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NOTES

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 exsists 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)

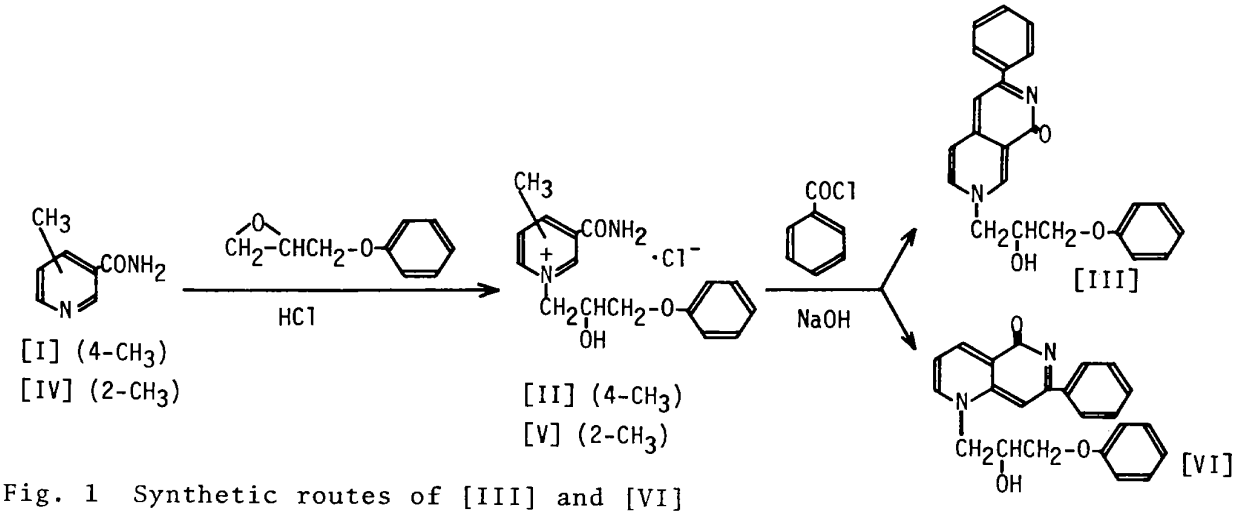

Fig. 1 Synthetic routes of [III] and [VI]

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][6] 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.

[II]: Mp. 166-168°(dec; uncorr.). Anal. Calcd for $C_{16}H_{19}N_2O_3Cl$: C, 59.54; H, 5.93; N, 8.68. Found: C, 59.52; H, 5.98; N, 8.74. EI-Mass m/z: 287(M^+-Cl, $C_{16}H_{19}N_2O_3$, Found 287.1397, Calcd 287.1395).

Preparation of compound [VI]

3-Carbamoyl-2-methyl-1-(2-hydroxy-3-phenoxypropyl)pyridinium chloride [V](970 mg), obtained from 2-methylnicotinamide [IV][7] 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%).

[V]: Mp. 149-151°(dec). Anal. Calcd for $C_{16}H_{19}N_2O_3Cl$: C, 59.54; H, 5.93; N, 8.68. Found: C, 59.51; H, 5.95; N, 8.73. EI-Mass m/z: 287(M^+-Cl, $C_{16}H_{19}N_2O_3$, Found 287.1412, Calcd 287.1395).

[VI]: Mp. 219-221°(dec). Anal. Calcd for $C_{23}H_{20}N_2O_3 \cdot H_2O$: C, 70.75; H, 5.68; N, 7.17. Found: C, 70.78; H, 5.66; N, 6.72. IR(KBr, cm^{-1}): 1635($\nu_{C=O}$), 1247 (ν_{C-O-C}). UV λ_{max}^{EtOH} nm(log ε): 244(4.10), 301(4.29). FD-Mass m/z: 373(M^+-H_2O+1, $C_{23}H_{21}N_2O_3$), 372(M^+-H_2O, $C_{23}H_{20}N_2O_3$), 236(M^+-$C_8H_{11}O_3$, $C_{15}H_{11}N_2O$), 222(M^+-$C_9H_{12}O_3$, $C_{14}H_{10}N_2O$). ^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). ^{13}C NMR δ (ppm): 61.936(t, splitting in ^1H-off

resonance decoupling measurement), 69.489(t), 70.998(d), 97.071(d), 116.076(d), 124.165(d), 124.750(d), 125.576(s), 128.984(d), 131.861(d), 132.055(d), 132.788(s), 135.416(d), 149.014(d), 150.721(s), 154.277(d), 155.641(s), 159.056(s), 163.095(s).

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 water of crystallization, was found to have a molecular formula of $C_{23}H_{20}N_2O_3 \cdot H_2O$ 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[8]. The ^{13}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 ^1H NMR spectral pattern on the heteroaromatic ring protons, namely, a triplet at δ 7.96(J=6.0 and 7.4 Hz) and two doublet peaks at δ 9.16 (J=6.0 Hz) and 9.44(J=7.4 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 Rf 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 Rf values of 0.23(a) and 0.27(b).

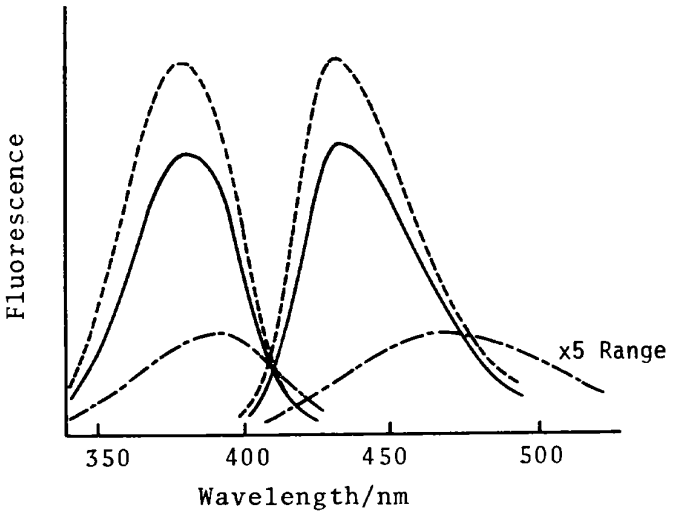

Fig. 2 Excitation and emission spectra of [B], [III], [VI] and reaction product of GPE

——GPE; -------[B] and [III]; —·—[VI]
Concentration: 3 nmol/tube.
[B], [III], and [VI]: measured in the blank solution obtained by the proposed procedure[1].

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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Keyword phrases

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