

RECENT ADVANCES ON ANTITUMOR-ACTIVE BENZO[C]PHENANTHRIDINE ALKALOIDS

Tsutomu Ishikawa* and the late Hisashi Ishii¹

Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi, Inage, Chiba
263-8522, Japan

Abstract-Recent advances on antitumor-active benzo[c]phenanthridine alkaloids mainly focused on our synthetic works are described.

1. Introduction

Benzo[c]phenanthridine alkaloids are distributed in Papaveraceous and Rutaceous plants.² They have long history, in which the first report³ appeared in 1839. Over a century later nitidine (**1**) and fagaronine (**2**), a nitidine (**1**) type O₄-bases isolated from Rutaceous plants, attracted much attention because of their antitumor-activities.⁴ Although serious cytotoxicities made them unsuccessful drugs, fagaridine (**3**), a chelerythrine (**5**) type O₄-base, is at present under examination in clinical level.⁵ (See Figure 1) This paper describes recent advances in the last decade on fully aromatized benzo[c]phenanthridine alkaloids mainly focused on our synthetic works.

2. Classification

Benzo[c]phenanthridine alkaloids are structurally classified into five categories based on both their oxidation level and the number of oxygen functionalities as shown in Figure 1. It must be noted that antitumor-activities have been shown only in the fully aromatized O₄-bases and that all of alkaloids obtained from Rutaceous plants are limited to the fully aromatized O₄-bases.

3. Biosynthesis

Benzo[c]phenanthridine alkaloids are biosynthesized from tyrosine through benzyloisoquinoline, tetrahydroprotoberberine, and protopine alkaloids.⁶ The pathway to sanguinarine (**4**) (a chelerythrine (**5**) type O₄-base), chelirubine (**6**) (a O₅-base), and macarpine (**7**) (a O₆-base) is shown in Scheme 1.

4. Synthesis

Many synthetic approaches to a benzo[c]phenanthridine skeleton have been reported.^{2a,2b} Although the most common route involves the construction of either ring B or C in the final step, nine routes are formally accessible in the methodology for the bond connections. (Figure 2) According to this methodology, selected recent synthetic works will be described. Among them no approaches through the bond

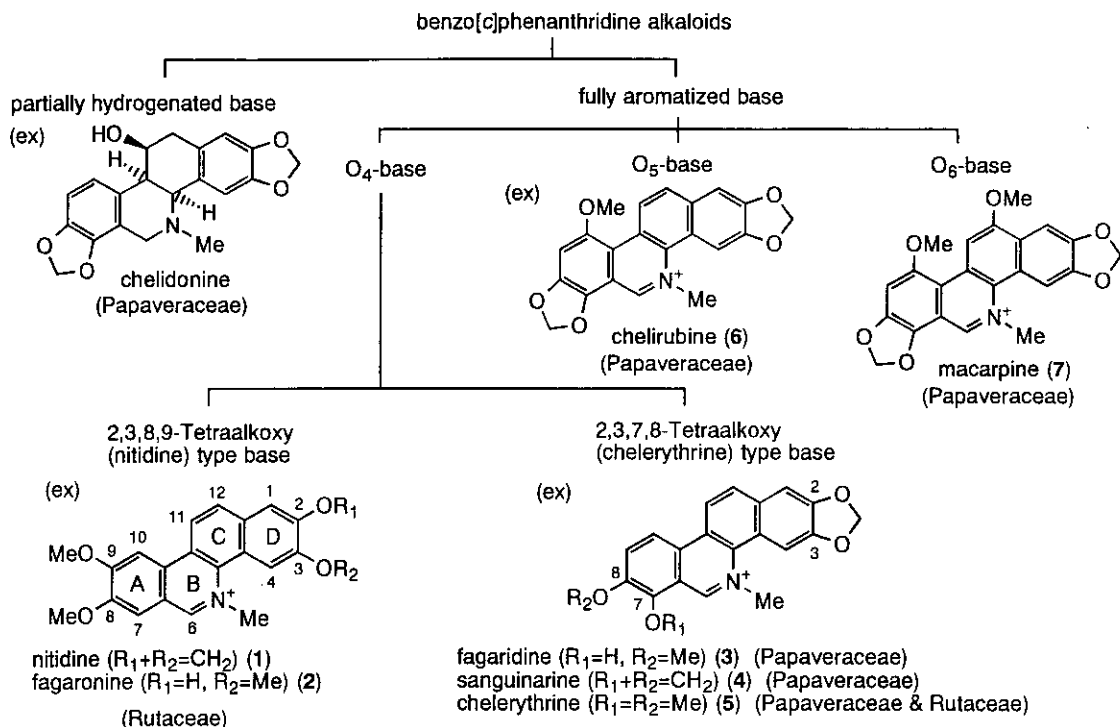
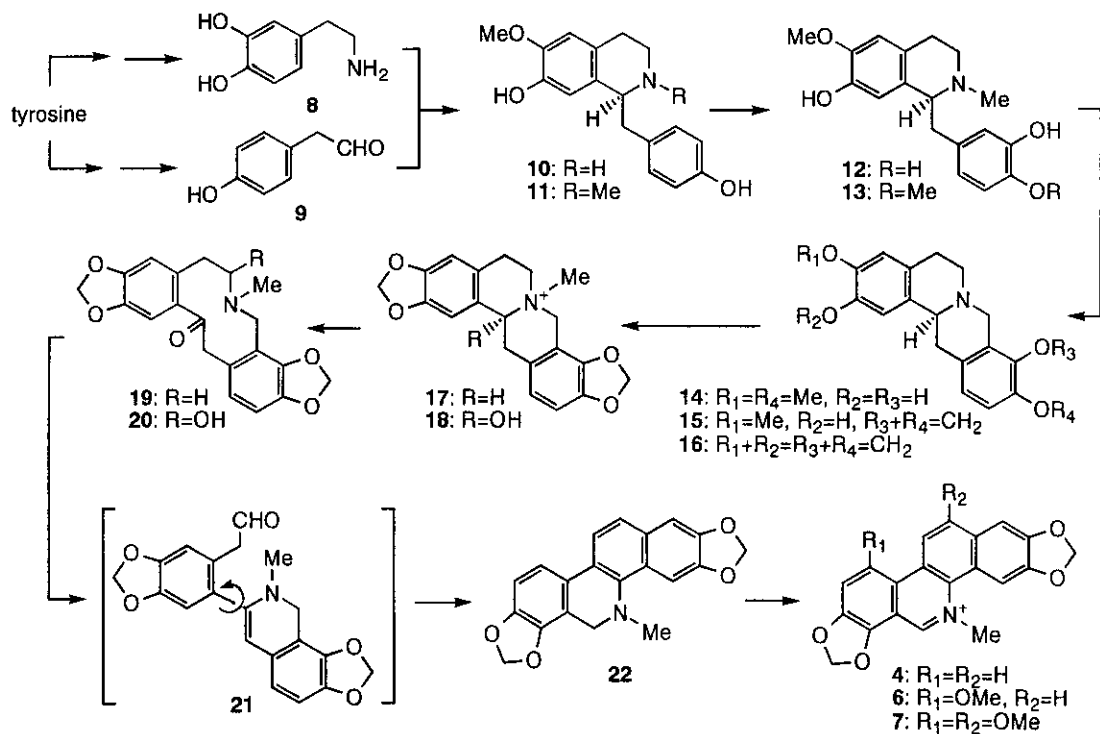


Figure 1 Classification of benzo[*c*]phenanthridine alkaloids



Scheme 1 A biosynthetic pathway for sanguinarine (4), chelirubine (6), and macarpine (7)

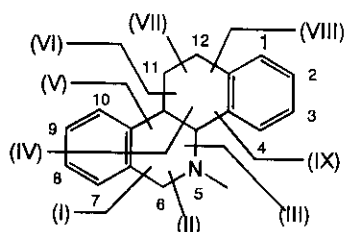
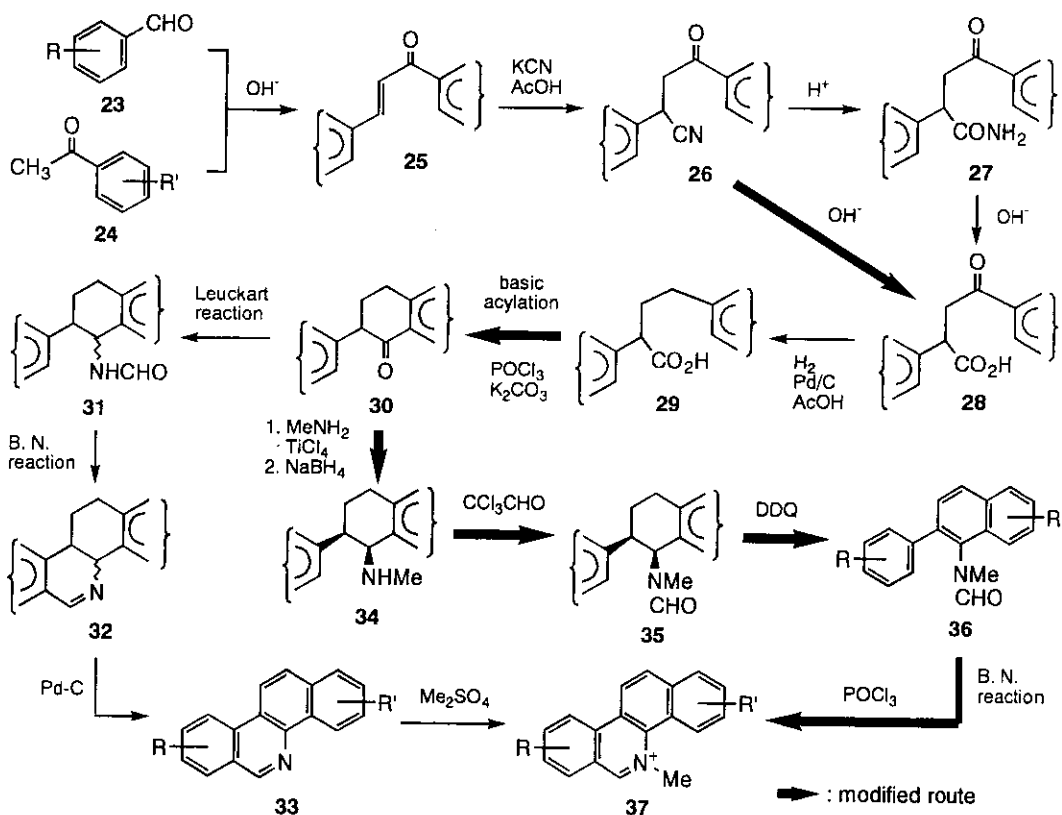


Figure 2 Nine possible bond connections

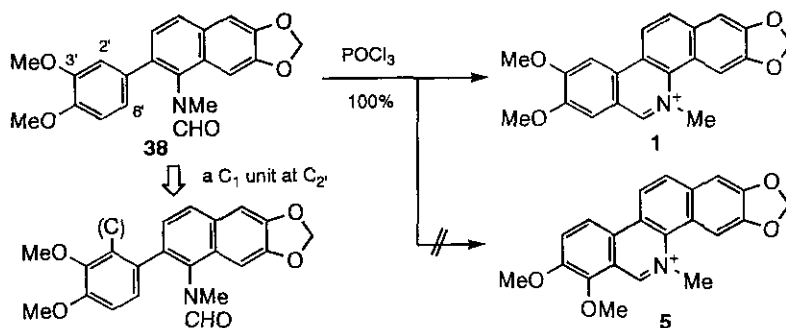
connections between $C_{4b}-N_5$ (III), $C_{11}-C_{12}$ (VII), and $C_{4a}-C_{4b}$ (IX) appeared, in spite of known routes^{2a} such as photocyclization, Pschorr reaction, and cyclization of isocarbostryls in the strategy for the last bond connection IX.

4-1. Bond connection between C_6-C_{6a} (I)

The most traditional synthetic route, which had been developed by Robinson *et al.*,⁷ for the bond connection between C_6-C_{6a} (I) is based on Bischler-Napieralski (B. N.) isoquinoline construction. We have established a general synthetic method for the fully aromatized quaternary benzo[*c*]phenanthridine bases by modification of the Robinson's method, one of key reactions in which is direct cyclization of aromatic *N*-methylnaphthylformamides (**36**), prepared from tetralones (**30**) by successive reactions of reductive



Scheme 2 Robinson's and the modified routes

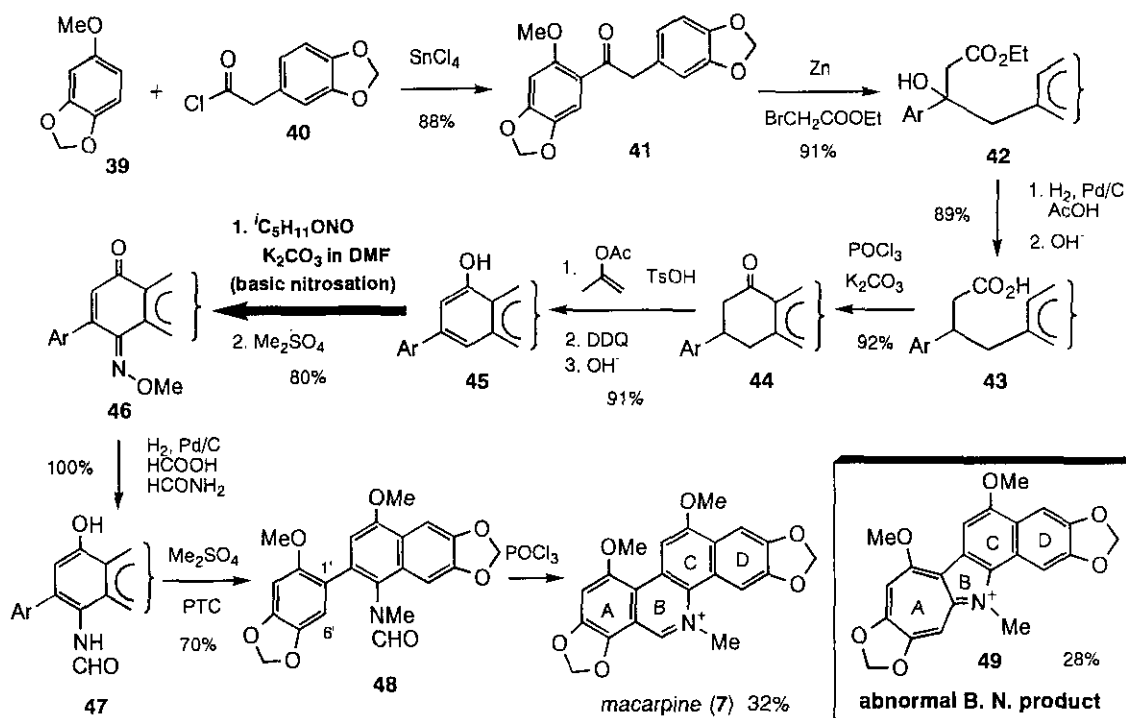


Scheme 3 Limitation of B. N. reaction of the aromatic formamide (38)

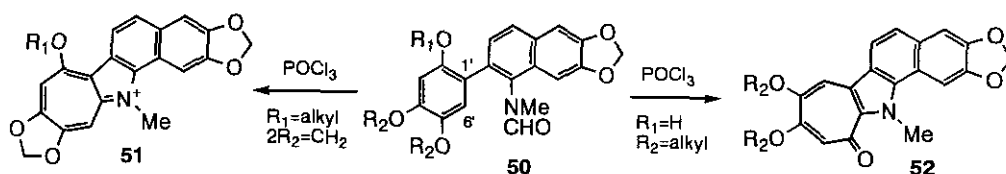
amination, formylation, and dehydrogenation, to quaternary bases.⁸ (Scheme 2) Many kinds of O₄- and O₅-bases were prepared by application of this general method.

However, when 2-(3,4-dialkoxyphenyl)-N-methylnaphthylformamides were used as the starting materials for O₄-bases, cyclization exclusively occurred at the *para* position (6') to the 3'-alkoxy group to afford 8,9-dialkoxy bases as sole products. (*para*-orientation) Thus, the 2-(3,4-dimethoxyphenyl)naphthylformamide (38) yielded nitidine (1), but not chelerythrine (5). (Scheme 3) Therefore, introduction of a C₁ unit to the *ortho* position (C₂) before the cyclization should be needed for preparation of 5 from 38. (See 4-2)

On the other hand, macarpine (7), a O₆-base, was synthesized by an alternative strategy based on B. N. reaction.⁹ (Scheme 4) In the course of this synthetic work some new findings were observed.



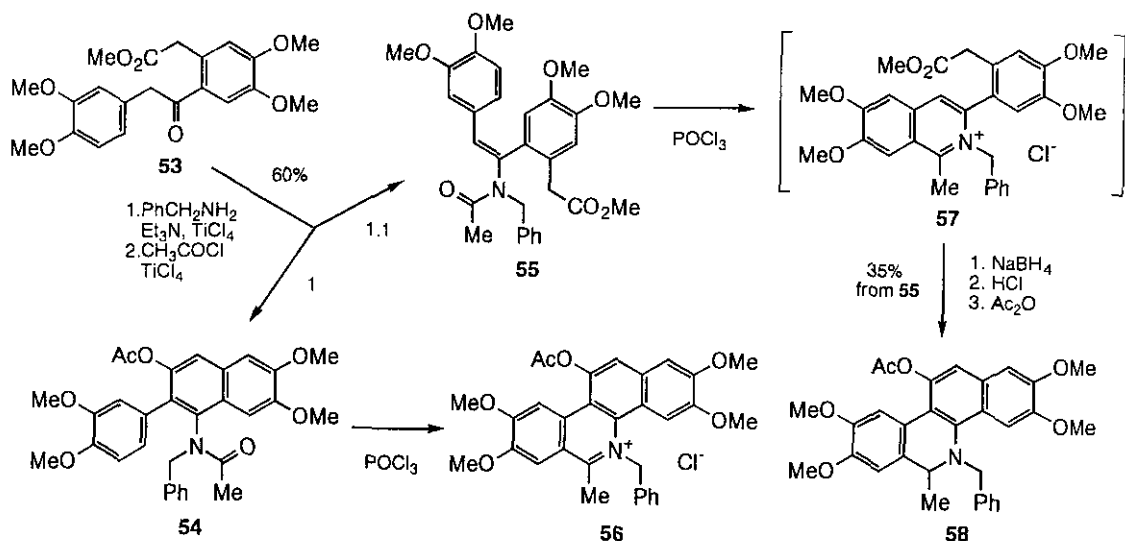
Scheme 4 Synthesis of macarpine (7)



Scheme 5 Direction of cyclization on abnormal B. N. reaction

Nitrosation of the naphthol (45) under mildly basic condition (${}^i\text{C}_5\text{H}_{11}\text{ONO}-\text{K}_2\text{CO}_3$ in DMF) led to regioselective introduction of a nitrogen function to the *para* position of the hydroxyl group, giving the quinone monoxime methyl ether (46) after methylation. The basic nitrosation¹⁰ with *para* selectivity was found to be applicable to a variety of phenol derivatives. Furthermore, in the final B. N. reaction abnormal cyclization (carbon insertion reaction into benzene ring)¹¹ occurred to produce an azoazulene skeleton (49) with a 7-5 ring system (rings A-B), together with macarpine (7), an expected cyclized product. Precise examination using various 2-phenylnaphthylformamides (50) as substrates indicated that the substitution pattern of oxygen functions on the 2-phenyl substituent restricts reaction paths to normal and/or abnormal cyclizations. The azoazulene products were given only when the naphthylformamides with a 2-alkoxy-4,5-methylenedioxyphenyl (50: $R_1 = \text{alkyl}$, $2R_2 = \text{CH}_2$) or 2-hydroxy-4,5-dialkoxyphenyl (50: $R_1 = \text{H}$, $R_2 = \text{alkyl}$) group at the 2 position were treated under the condition of B. N. reaction. Interestingly, the direction of carbon insertion could be dependent upon the character of the oxygen function on the 2 position (C_2) of 2-phenyl group.¹² (Scheme 5)

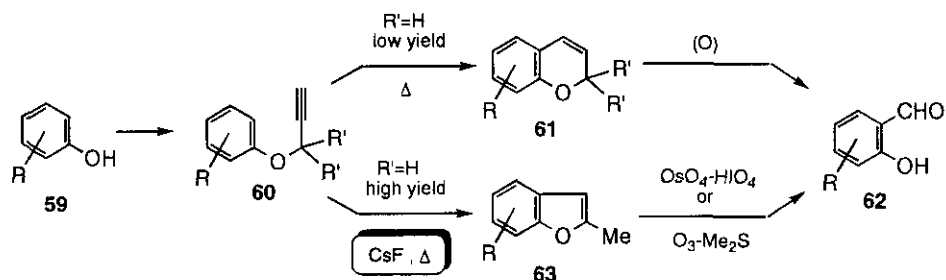
In the literature¹³ the 11-acetoxy-*N*-benzylbenzo[*c*]phenanthridine (56) was synthesized by the B. N. reaction of the corresponding naphthylamide (54) (bond connection I). The amide (54) was prepared by action of benzylamine to the benzyl phenyl ketone (53). (Scheme 6) The dihydrobenzo[*c*]phenanthridine (58) was also given by treatment of the stilbenamine (55), a side product in the synthesis of the starting

Scheme 6 The reported method¹³ through the bond connections I and IV

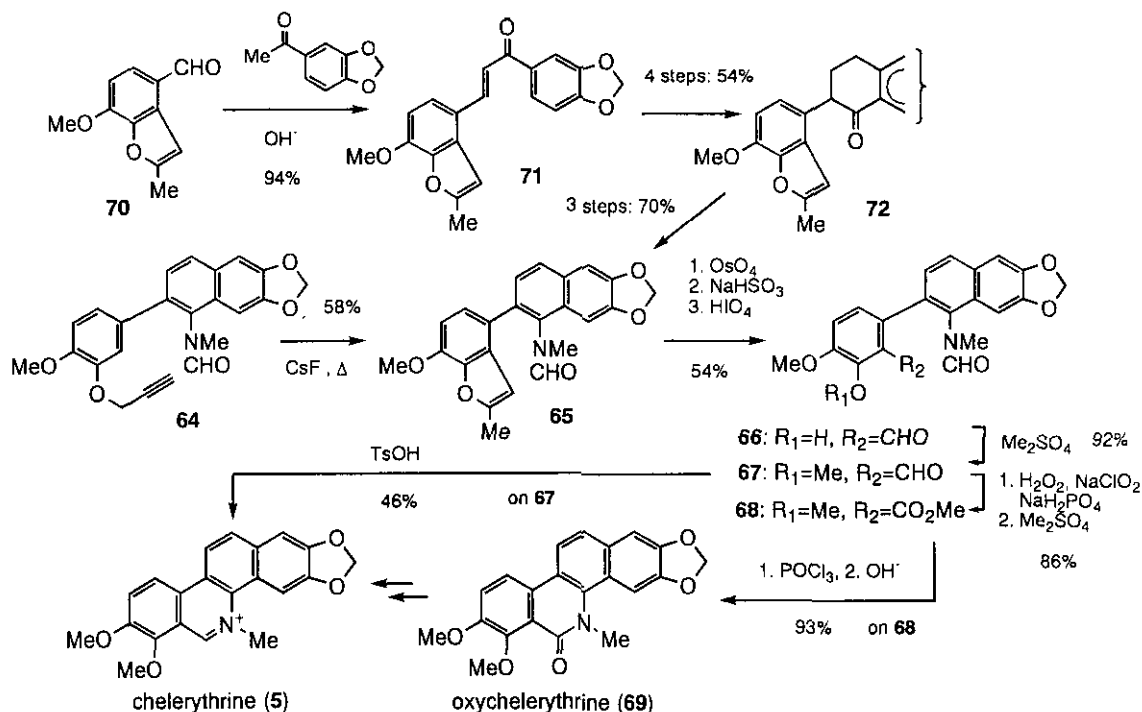
54, under the condition of B. N. reaction followed by reduction, in which a benzo[*c*]phenanthridine skeleton was constructed through the bond connection between C_{10b}-C₁₁ (VI) (See 4-3).

4-2. Bond connection between N₅-C₆ (II)

In our synthetic strategy for chelerythrine (5) type alkaloids using 2-phenyl-1-naphthylformamides, the ring construction could be achieved through the bond connection between N₅-C₆ (II). For the successful reaction the naphthylformamides with a 3,4-dialkoxy-2-substituted phenyl group at the 2 position should be effectively prepared. It is known that the Claisen rearrangement¹⁴ of aryl propargyl ethers afforded arylpyrans, which could be converted into salicylaldehyde derivatives by the bond cleavage of the double bond in the pyran ring. Thus, we examined Claisen rearrangement using primary and tertiary propargyl ethers. Heating primary ethers (60) (R' = H) in diethylaniline (thermal Claisen rearrangement) resulted in



Scheme 7 Aryl propargyl ethers : Potential precursors for salicylaldehydes ?



Scheme 8 Synthesis of chelerythrine (5) and oxychelerythrine (69)

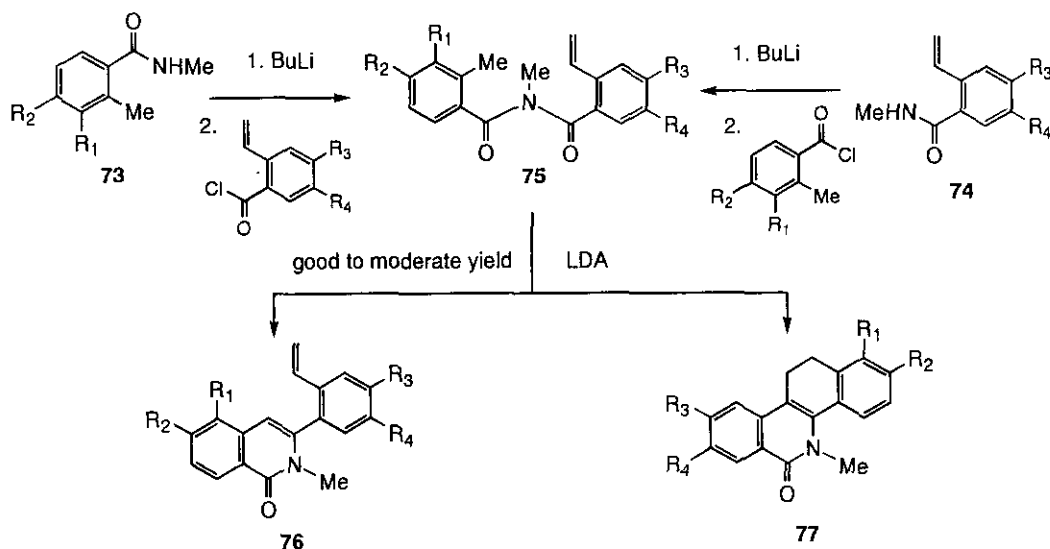
ineffective production of arylpyrans (**61**) ($R'=H$), whereas tertiary ethers (**60**) ($R'=Me$) smoothly afforded cyclized products (**61**) ($R'=Me$) under the same condition. However, more difficult preparation of the tertiary ethers than the primary ethers made us investigate the Claisen rearrangement using primary ethers (**60**) ($R'=H$) under various conditions in details.

After several trials, we succeeded in developing exclusive cyclization to an isomeric 2-methylarylfurans (**63**), but not arylpyran (**61**) ($R'=H$), when the Claisen rearrangement was subjected in the presence of cesium fluoride (CsF) (the CsF-mediated Claisen rearrangement). Treatment of the formed arylfurans (**63**) with a combination of either osmium tetroxide-periodic acid or ozone-dimethyl sulfide gave an intended salicylaldehyde derivatives (**62**). Thus, a new method for the effective introduction of a C_1 unit to the *ortho* position of a phenolic function was explored.¹⁵ (Scheme 7)

Application of the newly developed method to the phenyl propargyl ether (**64**) gave the aldehyde (**67**) after methylation, which was transformed into chelerythrine (**5**) in moderate yield under acidic condition.¹⁶ Improvement of the cyclization step was achieved by treatment of the 2-(3,4-dimethoxy-2-methoxycarbonylphenyl)-1-naphthylformamide (**68**) under the condition of Vilsmeier-Haack reaction ($POCl_3$ -1,3-dimethoxybenzene), giving oxychelerythrine (**69**) in nearly quantitative yield.¹⁷ (Scheme 8) The benzofuranyl naphthylformamide (**65**) was also easily accessible from 4-formyl-7-methoxy-2-methylbenzofuran (**70**), derived from isovanillin,¹⁶ as shown in Scheme 8. Thus, the 2-methylfuran moiety in a benzofuran skeleton was found to act as not only a source of a C_1 unit but also the protecting group of a phenolic function.

4-3. Bond connection between C_{4b} - C_{10b} (IV)

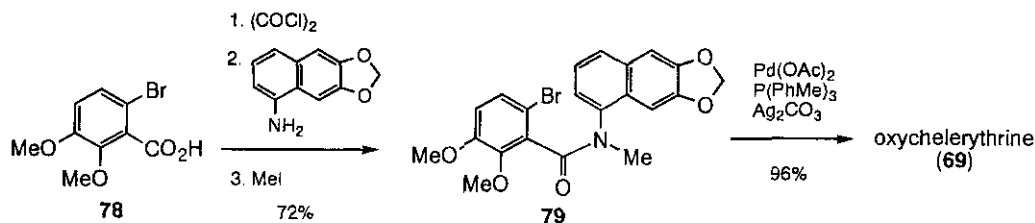
One step construction¹⁸ of the B-C ring of a benzo[*c*]phenanthridine base through the bond connection between C_{4b} - C_{10b} (IV) was reported in the base-induced intramolecular cyclization of the *N*-(2-ethenylbenzoyl)-*N*, 2-dimethylbenzamide derivative **75**. (Scheme 9)



Scheme 9 The reported method¹⁸ through the bond connection IV

4-4. Bond connection between C_{10a}-C_{10b} (V)

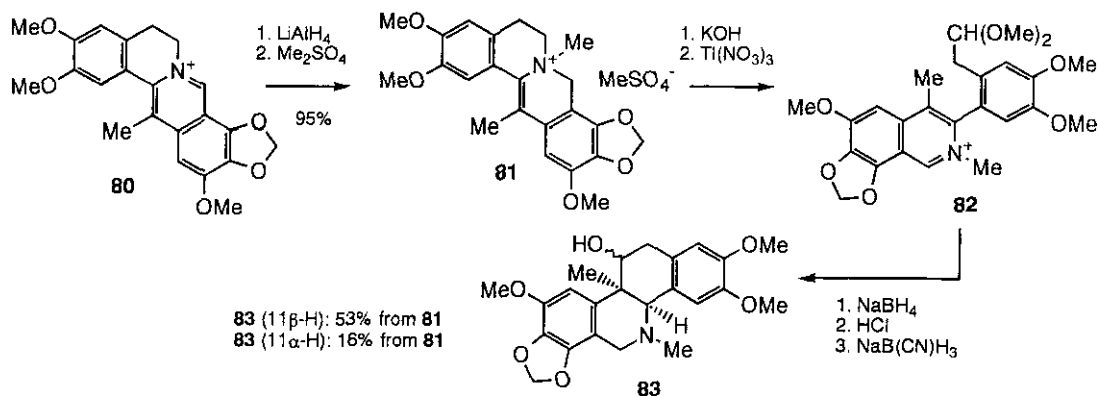
Cyclizations using enamides or benzynes were known as the bond connection between C_{10a}-C_{10b} (V) in traditional methods.^{2a} Recently palladium-catalyzed intramolecular coupling reaction¹⁹ was skilfully applied to this bond formation by Harayama *et al.* (Scheme 10)



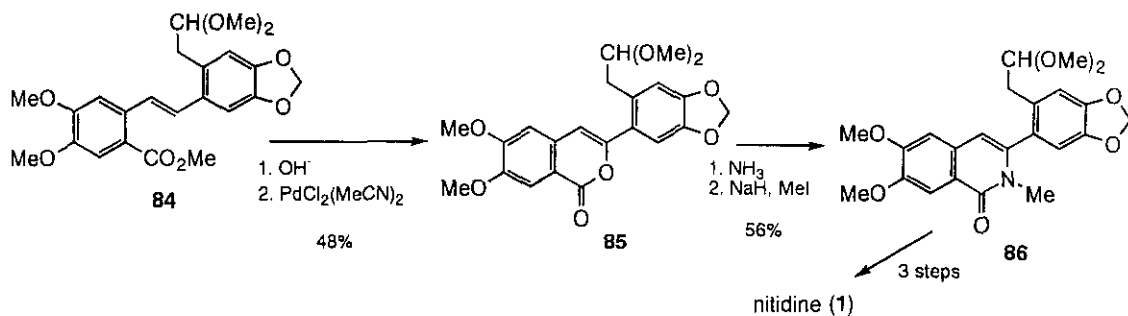
Scheme 10 The reported method¹⁹ through the bond connection V

4-5. Bond connection between C_{10b}-C₁₁ (VI)

As noted in Scheme 1 benzo[*c*]phenanthridine bases are biosynthesized through tetrahydroprotoberberines, in which the C-N bond in the B-C rings of a tetrahydroprotoberberine skeleton is cleaved to yield an intermediacy enamino aldehyde (**21**). Recyclization of it by attacking of the enamine function to the formyl



Scheme 11 The reported method^{20a} through the bond connection VI (1)



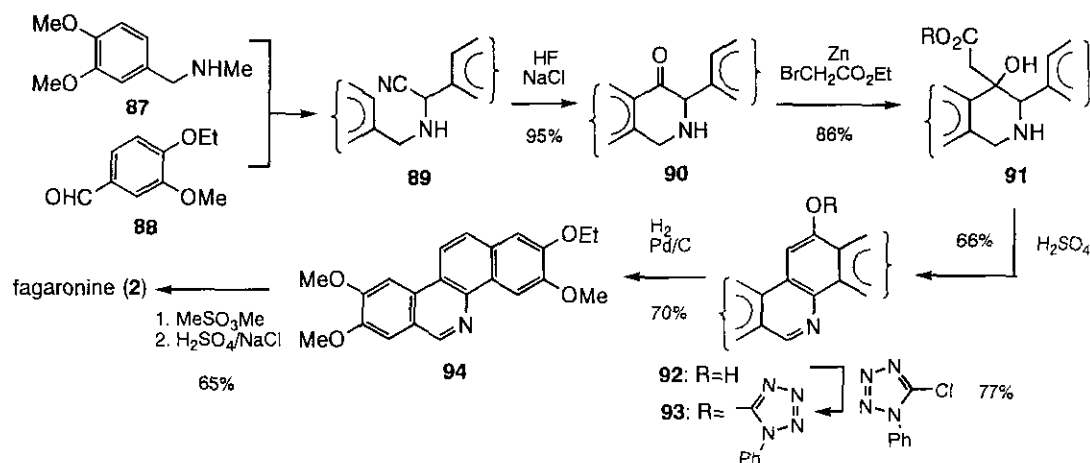
Scheme 12 The reported method^{20b} through the bond connection VI (2)

function produces a benzo[*c*]phenanthridine skeleton (**22**). Although photocyclization^{2a} of anhydroprotoberberines had been reported, Hanaoka *et al.*^{2b,20} independently developed the biomimetic transformation of dihydroberberinium bases into benzo[*c*]phenanthridine ones through the bond connection between C_{10b}-C₁₁ (VI). The syntheses of ambinine (**83**),^{20a} a partially hydrogenated base, using an enamine derived from the isoquinolinium salt (**82**) and nitidine (**1**)^{20b} using the enamide (**86**) were exemplified in Schemes 11 and 12, respectively.

The related biomimetic cyclization²¹ was also reported.

4-6. Bond connection between C₁₂-C_{12a} (VIII)

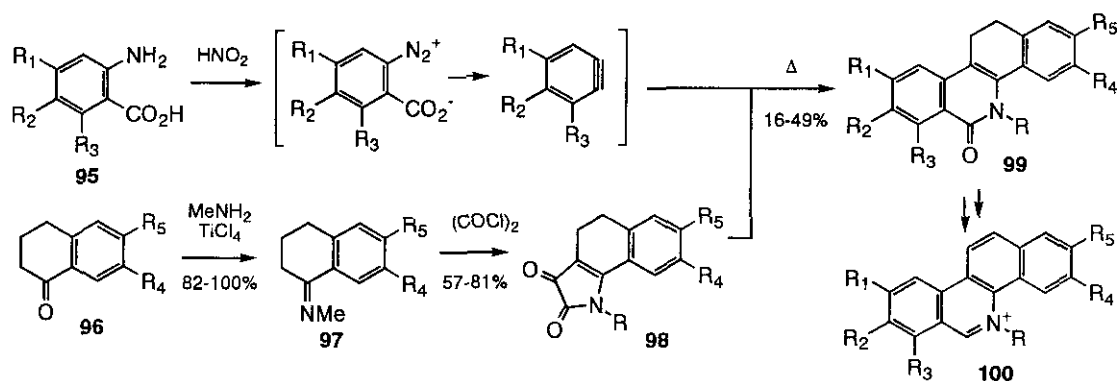
Cyclization of the (3-phenyltetrahydroisoquinolinyl)acetate derivative (**91**) afforded the 12-hydroxybenzo[*c*]phenanthridine skeleton (**92**) through the bond connection between C₁₂-C_{12a} (VIII). Fagarone (**2**) was prepared by the reductive removal of the phenolic function of **92**²² as shown in Scheme 13. Replacing the acetate residue in **91** into a vinyl one resulted in *more straightforward preparation*²³ of a benzo[*c*]phenanthridine base (**94**) (2 steps: 77-84%).



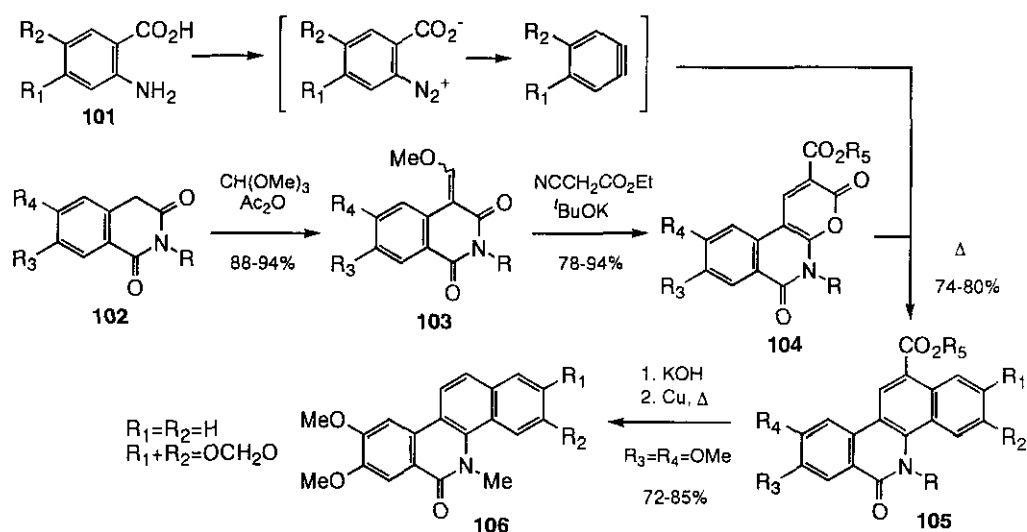
Scheme 13 The reported method²² through the bond connection VIII

4-7. Others: Direct ring construction

Diels-Alder reaction could lead to the direct construction of rings B or C when benzyne were used as dienophiles and either enamides or isocyanates as dienes, respectively. The benzyne derivatives were generated *in situ* by either diazotization of anthranilic acid derivatives (**95**) and (**101**) or oxidation of the benzotriazole derivative (**109**). The reactions using benzyne generated by the former method²⁴ were shown in Schemes 14 and 15, whereas the reaction using a benzyne generated by the latter method²⁵ in Scheme 16. Similar reaction²⁶ using a naphthyl isocyanate (**118**) and a morpholino enamine (**119**) afforded a benzo[*c*]phenanthridine skeleton (**120**) with a hydrogenated ring A by a stepwise construction of the ring B through the addition of **119** to **118** followed by cycloaddition. The corresponding aromatic base (**121**) was given by dehydrogenation of the ring A. (Scheme 17)



Scheme 14 The reported method²⁴ through direct ring construction (1)

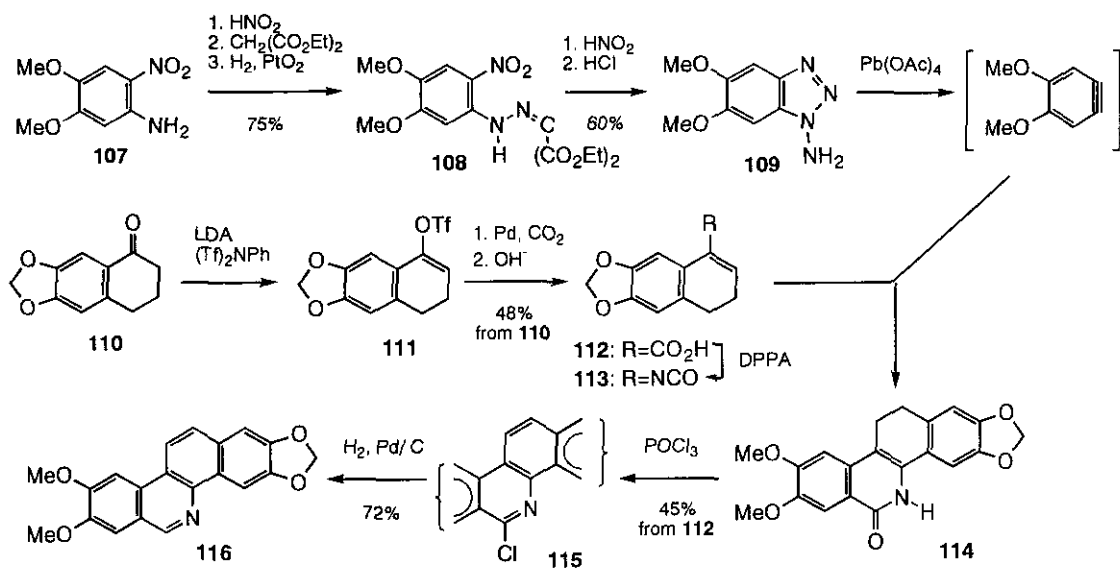


Scheme 15 The reported method²⁴ through direct ring construction (2)

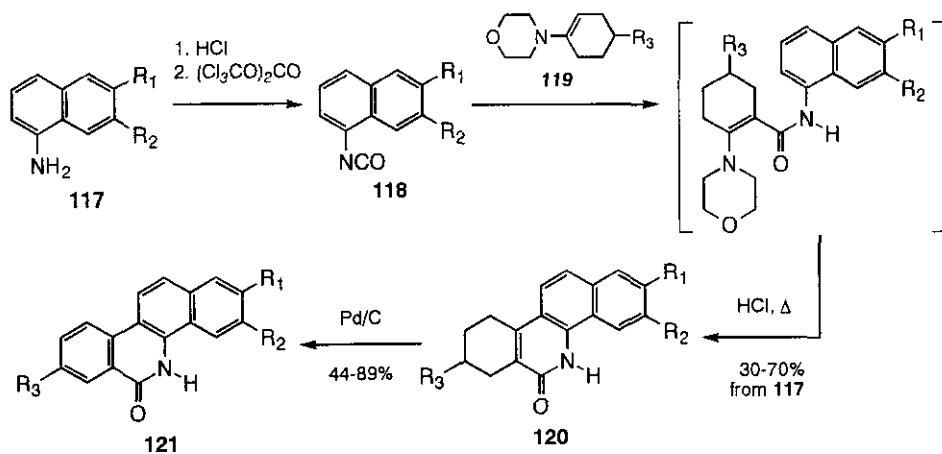
5. Pharmacological Activity

Fully aromatized benzo[*c*]phenanthridine bases show potent pharmacological activities²⁷ including antitumor activity. Recently it was reported that the antitumor activity of nitidine (**1**) and fagaronine (**2**) was due to the inhibition of DNA topoisomerases.^{26,28} On the other hand chelerythrine (**5**) inhibited protein kinase C (PKC).²⁹ Sanguinarine (**4**), a methylenedioxy analog of **5** in the ring A, also inhibited PKC (IC_{50} : 217 μ M), but it more effectively inhibited catalytic subunit of the cyclic-AMP dependent protein kinase (IC_{50} : 6 μ M).³⁰ It was pointed that sanguinarine (**4**) mediated chemical defense against microorganisms, viruses, and herbivores in the plants, in which choline acetyl-transferase was strongly inhibited (IC_{50} : 284 nM).³¹

Cho *et al.*³² found that the 3-arylisquinolone derivative (**122**) showed that the strong antitumor activity against human melanoma (SK-MEL-2) (IC_{50} : 0.2 nM). The isoquinolone (**122**) could be a bioisostere of

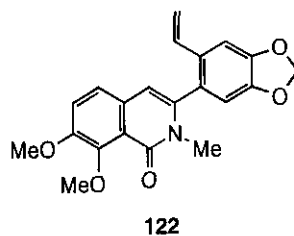


Scheme 16 The reported method²⁵ through direct ring construction (3)



Scheme 17 The reported method²⁶ through direct ring construction (4)

chelerythrine (5) via its C₁₀₆-C₁₁ bond cleavage of the ring C. Removal of the vinyl group on the 3-phenyl substituent in **122** resulted in lowering activity.³³



6. Conclusion

Antitumor activity of fully aromatized benzo[*c*]phenanthridine bases caused to develop possible synthetic approaches to their basic skeleton. While limited strategies have been available for the synthesis of partially hydrogenated bases because of incomplete examination for their pharmacological activities. Furthermore, no reports on their asymmetric synthesis have not appeared as possible as we know. Thus, main attention should direct to establishment of general synthetic methods of partially hydrogenated benzo[*c*]phenanthridine bases including asymmetric synthesis.

ACKNOWLEDGEMENT

We thank graduate and undergraduate students in our group for their experimental assistances. We also thank Prof. Takashi Harayama of Okayama university for his heartfelt discussion.

REFERENCES AND NOTES

1. Unfortunately Prof. Hisashi Ishii was died on 24th April, 1998 during preparation of this review.
2. a) I. Ninomiya and T. Naito, 'Recent Developments in the Chemistry of Natural Carbon Compounds', Vol 10, ed. by R. Bognar and Cs. Szantay, Akademiai Kiado, Budapest, 1984, pp. 11-90; V. Simanek, 'The Alkaloids', Vol. 26, ed. by A. Brossi, Academic Press. Inc., New York, 1985, pp. 185-240; J. Dostal and M. Potacek, *Coll. Czech. Chem. Commun.*, 1990, **55**, 2840. b) M. Hanaoka, 'The Alkaloids', Vol. 33, ed. by A. Brossi, Academic Press. Inc., New York, 1988, pp. 141-233. c) K. W. Bentley, *Natural Product Reports*, 1991, **8**, 350-352; 1992, **9**, 374-375; 1993, **10**, 457-458; 1994, **11**, 562-563; 1995, **12**, 427; 1996, **13**, 135-136; 1997, **14**, 395-396.
3. Q. M. Probst, *Ann.*, 1839, **29**, 120.
4. W. M. Messmer, M. Tin-Wa, H. H. S. Fong, C. Bevelle, N. R. Farnsworth, D. J. Abraham, and J. Trojanek, *J. Pharm. Sci.*, 1972, **61**, 1858; M. Tin-Wa, C. L. Bell, C. Bevelle, H. H. S. Fong, and N. R. Farnsworth, *ibid.*, 1974, **63**, 1476; R. K.-Y. Zee-Cheng and C. C. Cheng, *J. Med. Chem.*, 1975, **18**, 66; F. R. Stermitz, J. P. Gillespie, L. G. Amoros, R. Romero, and T. A. Stermitz, *ibid.*, 1975, **18**, 708.
5. M. Suzuki, T. Nakanishi, O. Kogawa, K. Ishikawa, F. Kobayashi, and H. Ekimoto, *Eur. Pat. Appl. EP 487,930*, 1992 (*Chem. Abstr.*, 1992, **117**, P191,706b); F. Kobayashi, H. Yokumoto, M. Suzuki, and M. Tsubuki, *Jpn. Kokai Tokkyo Koho, JP 04,364,128*, 1992 (*Chem. Abstr.*, 1993, **118**, P219,845t); M. Fukuda, M. Inomata, K. Nishino, K. Fukuoka, F. Kanzawa, H. Arioka, T. Ishida, H. Fukumoto, H. Kurokawa, M. Oka, and N. Saijo, *Jpn. J. Cancer Res.*, 1996, **87**, 1086.
6. K. Iwasa, 'The Alkaloids', Vol. 46, ed. by G. A. Cordell, Academic Press. Inc., New York, 1995, pp. 273-346.
7. T. Richardson, R. Robinson, and E. Seijo, *J. Chem. Soc.*, 1937, 835; A. S. Bailey and R. Robinson, *ibid.*, 1950, 1375.
8. H. Ishii, Y.-I. Ichikawa, E. Kawanabe, M. Ishikawa, T. Ishikawa, K. Kuretani, M. Inomata, and A. Hoshi, *Chem. Pharm. Bull.*, 1985, **33**, 4139.

9. T. Ishikawa, T. Saito, and H. Ishii, *Tetrahedron*, 1995, **51**, 8447.
10. T. Ishikawa, T. Watanabe, H. Tanigawa, T. Saito, K.-I. Kotake, Y. Ohashi, and H. Ishii, *J. Org. Chem.*, 1996, **61**, 1774.
11. T. Ishikawa, T. Saito, S. Noguchi, H. Ishii, S. Ito, and T. Hata, *Tetrahedron Lett.*, 1995, **36**, 2795.
12. T. Ishikawa, K. Simooka, S. Noguchi, A. Ishikawa, E. Yamazaki, T. Narioka, T. Harayama, and H. Ishii, unpublished results.
13. N. Sotomayor, E. Dominguez, and E. Lete, *Tetrahedron Lett.*, 1994, **35**, 2973; *idem*, *J. Org. Chem.*, 1996, **61**, 4062.
14. I. Iwai and J. Ide, *Chem. Pharm. Bull.*, 1962, **10**, 926; 1963, **11**, 1042.
15. H. Ishii, T. Ishikawa, S. Takeda, S. Ueki, and M. Suzuki, *Chem. Pharm. Bull.*, 1992, **40**, 1148.
16. H. Ishii, T. Ishikawa, S. Takeda, M. Suzuki, and T. Harayama, *Chem. Pharm. Bull.*, 1992, **40**, 2002.
17. T. Ishikawa, A. Takami, M. Abe, I.-S. Chen, T. Harayama, and H. Ishii, *Chem. Pharm. Bull.*, 1995, **43**, 766.
18. A. Couture, H. Cornet, and P. Grandclaoudon, *Tetrahedron Lett.*, 1993, **34**, 8097.
19. T. Harayama, T. Akiyama, and K. Kawano, *Chem. Pharm. Bull.*, 1996, **44**, 1634; T. Harayama, T. Akiyama, and Y. Nakano, *ibid.*, 1997, **45**, 1723.
20. a) M. Hanaoka, W. J. Cho, Y. Sugiura, and C. Mukai, *Chem. Pharm. Bull.*, 1991, **39**, 242; b) T. Minami, A. Nishimoto, and M. Hanaoka, *Tetrahedron Lett.*, 1995, **36**, 9505.
21. R. Beugelmanns and M. Bois-Choussy, *Tetrahedron*, 1992, **48**, 8285.
22. M. A. Lynch, O. Duval, P. Pochet, and R. D. Waigh, *Bull. Soc. Chim. Fr.*, 1994, **131**, 718.
23. D. Seraphin, M. A. Lynch, and O. Duval, *Tetrahedron Lett.*, 1995, **36**, 5731.
24. G. Martin, E. Guitian, L. Castedo, and J. M. Saa, *J. Org. Chem.*, 1992, **57**, 5907; D. Perez, E. Guitian, and L. Castedo, *ibid.*, 1992, **57**, 5911.
25. J. H. Rigby and D. D. Holsworth, *Tetrahedron Lett.*, 1991, **32**, 5757.
26. Y. L. Janin, A. Croisy, J.-F. Riou, and E. Bisagni, *J. Med. Chem.*, 1993, **36**, 3686.
27. M. Suffness and G. A. Cordell, 'The Alkaloids', Vol. 25, ed. by A. Brossi, Academic Press. Inc., New York, 1985, pp. 178-187.
28. S.-D. Fang, L.-K. Wang, and S. M. Hecht, *J. Org. Chem.*, 1993, **58**, 5025.
29. J. M. Herbert, J. M. Augereau, J. Gleye, and J. P. Maffrand, *Biochem. Biophys. Res. Commun.*, 1990, **172**, 993.
30. B. H. Wang, Z. X. Lu, and G. M. Polya, *Planta Med.*, 1997, **63**, 494.
31. T. Schmeller, B. Latz-Bruning, and M. Wink, *Phytochemistry*, 1997, **44**, 257.
32. W.-J. Cho, S.-J. Yoo, B.-H. Chung, S.-H. Whang, S.-K. Kim, B.-H. Kang, and C.-O. Lee, *Arch. Pharm. Res.*, 1996, **19**, 321.
33. W.-J. Cho, M.-J. Park, B.-H. Chung, and C.-O. Lee, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 41.