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Synthesis and Cytotoxic Activities of a New Benzo[*c*]phenanthridine Alkaloid, 7-Hydroxynitidine, and Some 9-Oxygenated Benzo[*c*]phenanthridine Derivatives

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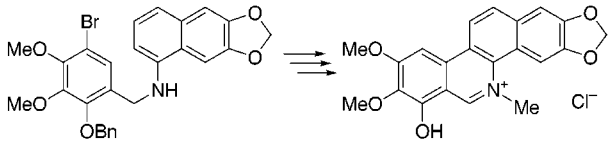
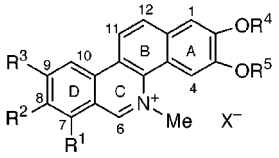
ABSTRACT



A new benzo[*c*]phenanthridine alkaloid, 7-hydroxynitidine, was synthesized by a novel synthetic procedure. The cytotoxic activity of this compound against HeLa S3 cells was strong, but not greater than those of its mother compounds, nitidine and NK109. We also synthesized other 9-oxygenated benzo[*c*]phenanthridine alkaloids, 7-methoxynitidine, 9-demethylnitidine, nitidine, and fagaronine, and tested their cytotoxic activities. These results suggest that the 7-hydroxy group enhances antitumor activity and an 8- or 9-hydroxy group weakens this activity.

Quaternary benzo[*c*]phenanthridiniums are widely known alkaloids with some antitumor activities.[1] Among them, nitidine (**1a**) and fagaronine (**1b**) have shown the most promise as practical antitumor drugs and were the subject of preclinical studies at the National Cancer Institute in the 1970s.[2] In 1992, we reported that NK109 (**1c**) had antitumor activity[3] that was superior to those of **1a** and **1b**.[4] We are now conducting clinical evaluations of NK109 in Japan. The superior activity of NK109 is due to its structure under biological conditions; i.e., NK109 exists as a resonance hybrid due to the loss of its acid moiety (Scheme 1). The resonance hybrid retains molecular planarity and a cationic property, both of which are essential for the expression of antitumor activity. This structural property of NK109 is derived from the hydroxy group at the 7-position. We were interested in a compound with both NK109 and nitidine substituents and therefore investigated a novel procedure for synthesizing a new benzo[*c*]phenanthridine alkaloid, 7-hydroxynitidine (**1d**, Figure 1). Since our procedure can be



nitidine (**1a**)
fagaronine (**1b**)
NK109 (**1c**)
7-hydroxynitidine (**1d**)
7-methoxynitidine (**1e**)
9-demethylnitidine (**1f**)

Figure 1. Structures of benzo[*c*]phenanthridine alkaloids.

(1) Suffness, M.; Cordell, G. A. *The Alkaloids*; Academic: New York, 1985; Vol. 25, pp 178−189.
(2) (a) Cordell, G. A.; Farnsworth, N. R. *Heterocycles* **1976**, *4*, 393−427. (b) Phillips, S. D.; Castle, R. N. *J. Heterocycl. Chem.* **1981**, *18*, 223−232.
(3) Hanaoka, M.; Ekimoto, H.; Kobayashi, M. F.; Irie, Y.; Takahashi, K.*Chem. Abstr.* **1992**, *116*, 718d.
(4) Nakanishi, T.; Suzuki, M.; Saimoto, A.; Kabasawa, T. *J. Nat. Prod.* **1999**, *62*, 864−867.

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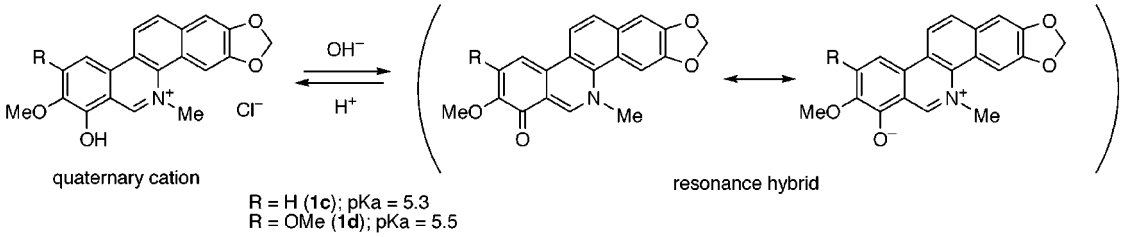
Scheme 1. Equilibrium of 7-Hydroxybenzo[*c*]phenanthridine Alkaloids



applied to other 9-oxygenated derivatives, we also synthesized 7-methoxynitidine (**1e**), 9-demethylnitidine (**1f**), and the natural benzo[*c*]phenanthridine alkaloids **1a** and **1b**. In this paper, we report the synthetic investigation and the cytotoxic activity of these compounds (Table 1).

Table 1. Substituent Patterns[a]

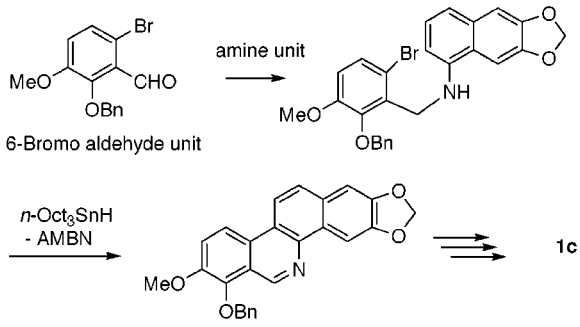
	R^1	R^2	R^3	R^4	R^5
a	H	OMe	OMe	-CH_2-	
b	H	OMe	OMe	H	Me
c	OH	OMe	H	-CH_2-	
d	OH	OMe	OMe	-CH_2-	
e	OMe	OMe	OMe	-CH_2-	
f	H	OMe	OH	-CH_2-	
g	OBzl	OMe	OMe	-CH_2-	
h	H	OMe	OBzl	-CH_2-	
i	H	OMe	OMe	^{i}Pr	Me

[a] This pattern is applied to all compounds.

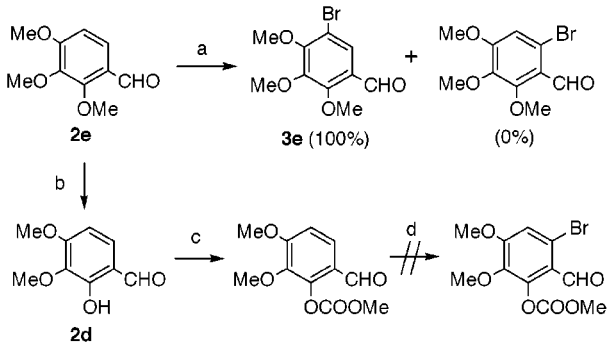
7,8,9-Trialkoxybenzo[*c*]phenanthridine alkaloids have never been isolated from plants and have only been reported by Ishii et al. as a synthetic compound, 7-methoxynitidine (**1e**).[5] Since it is difficult to apply their method to our target compound **1d**, we decided to develop a procedure for synthesizing **1d**.

We previously established the synthetic procedure for NK109 outlined in Scheme 2.[6] We initially attempted to obtain a corresponding 6-bromoaldehyde unit for the synthesis of **1d** (Scheme 3). When 2,3,4-trimethoxybenzaldehyde (**2e**) was reacted with bromine, it was brominated at the 5-position, rather than 6-position, to yield **3e**. To change the reaction site, we introduced an electron-withdrawing group at the 2-position. First, by treatment with lithium chloride,[7] **2e** was demethylated selectively to give 2-hydroxy-3,4-dimethoxybenzaldehyde **2d**. After phenolic hydroxide was protected by methyl carbonate, it was treated with bromine. However, no brominated compound was obtained and the starting material was recovered. Bromination also did not proceed when the temperature was increased to 80 °C. Thus, bromination at the 6-position of 2,3,4-trialkoxy aldehyde was very difficult. We could not obtain a precursor for the radical cyclization and did not continue the synthesis by this route.

Scheme 2. Synthetic Procedure of NK109 (**1c**)



Scheme 3. Attempt To Synthesize the 6-Bromoaldehyde Unit[a]



[a] Reagents and yield: (a) Br_2, NaOAc, AcOH, 50 °C; (b) LiCl, DMSO, 110 °C, 67%; (c) MeOCOCl, Et_3N, toluene, rt, 98%; (d) Br_2, NaOAc, AcOH, 50−80 °C.

As noted above, **2e** was easily brominated to **3e**. We decided to use **3e** as a new aldehyde unit (Scheme 4). Accordingly, to obtain the aldehyde unit for **1d**, phenolic benzaldehyde **2d** was protected by a benzyl group. The resulting trialkoxybenzaldehyde **2g** was converted into the aldehyde unit **3g** (Scheme 5). Synthesis of the aldehyde units for **1a**, **1b**, and **1f** was much simpler. 5-Bromo-4-hydroxy-

(5) Ishii, H.; Ichikawa, Y.; Kawanabe, E.; Ishikawa, M.; Ishikawa, T.; Kuretanim K.; Inomata, M.; Hoshi, A. *Chem. Pharm. Bull.* **1985**, *33*, 4139−4151.
(6) Nakanishi, T.; Suzuki, M.; Mashiba, A.; Ishikawa, K.; Yokotsuka, T. *J. Org. Chem.* **1998**, *63*, 4235−4239.
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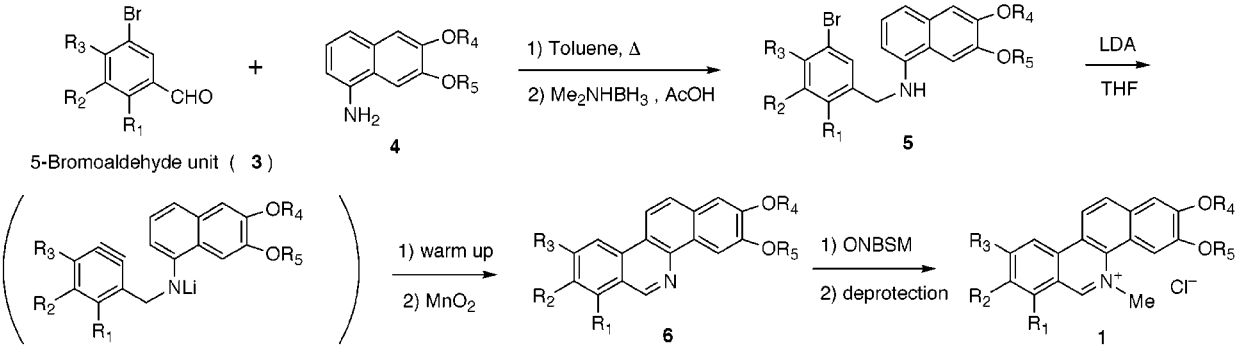
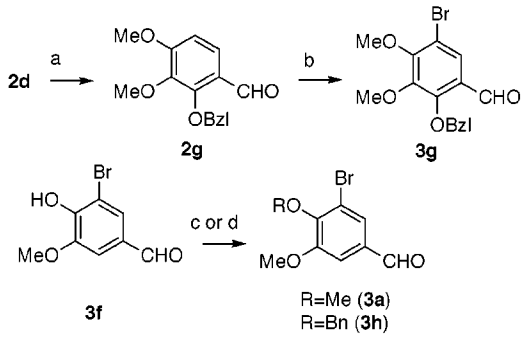
(8) (a) Stermitz, F. R.; Gillespie, J. P.; Amoros, L. G.; Romero, R.; Stermitz, T. A. *J. Med. Chem.* **1975**, *18*, 708−713. (b) Clark, J. H.; Holland, H. L.; Miller, J. M. *Tetrahedron Lett.* **1976**, *41*, 3361−3364.

Scheme 4. Synthetic Procedure of 9-Oxygenated Benzo[*c*]phenanthridine Alkaloids



3-methoxybenzaldehyde **3f** is readily available from commercial suppliers, and **3a** and **3h** were obtained by methylation and benzylation of **3f**, respectively.

Scheme 5. Synthesis of 5-Bromoaldehyde Units[a]



[a] Reagents and yield: (a) BzlCl, KI, K_2CO_3, MeOH, 80 °C, 98%; (b) Br_2, NaOAc, AcOH, 50 °C, 93%; (c) MeI, K_2CO_3, DMF, 50 °C, 100%; (d) BzlBr, K_2CO_3, DMF, 50 °C, 94%.

The aldehyde unit (**3**) and amine units (**4**), which were derived from 2,3-dihydroxynaphthalene,[8] were condensed in toluene to Schiff base. The imine portion of the Schiff base was reduced to amine with dimethylamineborane[9] to give the key intermediate **5** in good yield (Table 2).

Table 2. Yield of Each Process

starting materials		product (yield, %)		
3	**4**	**5**	**6**	**1**
3a	**4a**	**5a** (76)	**6a** (17)	**1a** (55)
3a	**4i**	**5i** (58)	**6i** (74)	**1i** (65), **1b** (92)
3g	**4a**	**5g** (79)	**6g** (17)	**1g** (82), **1d** (99)
3e	**4a**	**5** (83)	**6e** (29)	**1e** (77)
3h	**4a**	**5** (70)	**6h** (47)	**1h** (66), **1f** (92)

We previously examined the cyclization of the precursor of NK109 with LDA.[6] This reaction proceeds via benzyne as an intermediate. Accordingly, it is reasonable to use 5-bromobenzylamine **5** as a precursor instead of 6-bromobenzylamine (precursor of the radical cyclization). In this study, **5** was lithiated at −78 °C. This intermediate was allowed to stand at room temperature to give a cyclized product. Subsequent oxidization with manganese dioxide gave benzo[*c*]phenanthridine **6**. The resulting reaction mixture contained many unknown byproducts, and the yield of **6** was 17−47%. However, with **5i**, the yield of cyclization reached 74%. Since **6i** was precipitated from the reaction medium, undesirable reactions did not proceed and **6i** was obtained in good yield.

We also attempted cyclization of **5** by a radical-mediated reaction. Although **5c** was treated with *n*-Oct_3SnH and azobis(2-methylbutyronitrile),[6] no cyclized product was obtained. The product was mainly a 5-debromo compound. Thus, **5** was not available for radical cyclization; this supported the notion that the reaction with LDA proceeded via benzyne as an intermediate.

Benzo[*c*]phenanthridine **6** was methylated with methyl 2-nitrobenzenesulfonate. The resulting product was neutralized with sodium hydroxide to remove acid residue. After purification, it was acidified with dilute hydrochloric acid to give **1a**, **1e**, and **1g**−**i**. Finally, the benzyl or isopropyl group was deprotected with concentrated hydrochloric acid to give **1b**, **1d**, and **1f**.

A growth inhibition assay against HeLa S3 cells was performed as described previously.[4] Table 3 shows the cytotoxic activities of some phenolic benzo[*c*]phenanthridine

Table 3. Cytotoxic Activities of Several Benzo[*c*]phenanthridine Alkaloids against HeLa S3

phenolic alkaloid	(IC_{50}, μg/mL)	methylated alkaloid	(IC_{50}, μg/mL)
1d	0.23	**1e**	0.82
1f	>10	**1a**	0.05
fagaridine[a]	3.99	chelerythrine[b]	1.24
1c	0.08	chelerythrine[b]	1.24
1b	0.39		

[a] Synthesized in our laboratory (R^1 = OMe, R^2 = OH, R^3 = H, $R^4 + R^5$ = -CH_2-).[11] [b] Perchased from Sigma Chemical Company ($R^1 = R^2$ = OMe, R^3 = H, $R^4 + R^5$ = -CH_2-).

alkaloids compared with those of the corresponding methylated derivatives. The derivative with a hydroxy group at the 9-position had no activity (**1f**), and that with 8-OH had merely weaker activity (fagaridine). On the other hand, that with 7-OH showed stronger activity (**1c**). Compound **1c** has been reported to possess a unique property in its structure due to its 7-OH,[4] and the cytotoxic activity of **1d** is greater than that of **1e**. These facts suggest that the effect of the 7-hydroxide of **1d** is similar to that of **1c**. We measured UV spectra of **1d** under several pH conditions and observed some isosbestic points. The results show that **1d** is in acid−base equilibrium, the same as **1c** (Scheme 1).[10] We determined the pK_a value of **1d** (5.5) by an absorbance method. On the other hand, no isosbestic point was observed in UV spectra of phenolic alkaloid **1f**. This suggests that **1f** dose not exhibit acid−base equilibrium like **1c** and **1d**. This could explain why **1f** had no cytotoxic activity.

In conclusion, we have established a new procedure for synthesizing 7,8,9-trioxygenated-benzo[*c*]phenanthridiniums, which have not yet been isolated from plants. This procedure is very useful, since the aldehyde units are readily available; they were easily obtained either by bromination of the corresponding benzaldehyde or by alkylation of a 5-bromo aldehyde obtained from commercial sources. The benzo[*c*]-phenanthridine rings were constructed via benzyne as intermediates which were produced by treatment of substituted benzylamine with LDA. The yield of this reaction was about 20%, while the yield of **6i**, an intermediate of fagaronine, was excellent. Accordingly, our procedure may also be a practical approach to fagaronine. Subsequently, we tested the cytotoxicity of our synthetic benzo[*c*]phenanthridiniums **1d**−**f** and confirmed the effect of the 7-hydroxide on the antitumor activity of benzo[*c*]phenanthridiniums. While **1d** showed strong antitumor activity, it was not greater than those of its mother compounds nitidine and NK109.

Supporting Information Available: Synthetic procedures for all compounds and ^{1}H NMR and HPLC charts of evaluated compounds **1a**−**f**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Billman, J. H.; McDowell, J. W. *J. Org. Chem.* **1961**, *26*, 1437−1440.

(10) NMR data of **1d**: quaternary cation, ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H), 4.23 (s, 3H), 4.86 (s, 3H), 6.34 (s, 2H), 7.75 (s, 1H), 7.96 (s, 1H), 8.25 (d, 1H, J = 9.1 Hz), 8.27 (s, 1H), 8.83 (d, 1H, J = 9.1 Hz), 9.85 (s, 1H), 11.65−11.80 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 51.7, 57.9, 61.5, 96.9, 103.2, 104.9, 106.1, 112.3, 119.9, 120.5, 124.3, 130.3, 133.1, 133.2, 133.3, 136.6, 148.9, 149.2, 149.4, 150.4, 163.2; resonance hybrid, ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 3H), 4.01 (s, 3H), 4.46 (s, 3H), 6.26 (s, 2H), 7.03 (s, 1H), 7.59 (s, 1H), 7.94 (d, 1H, J = 9.0 Hz), 8.03 (s, 1H), 8.42 (d, 1H, J = 9.0 Hz), 8.99 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 48.2, 56.0, 58.4, 88.3, 102.0, 103.4, 105.1, 117.2, 119.3, 120.1, 123.6, 127.1, 130.9, 131.7, 132.6, 137.7, 147.5, 147.7, 148.2, 161.0, 168.8.

(11) Nakanishi, T.; Suzuki, M. *J. Nat. Prod.* **1998**, *61*, 1263−1267.