SUPPLEMENTAL STUDIES ON FLUOROPHORE IN REACTIONS OF EPOXIDES WITH NICOTINAMIDE AND ACETOPHENONE

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As supplemental studies on fluorophore in the reaction of epoxides with nicotinamide and acetophenone, 1,6- and 2,7-naphthyridine derivatives were prepared, and their fluorescence properties were compared. The result confirmed that the fluorophore has a 2,7-naphthyridine structure, but no 1,6-isomer.

INTRODUCTION

In the previous paper\(^1\), we proposed a new reaction mechanism for a fluorescence reaction of common epoxides with nicotinamide and acetophenone: The final fluorophore was considered to have a 2,7-naphthyridine structure, based on the instrumental analysis of the reaction intermediate [A] and the final fluorophore [B] using glycidyl phenyl ether (GPE) as a model epoxide. A 1,6-naphthyridine structure had been cited as the fluorophore in past papers\(^2\)-\(^4\). As a supplemental study, in the present work we aimed at the preparations of 1,6- and 2,7-naphthyridine derivatives in order to get some synthetical proof on the chemical structure of the final fluorophore and also to compare the fluorescence properties of the two compounds.

Since a methyl group at 2- or 4-position on the pyridine ring of pyridinium compounds is reactive, Baker et al.\(^5\) have synthesized phenacylidene derivative by the reaction between 1-benzyl-2-picolinium chloride and benzoyl chloride. In this reaction, if a carbamide group exists at 3-position on the pyridine ring, the resulting phenacylidene derivative may be subsequently subjected to a dehydration reaction. Therefore, a 1,6- or 2,7-naphthyridine derivative was expected to form as the final product. Thus, we constructed the synthetic route given in Fig. 1, and we could obtain both 2,7-naphthyridine derivative [III] and its 1,6-isomer [VI] from 4-methyl- and 2-methylnicotinamide, respectively.

EXPERIMENTAL

Apparatus

Excitation and emission spectra (uncorr.) were measured with a Shimadzu RF-502 spectrofluorometer. UV spectra were measured with a Hitachi 323 spectrophotometer in ethanol. IR spectra were measured with a Hitachi Perkin-Elmer 225 spectrometer in KBr disk. \(^1\)H and \(^13\)C NMR spectra were measured with a JNM FX-100 spectrometer in trifluoroacetic acid-d. Mass spectra were measured with a Hitachi M-80 double focusing mass spectrometer equipped with EI and FD ion source. Thin-layer chromatography (TLC) was carried out with pre-coated silica gel 60 HF\(_{254}\) TLC plates (Merck)
and with solvent systems such as (a) acetone-methanol-formic acid (17:2:1) and (b) ethyl acetate-methanol-formic acid (10:5:1).

**Preparation of compound [III]**

To a mixture of 2.7 ml of water, 5.6 ml of dichloromethane and 970 mg of 3-carbamoyl-4-methyl-1-(2-hydroxy-3-phenoxypropyl)pyridinium chloride [II], obtained from 4-methylnicotinamide [I] and GPE by the previously reported procedure for compound [A], were added 0.5 ml of benzoyl chloride and then 4 ml of 25% sodium hydroxide within 5 min under a nitrogen stream with vigorous stirring. After 30 min, the organic layer was evaporated to dryness under a reduced pressure. The crude product was recrystallized from methanol. Thus, 7-(2-hydroxy-3-phenoxypropyl)-3-phenyl-2,7-naphthyridin-1(7H)-one [III] was obtained as yellow needles (yield, 13.9%); melting point and all instrumental data were identical with those of [B], previously reported.


**Preparation of compound [VI]**

3-Carbamoyl-2-methyl-1-(2-hydroxy-3-phenoxypropyl)pyridinium chloride [V] (970 mg), obtained from 2-methylnicotinamide [IV] and GPE, was treated in the manner described for [III]; 1-(2-hydroxy-3-phenoxypropyl)-7-phenyl-1,6-naphthyridin-5(1H)-one [VI] monohydrate was obtained as orange needles (yield, 7.3%).


**[VI]**: Mp. 219-221° (dec). Anal. Calcd for C₂₃H₂₀N₂O₃.H₂O: C, 70.75; H, 5.68; N, 7.17. Found: C, 70.78; H, 5.66; N, 6.72. IR(KBr, cm⁻¹): 1635(vC=O), 1247 (vC-O-C). UV λₘₐₓ nm(log ε): 244(4.10), 301(4.29). FD-Mass m/z: 373(M⁺-H₂O⁺, C₂₃H₂₀N₂O₃), 372(M⁺-H₂O, C₂₃H₂₀N₂O₃), 236(M⁺-C₈H₁₁O₅, C₁₅H₁₁N₂O), 222(M⁺-C₉H₁₂O₃, C₁₄H₁₀N₂O). ¹H NMR δ (ppm): 4.23-5.50 (multiplet, 5H), 6.80-7.33 (m, 5H), 7.44 (singlet, 1H), 7.51-7.78 (m, 5H), 7.96 (triplet, 1H, J=6.0 and 7.4 Hz), 9.16 (doublet, 1H, J=6.0 Hz), 9.44 (d, 1H, J=7.4 Hz). ¹³C NMR δ (ppm): 61.936 (t, splitting in ¹H-off
RESULTS AND DISCUSSION

Structures of the N^1-alkylated derivatives [II] and [V] of methylnicotinamides were confirmed mainly by their instrumental data as well as by comparison of the physical properties of these compounds and those of compound [A]. [III] was confirmed in the same way as previously reported for [B]. [VI], which crystallizes with one molecule of water of crystallization, was found to have a molecular formula of \(\text{C}_{23}\text{H}_{20}\text{N}_{2}\text{O}_{3}\cdot\text{H}_{2}\text{O}\) by elementary analysis and by study of its FD-mass spectrum. [VI] has a carbonyl group and ether bond but no primary or secondary carbamide group in the IR spectrum. The absorption spectrum of [VI] was similar to that of 1,6-naphthyridine derivatives which was reported by Ikekawa. The \(^{13}\text{C}\) NMR spectra showed that the molecule has five tertiary carbon atoms and one carbonyl carbon atom, and also that the methyl and carbonyl carbon of the raw materials form a part of a heteroaromatic ring. The \(^{1}H\) NMR spectral pattern on the heteroaromatic ring protons, namely, a triplet at \(\delta 7.96(J=6.0 \text{ and } 7.4 \text{ Hz})\) and two doublet peaks at \(\delta 9.16(J=6.0 \text{ Hz})\) and \(9.44(J=7.4 \text{ Hz})\), showed the characteristic pattern of 1,5,7-trisubstituted 1,6-naphthyridine structure. Thus, [VI] was confirmed to be 1-(2-hydroxy-3-phenoxypropyl)-7-phenyl-1,6-naphthyridin-5(1H)-one.

The fluorescence properties of [III] and [VI] were apparently different: The former gave a blue fluorescence and the latter a green fluorescence in the blank solution of the proposed procedure\(^{1}\). As is shown in Fig. 2, the maximum fluorescence was obtained with excitation at 382 nm and emission at 432 nm for [III]. This maximum was obtained with excitation at 393 nm and emission at 468 nm for [VI]. Further, the fluorescence intensity of [VI] was very weak in comparison with that of [III] or of [B]; the intensity was only 2.8% against [III] or [B]. In TLC experiment, the quantitative reaction solution of GPE gave a single blue fluorescent spot with \(R_f\) values of 0.33(solvent system a) and 0.40(solvent system b), this spot was the same as those of [III] and [B]. [VI] showed a green fluorescent spot with \(R_f\) values of 0.23(a) and 0.27(b).
On the basis of the above results, the final fluorophore of the reaction of common epoxides with nicotinamide and acetophenone could be confirmed as 2,7-naphthyridine derivatives, but not its 1,6-isomer. Furthermore, the fluorophores obtained by the reaction of quarternary pyridinium derivatives of nicotinamide with active methylenes\(^2\)\(^3\) and obtained by Nelis's method\(^4\) for some epoxides were also found to be 2,7-naphthyridine derivatives on the basis of the characteristics of those fluorescence spectra.

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REFERENCES
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Keyword phrases
fluorophore in the reaction of epoxides with nicotinamide and acetophenone; synthesis of 2,7- and 1,6-naphthyridine fluorophores for the elucidation of the reaction mechanism; fluorescent properties of naphthyridines.

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