12}NO$) (the counterpart of *m/z* 110 of Scheme 1), an ion which is also present in the mass spectrum of castoramine [6,7]. Other ions characteristic of the $C_{15}N$ quinolizidine system found in the *Nuphar* alkaloids are present at *m/z* 107 and 94, while still other ions listed in the Experimental section may be accommodated by fragmentation pathways proposed for related alkaloids [6,7].

The two hydroxymethyl groups of **2** were converted to methyl groups by treatment first with $(C_6H_5O)_3P^+$-MeI^- in HMPA followed, without isolation of the intermediate, by reduction with sodium cyanoborohydride according to the procedure of Hutchins *et al.* [9]. The product of reduction was a mixture of deoxynupharidine **3** and 7-epideoxynupharidine **4** in a ratio of 3 to 2. Compound **3** would be expected to form from **2** through the intermediacy of a di-iodide. The presence of substantial quantities of **4** was unexpected and is not readily explicable in terms of a di-iodide intermediate. The lack of material precluded a more thorough investigaton of this interesting epimerization at C-7.

These studies have established beyond reasonable doubt the structure and relative stereochemistry of nuphacristine **1**.

EXPERIMENTAL

The ^{1}H NMR (100 MHz) and ^{13}C NMR (22.5 MHz) spectra were measured in $CDCl_3$; chemical shifts are reported in ppm (δ) downfield from the signal of TMS used as an int. standard. The EI MS were recorded at 70 eV; samples were introduced directly into the ion source by a probe. Accurate mass measurements agreed with calculated value within $\pm$ 5 mmu. Optical rotations were measured in EtOH in a 1-dm cell. Separation by column chromatography was carried out on alumina (Fluka 507C, Act. II or III) and silica gel (MN 100, 200 mesh) or (Merck, 230–400 mesh).

Isolation of nuphacristine The crude alkaloids (60 g) obtained as described earlier [10], were dissolved in C_6H_6, adsorbed on an Al_2O_3 column (act. II, Fluka 507C, 1 kg) and eluted in order with C_6H_6, $CHCl_3$, and $CHCl_3$/MeOH. The $CHCl_3$–MeOH fraction (3.6 g) was adsorbed on silica gel (70 g, 100–200 mesh, MN) and eluted with $CHCl_3$ to give a 2 g fraction. Rechromatography of this fraction on silica gel (60 g, 230–400 mesh, Merck) gave compound **1**, 0.114 g (0.19%); $[\alpha]_D^{20} = +65.9°$; ^{1}H NMR ($CDCl_3$) δ: 0.7–2.5 (10 H, *m*), 3.1–3.5 (1H, *m*, H-10), 3.69 (2H, *d*, *J* = 3 Hz, H-11), 4.1–4.3 (1H, *m*, H-4), 6.40 (1H, *s*, *br*, β-furanyl), 6.77 (1H, *s*, H-6), 7.47 (1H, *s*, *br*, α-furanyl), 7.52 (1H, *m*, α-furanyl), 8.71 (1H, *s*, -CHO); ^{13}C NMR ($CDCl_3$) δ: 15.7 (*t*, C-8), 23.3 (*t*, C-2), 28.0 (*t*, C-9), 32.2 (*t*, C-3), 40.6 (*d*, C-1), 58.1 (*d*, C-4), 59.3 (*d*, C-10), 63.2 (*t*, C-11), 109.8 (*d*, C-15), 122.3 (*s*, C-7), 136.5 (*s*, C-13), 141.4 (*d*, C-14), 144.3 (*d*, C-16), 152.0 (*d*, C-6), 187.7 (*s*, C-12). EIMS, *m/z* (rel. int.): 261 (M^+, 100), 244(6), 232(27), 230(11), 190(7), 136(6), 110(29), 94(66), 82(23), 81(19), 77(10); TLC (silica

gel $CHCl_3$–MeOH = 17:3); $R_f = 0.48$; TLC (alumina, $CHCl_3$–MeOH = 93:7); $R_f = 0.18$.

Preparation of compound 2. To a soln of **1** (0.063 g, 0.00023 mol) in EtOH (25 ml), $NaBH_4$ (0.035 g, 0.00093 mol) was added portionwise and the mixture was allowed to stand at room temp. The course of the reaction was monitored by means of TLC and when it was complete the EtOH was evapd. The residue was treated with H_2O (20 ml) and the mixture extracted with $CHCl_3$. The $CHCl_3$ extract was dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude residual oil was chromatographed on Al_2O_3 using $CHCl_3$–MeOH = 99:1 as eluant. Yield: 0.052 g (81.2%). $[\alpha]_D^{20} = -54.2°$; ^{1}H NMR ($CDCl_3$) δ: 0.7–2.5 (12H, *m*), 3.08 (2H, *m*, H-6e, H-4a), 3.67 (2H, *d*, $J = 2.5$ Hz, H-11), 3.78 (2H, *d*, $J = 2.5$ Hz, H-12), 6.43 (1H, *s*, *br*, β-furanyl), 7.39 (2H, *m*, α-furanyl); ^{13}C NMR ($CDCl_3$) δ: 27.7 (*t*, C-2), 28.2 (*t*, C-9), 29.6 (*t*, C-8), 34.1 (*t*, C-3), 34.9 (*d*, C-7), 44.3 (*d*, C-1), 56.5 (*t*, C-6), 60.3 (*d*, C-4), 64.2 (*t*, C-11), 64.5 (*d*, C-10), 68.5 (*t*, C-12), 108.9 (*d*, C-15), 128.7 (*s*, C-13), 139.5 (*d*, C-14), 143.5 (*d*, C-16). EIMS, *m/z* (rel. int.): 265 (M^+, 22), 248 (5), 234 (19), 220 (7), 206 (16), 194 (4), 193 (9), 178 (4), 152 (17), 121 (51), 114 (70), 107 (12), 94 (50), 81 (19), 77 (13), 31 (100). TLC (alumina, $CHCl_3$–MeOH = 93:7) $R_f = 0.29$.

Preparation of compounds **3** and **4**. To 54 mg (0.00020 mol) of **2** in hexamethylphosphoramide (1 ml) methyltriphenoxyphosphonium iodide (0.368 g, 0.0081 mol) [11] was added and the mixture was heated with stirring at 70° for 3 hr. Na cyanoborohydride (0.102 g, 0.0016 mol) was then added and the mixture was heated with stirring at 70° for 3 hr. Na cyanoborohydride (0.102 g, 0.0016 mol) was then added and the The hexane extract was washed with H_2O and dried (K_2CO_3). Evaporation of the hexane gave a crude residue which was chromatographed on Al_2O_3 affording from the hexane fraction 22 mg of **3** (46.2%) and 15.0 mg of **4** (31.5%). Spectral data (^{13}C NMR, ^{1}H NMR) for compound **3** were in agreement with those for deoxynupharidine, and for compound **4** with those for 7-*epi*-deoxynupharidine [2].

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