Phytochemistry, 1970, Vol. 9, pp. 1851 to 1854. Pergamon Press. Printed in England.

THE STRUCTURE OF 3-EPINUPHAMINE, A NEW ALKALOID FROM *NUPHAR LUTEUM* SUBSP. *VARIEGATUM**

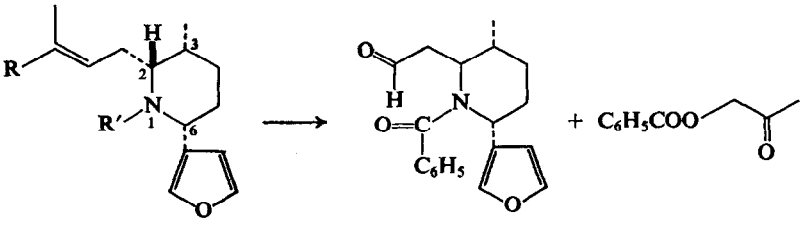
CHUN FOOK WONG and ROBERT T. LALONDE

Department of Chemistry, State University College of Forestry at Syracuse University, Syracuse, New York 13210, U.S.A.

(*Received* 11 *November* 1969)

Abstract—The structure of a new alkaloid from *N. luteum* subsp. *variegatum* is shown to be that of 3-epinuphamine, I (R = CH_2OH, R′ = H).

ALONG WITH a number of known alkaloids† of the water lily *Nuphar luteum* subsp. *variegatum* (Nymphaeaceae),[1] we have isolated a hitherto unknown minor constituent whose structure is expressed as I (R = CH_2OH, R′ = H) on the basis of the evidence given here.



(I) (II) (III)

The elemental analysis and mass spectrum (m.s.) correspond to a molecular formula $C_{15}H_{23}NO_2$. The m.s. was very similar to that of nuphenine,[2] I (R = CH_3, R′ = H), indicating that the same gross skeletal features were present in both alkaloids. One of the oxygen atoms was accounted for in a 3-furanyl group, whose presence was indicated by the i.r., NMR and mass spectra. Attachment of this group on carbon α to nitrogen (C_6) was indicated by a proton (3·58 δ) deshielded by both 3-furanyl group and nitrogen. This partial structure could be expanded to include the C_5 methylene since the m.s. showed an intense peak at *m/e* 94 ($C_6H_5O^+$).[3] The involvement of the two remaining heteroatoms in alcohol and secondary amine groups was established in the conversion of 3-epinuphamine to a *N*-,*O*-dibenzoyl derivative, I (R = $CH_2OCOC_6H_5$, R′ = C_6H_5CO).

* Support of this work by the U.S. Department of Interior, Federal Water Pollution Control Administration and the McIntire–Stennis Cooperative Forestry Research Program of the U.S. Department of Agriculture is gratefully acknowledged. Support of the National Science Foundation in the purchase of the mass spectrometer used in this work also is gratefully acknowledged.

† These alkaloids include nupharidine, deoxynupharidine, 7-epideoxynupharidine, nuphenine and nuphamine. The isolation and identification of nuphamine from *N. luteum* will be the topic of a subsequent publication.

[1] E. O. BEAL, *J. Elisha Mitchell Sci. Soc.* **72**, 317 (1956).
[2] R. BARCHET and T. P. FORREST, *Tetrahedron Letters* 4229 (1965).
[3] J. T. WRÓBEL, in *The Alkaloids* (edited by R. H. F. MANSKE), Vol. IX, p. 449. Academic Press, New York (1967).

The presence of a trisubstituted double bond was indicated by the i.r. and NMR; moreover, the latter showed that one substituent was a hydroxymethyl group (3·93 δ, 2H, broad singlet), the second was a vinyl methyl group (1·65 δ, 3H, broad singlet) and the third was a methylene group as evidenced by the single vinyl proton split into a broad triplet. The *trans* stereochemistry of this double bond was established by observing that the vinyl proton resonance at 5·41 δ is in good agreement with the chemical shift displayed by the vinyl proton of *trans*-2-methyl-2-penten-1-ol(5·35 δ) but was downfield from the vinyl proton of *cis*-2-methyl-2-penten-1-ol(5·25 δ).[6] Confirmation of the double bond stereochemistry came from MnO_2 oxidation of 3-epinuphamine to an aldehyde (I) (R = CHO, R′ = H) whose u.v. spectrum (λ_{max} 225 nm, ϵ 16,000) was in accord with that reported[4] for *trans*-2-methyl-2-pentenal (λ_{max} 228, ϵ 12,000) but different from that of the *cis* pentenal (λ_{max} 237, ϵ 8900). Attachment of the *trans*-$HOCH_2(CH_3)C{=}CHCH_2$-group to a second carbon α to nitrogen was consistent with the appearance of *m/e* 164 as the base peak in the m.s. Generation of *m/e* 164 would result through facile α-cleavage of an amine[5] accompanied by the loss of a stable allyl radical. Accordingly, the NMR showed the C_2 proton (2·82 δ) as a triplet of doublets, a splitting pattern generated by coupling to the side chain methylene ($J = 7$ Hz) and to a single proton at C_3 ($J = 2·5$ Hz). This interpretation implied substitution at C_3 and on this basis a methyl group, whose presence is indicated by a doublet ($J = 5·5$ Hz) at 0·99 δ, is attached to C_3. The remaining methylene not yet accounted for in the partial structure was incorporated as C_4 to complete the piperidine ring. That the piperidine substitution pattern expressed in I (R = CH_2OH, R′ = H) is correct was determined by converting both the *N*-,*O*-dibenzoyl derivative, I (R = $CH_2OCOC_6H_5$, R′ = C_6H_5CO), and nuphenine benzamide I (R = CH_3, R′ = C_6H_5CO) to the aldehyde II. Also isolated from the oxidation of I (R = $CH_2OCOC_6H_5$, R′ = C_6H_5CO) was benzoxyacetone III.

The results of the oxidation experiment also link 3-epinuphamine to nuphenine stereochemically. Since the NMR spectrum shows the C_3 methyl doublet (0·99 δ) at a lower field than the reported[6] resonance (0·91 δ) displayed by the equatorial methyl of nuphamine, an axial methyl group in 3-epinuphamine and nuphenine is implicated. This assignment is based on the correlation from NMR studies of methylquinolizidines[7] which shows axial methyl groups associated with lower field signals than their equatorial counterparts. Since the piperidines considered here are undoubtedly conformationally rigid because of the bulky furan group, the extention of the correlation seems warranted. Consistent with this assignment is the observed benzene-induced downfield shift ($^{\delta}CDCl_3{}^{-\delta}C_6H_5 = -3·0$ Hz) of the methyl group in 3-epinuphamine and the upfield shift (+4·0 Hz) in nuphamine. Also consistent with an axial C_3 methyl group is the equatorial–equatorial or axial–equatorial relationship between C_2 and C_3 protons indicated by the small coupling (2·5 Hz) observed for the proton at C_2. Based on the appearance of a single Bohlmann band,[8-10] at 3·58 μ we propose that 3-epinuphamine possesses an axial hydrogen at C_2. For comparison, the i.r. of nuphamine,†

† See Footnote p. 1851.

[4] K. C. CHAN, R. A. JEWELL, W. H. NUTTING and H. RAPOPORT, *J. Org. Chem.* **33**, 3382 (1968).
[5] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Mass Spectrometry of Organic Compounds*, Chapter 8. Holden-Day, San Francisco, (1967).
[6] Y. ARATA and J. OHASHI, *Chem. Pharm. Bull. Tokyo* **13**, 1247 (1965).
[7] T. M. MOYNEHAN, K. SCHOFIELD, R. A. Y. JONES and A. R. KATRITZKY, *J. Chem. Soc.* 2637 (1962).
[8] F. BOHLMANN, *Chem. Ber.* **91**, 2157 (1958).
[9] M. W. WIEWIOROWSKI and J. SKOLICK, *Bull. Acad. Polon. Sci. Ser. Sci. Chim.* **10**, 1 (1962).
[10] C. D. JOHNSON, R. A. Y. JONES, A. R. KATRITZKY, C. R. PALMER, K. SCHOFIELD and R. J. WELLS, *J. Chem. Soc.* 6797 (1965).

whose stereochemistry at C_2 and C_3 is known,[6] also exhibits a single Bohlmann band at 3·58 μ, equally intense as that of 3-epinuphamine. Accordingly the complete relative stereochemistry is expressed as I (R = CH_2OH, R′ = H).

EXPERIMENTAL

Isolation of 3-*Epinuphamine*, I (R = CH_2OH, R′ = H)

Rhizomes of *Nuphar luteum* subsp. *variegatum* were harvested from the north shore of Cazenovia Lake, New York during August, 1966.

Dried powdered rhizomes, 2 kg, were extracted with 10–12 l. of methanol (×6) for periods of 3–5 days. Concentration of the combined methanol extract furnished 553 g of residue which was mixed with 1·5 l. of 10% aq. acetic acid. The mixture was extracted successively with hexane, benzene and CH_2Cl_2. These extracts were set aside for other investigations. The acetic acid solution was neutralized with solid $NaHCO_3$, the solids removed by filtration and the filtrate was extracted repeatedly with $CHCl_3$. The $CHCl_3$ extract was dried and concentrated to afford 5·2 g of residue. The residue was chromatographed on 60 g Al_2O_3 (act. I), using cyclohexane (700 ml) cyclohexane–benzene (70:30, 1300 ml; 25:75, 700 ml) benzene–$CHCl_3$ (75:25, 200 ml; 25:75, 350 ml); and $CHCl_3$–CH_3OH (80:20, 200 ml). $CHCl_3$–CH_3OH and benzene–$CHCl_3$ (25:75) fractions were combined (2·9 g) and rechromatographed on 75 g Al_2O_3 (act. I) using cyclohexane (fraction number, volume of each fraction in ml. and total weight in mg: 1–4, 30, 0) cyclohexane–CH_2Cl_2, 1:1 (5–18, 30, 22) CH_2Cl_2 (19–42, 30, 170) CH_2Cl_2–2% CH_3OH (42–47, 25, 1978) CH_2Cl_2–5% CH_3OH (48–50, 25, 71) CH_2Cl_2–10% CH_3OH (51–57, 25, 106). Fractions 42, 44 and 45 were combined (1·005 g). This material was chromatographed three times on Al_2O_3 (act. I) eluted with CH_2Cl_2, CH_2Cl_2–0·3% CH_3OH and CH_2Cl_2–20% CH_3OH. In each case the fraction being rechromatographed was the CH_2Cl_2–0·3% CH_3OH fraction from the preceding chromatography. The CH_2Cl_2–0·3% CH_3OH fraction from the third such rechromatography on concentration gave 72 mg of slightly impure 3-epinuphamine. A 68 mg sample was chromatographed on 6·8 g of neutral Al_2O_3 (act. III) eluting with benzene (fraction 1, 150 ml, 6 mg), benzene–ether (4:1) (fraction 2, 25 ml, 39 mg; fraction 3, 25 ml, 10 mg; fraction 4, 25 ml, 2·6 mg) and CH_3OH (fraction 5, 25 ml, 13·7 mg). Fraction 2 contained pure 3-epinuphamine: TLC on Al_2O_3–G, $CHCl_3$, one spot, R_f 0·32; $[\alpha]_D^{25}$–47·5 (c, 0·52, 95% EtOH); i.r. (CCl_4) 2·74, 3·05, 3·43, 3·50, 3·58, 5·98, 6·68, 6·94, 7·28, 8·60, 9·43, 9·78, 9·91, 11·44 μ; i.r. (neat) 6·06, 11·47, 12·6, 13·2, 13·8, NMR ($CDCl_3$, TMS 1% 0·0 δ) 0·99 (d, 5·5 Hz, 3H, H–C–CH_3), 1·65(s, 3H, C=CCH_3), 2·82 (d of t, 7 and 2·5 Hz, 1 H, N–CHC), 3·58 (m, $W_{1/2h}$ 14·5 Hz, 1H, N–CH–C_4H_3O) 3·93(s, 2H, CH_2–0), 5·41 (br. t, 1H, C=CH), 6·41 (*m*, 1H, β-furanyl) and 7·35 δ (*m*, 2H, α-furanyl); NMR (C_6H_6) 1·02 δ; m.s. (70eV, all glass heated inlet, chamber temperature 160–165°) *m/e* (rel. intensity) 249 (1·9), 248 (2·5), 234 (1·9), 178 (5·5), 164 (100) 107 (53·8), 94 (34). Found*: C, 72·34, 72·15; H, 9·40, 9·48; N, 5·70, 5·70. Calc. for $C_{15}H_{23}NO_2$: C, 72·25; H, 9·30; N, 5·62%. Nuphamine: NMR ($CDCl_3$) 0·92, (C_6H_5) 0·85 δ; i.r. (CCl_4) 3·58, 5·98 μ.

N-,O-*Dibenzoyl*-3-*epinuphamine*, I(R = $CH_2OCOC_6H_5$, R′ = C_6H_5CO). A 39 mg sample of 3-epinuphamine in 1 ml of CH_2Cl_2 was treated with benzoyl chloride (0·073 ml) and excess pyridine at 25° overnight. The mixture was washed with dilute HCl and saturated $NaHCO_3$. The aqueous layers were back washed with CH_2Cl_2. The combined organic extract was dried and evaporated to dryness affording 172·7 mg of brown liquid which was dissolved in $CHCl_3$ and filtered through 6 g of neutral Al_2O_3. The filtrate and washings were combined and evaporated to 72 mg of residue which was chromatographed on 6 g neutral Al_2O_3 (act. III) using benzene, fractions 1–6 (fraction number, vol. in ml, and wt. in mg are as follows: 1, 10, 3; 2, 25, 26; 3, 24, 15; 4, 24, 7; 5, 25, 4; 6, 25, 0) and $CHCl_3$, fraction 7 (25, 6). Fractions, 2, 3, 4 and 5 were combined and rechromatographed on a similar column using benzene, fractions 1–5 (1, 25, 22·6; 2, 12, 10·7; 3, 20, 4·6; 4, 17, 2; 5, 13, 0·5) and CH_2Cl_2, fraction 6 (17, 0·4). Fractions 1 and 2 were pure (TLC Al_2O_3 G, $CHCl_3$, one spot, R_f 0·9) *O*-,*N*-dibenzoyl-3-epinuphamine: $[\alpha]_D^{25}$ –82° (c, 0·67, CH_2Cl_2); i.r. (CH_2Cl_2) 5·85 (C_6H_5COO-), 6·18 μ ($C_6H_5CON<$); NMR($CDCl_3$) 8·1 (m, 2H, *o*-C_6H_5COO-) 7·55 (m, 3H, *m*-and *p*-C_6H_5COO-), 7·40 (s and m, 7H, C_6H_5 and α-furanyl), 6·58 and 6·50 (2 m, 1H, β-furanyl), 4·63 (br. s, 2H, $CH_2OCOC_6H_5$) m.s. *m/e* (rel. intensity) 457 (0·2), 352 (0·2), 336 (0·2), 177 (12), 176 (16), 105 (100).

Oxidation of O-,N-*Dibenzoyl*-3-*epinuphamine*, I (R = $CH_2OCOC_6H_5$, R′ = C_6H_5CO). A 41 mg sample of the dibenzoyl derivative in 0·5 ml of dioxane was treated with 1 mg of osmium tetroxide, a drop of pyridine and 41 mg of paraperiodic acid at 25° overnight. The mixture was stirred with 110 mg of sodium sulfite for 30 min then extracted with $CHCl_3$. The extract was dried and concentrated to 41 mg of liquid which was chromatographed on 6 g of neutral Al_2O_3 (act. III) using benzene, fractions 1 and 2 (15 ml, 8·1 mg; 5 ml, 2·8 mg), $CHCl_3$–benzene 1:9, fractions 3 and 4 (50 ml, 3 mg; 50 ml, 13 mg), $CHCl_3$–benzene 2:8, fraction 5 (50 ml, 1·4 mg) and CH_3OH (25 ml, 7·4 mg). Fractions 1 and 2 consisted of III, identical with authentic material prepared as described in another section.

* The elemental analysis was determined by Miss Betty McCarthy, Stanford Research Institute, Menlo Park, California, U.S.A.

Fractions 3, 4 and 5 were combined and chromatographed on a plate of Al_2O_3 HF_{254}(E) (20 × 20 cm, 0·5 mm, $CHCl_3$) to afford 3·8 mg of the aldehyde II (R_f 0·5, u.v. $\lambda_{max\,254}$ blue): $[\alpha]_D^{25}$ 245 ± 7° (*c*, 0·38, CH_2Cl_2) and showing i.r. and TLC properties the same as the aldehyde obtained from the osmium tetroxide–paraperiodate oxidation of *N*-benzoylnuphenine I (R = CH_3, R′ = C_6H_5CO).

Manganese dioxide oxidation of 3-*epinuphamine.* A 2·2 mg sample of 3-epinuphamine in 0·5 ml of CH_2Cl_2 was treated with 21 mg of active MnO_2 at 25° for 12 hr. The solids were removed by filtration and the filtrate was evaporated to afford 1·6 mg of residue: TLC (Al_2O_3 G, $CHCl_3$) R_f 0·5 (one spot); i.r. (CH_2Cl_2) 3·68 and 5·94 (—CHO), 6·10 (C=C), 6·30 (NH def.); u.v. λ_{max} (95% EtOH) 225 (16,500).

N-*Benzoylnuphenine*, I (R = CH_3, R′ = C_6H_5CO). A 47 mg sample of nuphenine (0·202) isolated from a benzene extract of a 10% aq. acetic acid solution of Nuphar alkaloids,[11] in 0·4 ml of pyridine was treated with 0·5 ml of benzoyl chloride in 1 ml of CH_2Cl_2 at 25° for 5 hr. The mixture was washed with aq. HCl and $KHCO_3$. The aqueous solutions were extracted with CH_2Cl_2. The combined CH_2Cl_2 extract was dried and concentrated to afford 126 mg of liquid which was chromatographed on 6·3 g of neutral Al_2O_3 (act. III). Crude *N*-benzoylnuphenine (68 mg) emerged on eluting with the first 25 ml of benzene. Rechromatography on 6·3 g of neutral Al_2O_3 (act. III) using 150 ml of hexane gave fraction 1 (1 mg); using benzene–hexane, 1:3, gave fractions 2–4, 25 ml each (2, 1 mg; 3, 0 mg; 4, 18 mg); using benzene–hexane, 1:1, gave fractions 5–9, 25 ml each (5, 19 mg; 6, 15 mg; 7, 8 mg; 8, 0 mg; 9, 0 mg) using benzene, 50 ml gave fraction 10, 2·5 mg.

Fractions 4–7 contained pure *N*-benzoylnuphenine I(R = CH_3, R′ = C_6H_5CO): TLC Al_2O_3 G, CH_2Cl_2 R_f 0·25, one spot; $[\alpha]_D^{25°}$ –128·5 (*ca.* 0·6 CH_3OH); i.r. (neat) 6·12 (C_6H_5CON <); NMR ($CDCl_3$) 7·4 (s and m, 7H, phenyl and α-furanyl), 6·41 (m, 1H, β-furanyl), 5·41 (m, 1H, C=C—H), 1·58 and 1·03 (2 s, 6H, C=C—CH_3), 0·87 δ (d, 6 Hz, 3H, C_3-CH_3); m.s. (70 ev) *m/e* (rel. intensity) 337 (2), 268 (52), 232 (3), 107 (23), 105 (100) 94 (0·5).

Osmium tetroxide–paraperiodic acid oxidation of N-*benzoylnuphenine*, I (R = CH_3, R′ = C_6H_5CO). A 57 mg sample of *N*-benzoylnuphenine (0·17 μmole) in 0·5 ml of dioxane was treated with 7 mg (0·027 mmole) of osmium tetroxide, 5 drops of pyridine, and 81 mg (0·36 mmole) of paraperiodic acid in a few drops of water at 25° for 12 hr. The mixture was stirred with 200 mg of Na_2SO_3 and extracted with $CHCl_3$ (5 × 15 ml.) The combined $CHCl_3$ extract was dried and evaporated to afford 40·7 mg of residue which was chromatographed on 6 g of neutral Alumina (act. III) using 250 ml of benzene, fraction 1 15 mg, 100 ml of $CHCl_3$, fraction 2 29 mg and 30 ml of CH_3OH, fraction 3 36 mg. Fraction 1 contained pure aldehyde II which was unstable on storing: TLC Al_2O_3, $CHCl_3$, R_f 0·56, one spot; $[\alpha]_D^{25}$ –254 ± 7° (*c*, 0·35, CH_2Cl_2); i.r. (CH_2Cl_2) 3·29, 3·40, 3·49, 3·51(sh), 3·65, 5·80, 6·14, 6·18(d), 6·92, 7·12, 7·34, 7·60, 8·62, 9·78; 11·42 μ; m.s. (70 eV) *m/e* (rel. intensity) 311(6), 282(3), 268(2), 267(3), 206(19), 190(8), 162(18), 105(100), 94(23).

Benzolyacetone III. Acetol,* 451 mg in 10 ml of CH_2Cl_2 and 480 mg of pyridine was treated with 850 mg of benzoyl chloride at 25° for 24 hr. The mixture was washed with 10 ml of 1 N aq. HCl, saturated Na_2CO_3 and then water. The CH_2Cl_2 extract was dried and evaporated giving 1·426 g of residue. Eluting a 205 mg sample on 10 g Al_2O_3 (act. III) with 25 ml of benzene afforded 143 mg of pure benzoxyacetone, III; TLC Al_2O_3-G, $CHCl_3$, R_f 0·8, one spot; i.r. (CH_2Cl_2) 3·29, 3·35, 5·81 (reported,[12] 5·80 μ) 6·24, 8·0, 9·02; NMR ($CDCl_3$) 8·15 (m, 2H, *o*-C_6H_5CO), 7·55 (m, 3H, *p* and *m* C_6H_5CO), 4·90 (s, 2H–CH_2–O–) and 2·22 δ (s, 3H, CH_3CO).

* Purchased from the Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.

[11] C. F. WONG and R. T. LALONDE, *Phytochem.* **9**, 659 (1970).

[12] E. SCHAETZLE, H. URHEIM, M. THUERKAUF and M. ROTTENBERG, *Helv. Chim. Acta*, **46**, 2418 (1963).