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QUINOLINE ALKALOIDS OF *ORIXA JAPONICA*[1†]

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Abstract - Research works on the isolation, classification, biosynthesis, and biological activities of quinoline alkaloids from *Orixa japonica* (Rutaceae), done mainly by these authors, are reviewed.

1. Introduction

Orixa japonica THUNB. (Rutaceae) is one of the materials of the crude drug "JOHZAN". *Dichroa febrifuga* LOUR. (Hydrangeaceae) is also used as a material of a crude drug of the same name. It seems that currently the latter is mainly used as the material of "JOHZAN". However, it was pointed out that the description of the material of "JOHZAN" in the literature formerly corresponds to *O. japonica*.[2]

"JOHZAN" has been mainly applied for symptoms corresponding to malaria. For this purpose, the material of the crude drug "JOHZAN" was changed gradually from *O. japonica*

[†]Dedicated to Professor Sho Ito on the occasion of his 77th birthday.

to *D. febrifuga*, which was previously called "KEIKOTSU-JOHZAN".

As a part of a program on the medicinal resources of natural products origin, chemical research of the crude drug "JOHZAN" was planned. As a result, research on the chemical components of *O. japonica*, one of the materials of "JOHZAN", was initiated.

It was established that this plant contained many kinds of have been alkaloids, and, including our research results,[3~8] 24 different quinoline alkaloids were reported to date. Through this research, we succeeded in the isolation of a quinoline alkaloid which seems to be an important biosynthetic intermediate of certain quinoline alkaloids, and determined the absolute stereochemistry of this alkaloid. Antimalarial and rat small intestine muscle relaxation activities of some of these alkaloids were investigated. This review describes the research works on the isolation, classification, biosynthesis and biological activities of quinoline alkaloids from *Orixa japonica*, done mainly by these authors.

Other than alkaloids, bergapten, xanthotoxin, friedelin, isoarborinol, spathulenol, carvomenthol, a-terpineol, a- and b-pinenes, camphene, g-terpinene, limonene, cineol, and five unidentified compounds were reported as neutral constituents of *O. japonica*.[9]

2. The isolation of quinoline alkaloids from *Orixa japonica*

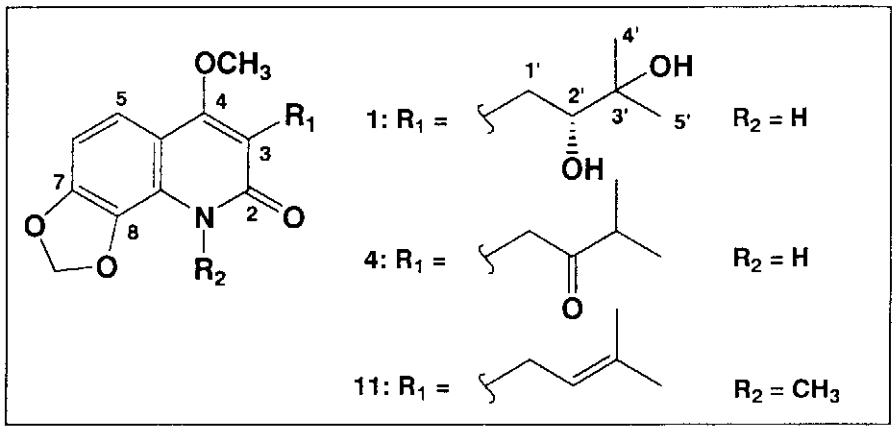
From an investigation of the chemical constituents of *Orixa japonica*, 14 quinoline alkaloids, including 7 new components, were isolated from the stems, roots and leaves of this plant material by us.[3~8] Thus, totally 24 quinoline alkaloids, including 10 other quinoline alkaloids as will be described below, have been isolated from this plant to date. These 24 quinoline alkaloids can be divided into following four categories.

1) Alkaloids in which a C_5 unit is combined with the quinoline moiety
2) Alkaloids which are generated by cyclizing the C_5 unit to the quinoline moiety
3) Alkaloids which are generated by the elimination of the C_3 unit from the cyclized C_5 unit on the quinoline moiety
4) Alkaloids in which a benzene ring is combined with the quinoline moiety

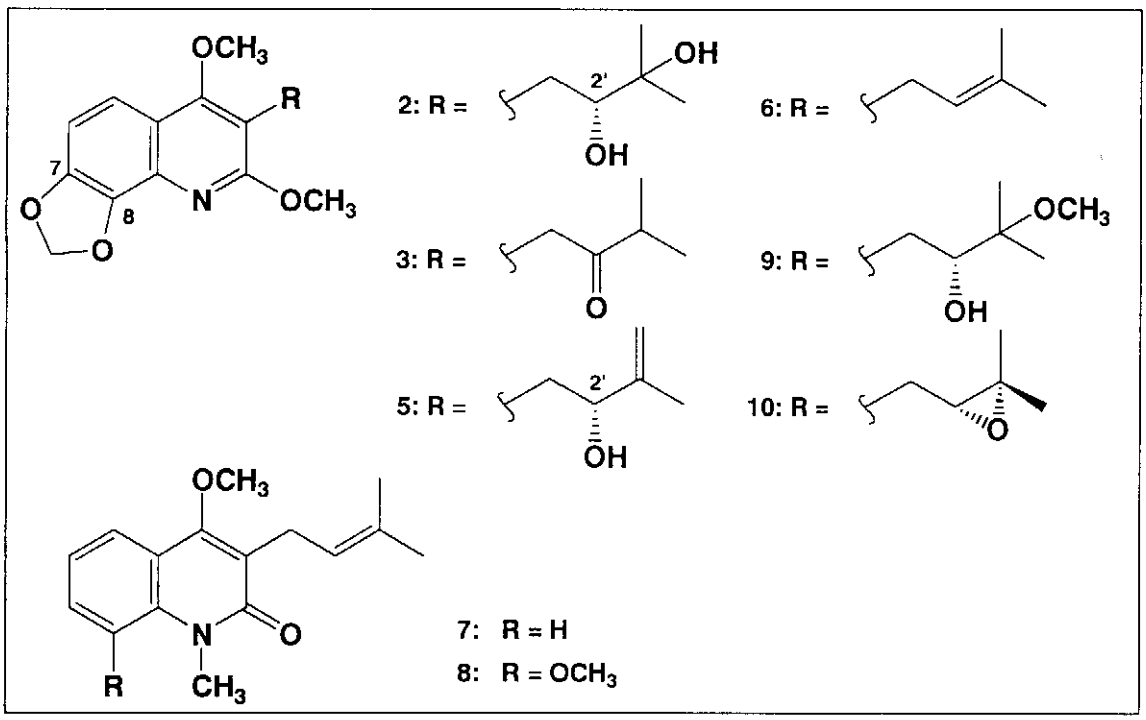
1) *Alkaloids in which a C_5 unit is combined with the quinoline moiety*

The alkaloids in this category possess a C_5 unit combined with a quinoline skelton which has not cyclized. Quinoline alkaloids of this category isolated from *O. japonica* included dihydroxylunidonine (nororixine, **1**),[10] (+)-orixine (**2**),[11] and orixinone (**3**).[12] Recently, we have isolated eight such quinoline alkaloids from *O. japonica*, *i.e.*, *N*-demethyl lunidonine (**4**),[4] (+)-isoptelefolidine (**5**),[4] isoptelepreneine (**6**),[4] *N*-methylatanine (**7**),[5] 8-*O*-methyl-

glycosolone (**8**),[5] (+)-3'-*O*-methylorixine (**9**),[13] (-)-preorixine (**10**)[3] and pteleprenine (**11**).[4] Alkaloids (**4**)[14] and (**11**)[15] were formerly isolated from *Ptelea trifoliata* (Rutaceae), and



alkaloids (**7**) and (**8**) were previously isolated from *Almeidea guyanenis* (Rutaceae)[16] and *Citrus grandis* (Rutaceae),[17] respectively. Alkaloid (**10**) was synthesized previously with asymmetric induction.[18]

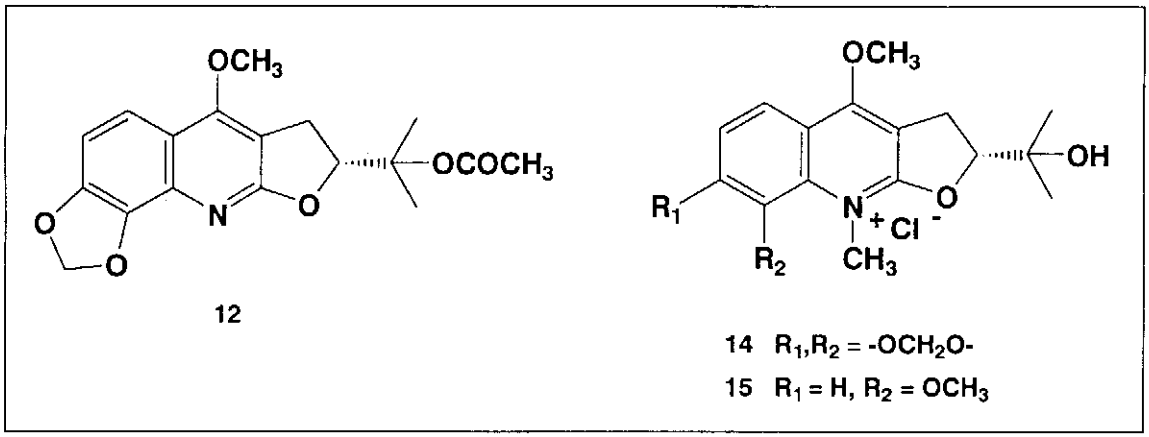


The quinoline alkaloids from *O. japonica* have two distinctive features. The first is that most of these alkaloids possess a methylenedioxy moiety at the C-7 and C-8 positions, and the second is that many of the alkaloids possess a methoxyl group at the C-2 position.

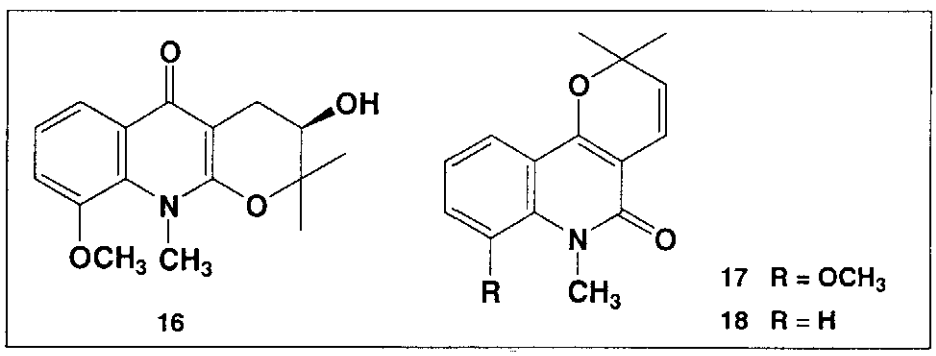
As described subsequently, (-)-preorixine (**10**),[3] which possesses an epoxide moiety in the side-chain, is considered to be a very important biosynthetic intermediate for the formation of various quinoline alkaloids such as (+)-orixine (**2**),[11] orixinone (**3**),[12] (+)-3'-*O*-acetylisopteleflorine (**12**),[6] and kokusagine (**13**).[19]

2) *Alkaloids which are generated by cyclizing the C5 unit to the quinoline moiety*

The alkaloids of this category seem to be formed by cyclization of the C5 unit attached to the quinoline moiety. Six alkaloids of this category were isolated from this plant material. Quinoline alkaloids possessing a furan ring formed by cyclization include (+)-3'-*O*-acetyl-isopteleflorine (**12**), 4-*O*-methylhydroxyluninium chloride (**14**),[5] and 4-*O*-methyl-balfourodinium chloride (**15**).[20]



In addition, isobalfourodine (**16**),[13] 8-methoxy-*N*-methylflindersine (**17**),[4] and *N*-methyl-flindersine (**18**)[4] were isolated in which a pyran ring was formed by cyclization. Among these alkaloids possessing a pyran ring, the third ring is attached to the C-2 and C-3 positions of the quinoline ring (linear type) in isobalfourodine (**16**) , and this third ring is attached to the C-3 and C-4 positions of the quinoline ring (angular type) in the case of alkaloids (**17**) and (**18**).

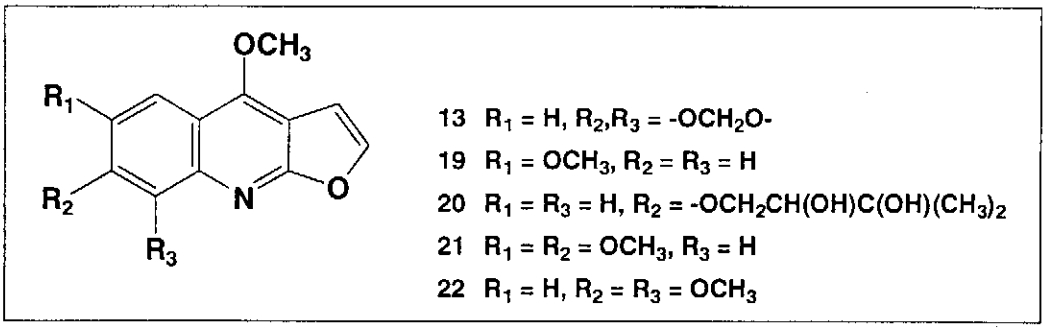


Alkaloid (**14**) was isolated previously from *Ptelea trifoliata*,[21] and alkaloids (**16**), (**17**), and (**18**) were isolated from *Balfourodendron riedelianum* (Rutaceae),[22] *Zanthoxylum simulance* (Rutaceae),[23] and *Spathelia sorbifolia* (Rutaceae),[24] respectively.

3) *Alkaloids which are generated by the elimination of the C3 unit from the cyclized C5 unit on the quinoline moiety*

The alkaloids classified into this category were biosynthesized by the elimination of a C3 unit from the furan ring formed as described above. Five such alkaloids, namely,

kokusagine (**13**),[19] evolitorine (**19**),[25] evoxine (**20**),[26] kokusaginine (**21**),[27] and skimmianine (**22**)[27] were isolated from *O. japonica*. Evolitorine (**19**) was also isolated from *Evodia belahe*

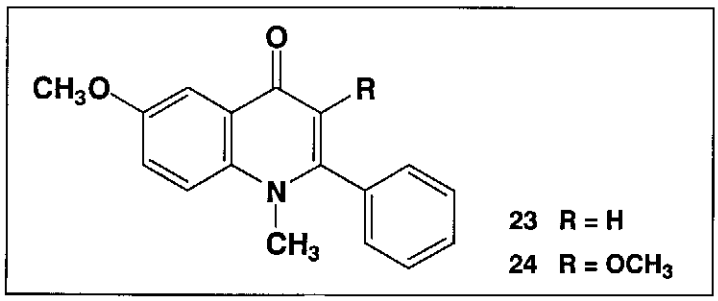


(Rutaceae).[15]

The alkaloids of this class were studied since the early part of the 20th century. For example, the first isolation of compound (**13**) from *Skimmia repens* (Rutaceae) was reported in 1930.[28] The alkaloids belonging to this class are also known as furoquinoline alkaloids.

4) *Alkaloids in which a benzene ring is combined with the quinoline moiety*

The alkaloids described above were mainly isolated from the stems, fruits and roots of *O. japonica*. On the other hand, eduline (**23**)[8] and japonine (**24**)[29] in this category were isolated only from the leaves of *O. japonica*. These alkaloids possess a unique structure in



which a benzene ring is attachd to a quinoline moiety at the C-2 position. Eduline (**23**), which had been isolated from the seeds of *Casimiroa edulis* (Rutaceae), was obtained as a constituent of *O. japonica*, and, as described later, these two alkaloids showed relaxation activity against the rat small intestine smooth muscle.

3. Biosynthesis of the quinoline alkaloids

The biosynthetic units of the quinoline alkaloids of categories 1) - 3) of *Orixa japonica* are considered to be each one molecule of anthranilic acid, acetic acid and isoprene pyrophosphate.[30]

The biosynthetic origin of the furan ring of the furoquinoline skeletone was examined in skimmianine (**22**).[31] It was established that the furan ring portion of this alkaloid was formed by the elimination of a C3 unit from the introduced isoprene (C5) unit.[31] That is to say, the alkaloids belonging to the category 3) are biosynthesized through the following

two steps. Initially, the alkaloids belonging to category 1), with an epoxide moiety in the side-chain, would become alkaloids of category 2) by epoxide ring opening and formation of the furan ring. Then, the alkaloids belonging to category 3) would be formed by the elimination of a C_3 unit attached to the furan ring.

Thus, the alkaloids of category 1) possessing an epoxide ring in the side-chain appeared to be a biosynthetic intermediate for the initial stage of the formation of alkaloids belonging to categories 2) and 3). However, such an alkaloid had not been isolated previously. One of the reasons for this was considered to be the following. For the quinoline alkaloids with a C_5 unit as the side-chain, in most cases, a carbonyl group also exists at the C-2 position. Therefore, an intermediate with an epoxide ring in the side-chain will be rapidly cyclized.

A research group in the United Kingdom noticed[18] the following two points. One is that many of the alkaloids isolated from *O. japonica* possess a methoxyl group at the C-2 position instead of a carbonyl group, and the other is that as an example of such alkaloids, orixine (**2**) was already isolated from this plant. Based on these two points, they synthesized (**10**)[20] as a postulated precursor of alkaloids such as (**2**), (**3**), (**12**) and (**13**). Then, the existence of this compound in *O. japonica* (cultivated in the United Kingdom) was investigated by the same group.[20] However, they failed in the isolation and/or detection of this alkaloid in the extraction of *O. japonica*.[20] Whereas, during the investigation of the alkaloidal constituents of this plant, we encountered just this alkaloid. Therefore, we named this alkaloid as (-)-preorixine (**10**) and reported it's existense in nature.[3]

4. Investigation of the absolute stereochemistry of an important biosynthetic intermediate, (-)-preorixine

Thus, (-)-preorixine (**10**) was considered to be both the biosynthetic precursor of alkaloids (**2**), (**3**), (**12**) and (**13**), and the first example of a very important type of alkaloid as a key precursor for the biosynthesis of various other quinoline alkaloids.

The absolute configuration of the C-2’ position was deduced. Two alcohols (**2**) and (**5**) were obtained by treating (-)-preorixine (**10**) with a dilute sulfuric acid. After preparing the Mosher's esters of these two alcohols, their stereochemistry was examined by the application of the extended Mosher's method. As a result, the absolute configuration of the C-2' position of these two alcohols was determined to be *R*.[7] The summary of the NMR spectral data of the Mosher's esters of orixine (**2**) is shown in Figure 1. In addition, (+)-

orixine (**2**) and (+)-isoptelefolidine (**5**) were obtained by hydrolysing the Mosher's esters of each of the derivatives mentioned above.

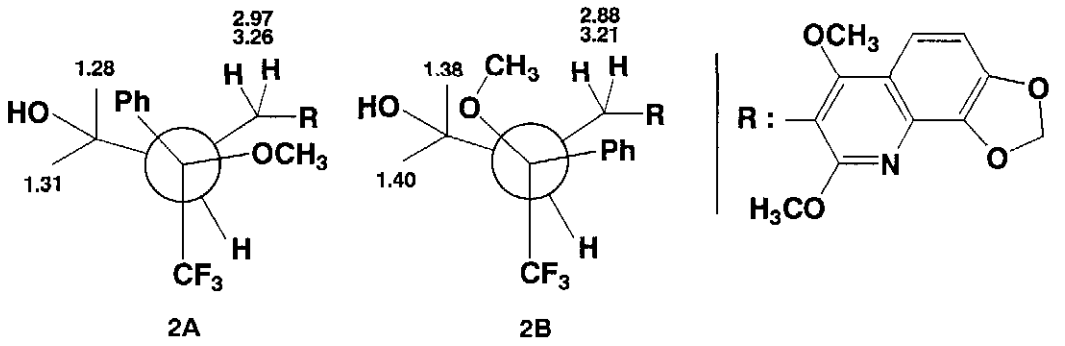


Figure 1. Extended Newman Projection of the (*S*)- and (*R*)-Mosher's Esters (2A and 2B) of (+)-Orixine (2).

On the other hand, when (-)-preorixine (**10**) was treated with dilute hydrochloric acid in methanol, compound (**9**), which possessed a methoxyl group at the C-3' position, was obtained. From this fact, it was clear that an inversion of the stereochemistry at the C-2' position, as proposed by Bowman *et al.*,[18] did not occur by treating with a dilute sulfuric acid. They carried out the ring-opening reaction on the epoxide ring of the stereoselectively synthesized (-)-**10** using formic acid, and an erroneus judgment (stereoinversion of the C-2' position) was made. It appears that an ester interchange from the C-3' to C-2' position occurred during the ring-opening reaction of the epoxide by treating (-)-**10** with formic acid. Thus, we succeeded in the determination of the absolute configuration at the C-2' position of the epoxide moiety of a key biosynthetic intermediate quinoline alkaloid. It was accomplished because of the success in isolating of (-)-preorixine (**10**) from *O. japonica*. Thus it was estimated that alkaloids (**1**), (**12**), (**14**), (**15**) and (**16**) possess *R* configurations at C-2' position.

Many of the quinoline alkaloids isolated from the Rutaceae possess a structure in which a C_5 unit is attached to the quinoline skeleton. Among these alkaloids, those which possess an epoxide ring on the C_5 unit appear to be the biosynthetic intermediates of those quinoline alkaloids with a furan or a pyran ring. Also, it was considered that the so-called furoquinoline alkaloids were formed by the elimination of a C_3 unit from the quinoline alkaloids with a furan ring.

5. Antimalarial activity of the quinoline alkaloids

The *in vitro* antimalarial activity of the quinoline alkaloids isolated from *Orixa japonica* was examined. Among them, *N*-methylatanine (**7**) and 4-*O*-methylbalfourodinium Cl (**15**)

showed antimalarial activity (EC_{50} values: 3 mg/mL and 21 mg/mL, respectively) for falciparum (*Plasmodium falciparum*).[32]

Concerning the antimalarial activity, the methanol extract of the roots of *Dichroa febrifuga*, which also comprises the crude drug "JOHZAN", showed stronger activity (EC_{50} value: 0.025 μg/mL) against *Plasmodium falciparum*.[32] Therefore, the history that *D. febrifuga* gradually replaced *O. japonica* as the base material plant of "JOHZAN" is understandable, if it is only judged from the viewpoint of direct activity for the disease which corresponds to malaria in modern medicine.

6. Rat small intestine muscle relaxation activity of the quinoline alkaloids

Eduline (**23**) and japonine (**24**) were found to possess rat small intestine relaxation activity (**23**: 0.17 ± 0.00 g relaxative tention/10 μM, and **24**: 0.12 ± 0.03 g relaxative tention/5 μM, respectively), and it was found that this activity was comparable to that of papaverine[8] (0.16 ± 0.03 g relaxative tention/10 mM). The same kind of activity of pteleprenine (**11**), was reported separately.[33]

7. Conclusion

Recently, it appears that few scientists conduct research on the chemical constituents of the extract of a plant which is not connected with any biological activity. On the other hand, we attempted to clarify the chemical constituents of the crude drug "JOHZAN", which has been used in Chinese Traditional Medicine for a long time. That is to say, this research was undertaken from the viewpoint to establish a monograph of the chemical aspects of "JOHZAN". Then, as a prelude to this research, we undertook the investigation of the alkaloidal constituents of *Orixa japonica*, which is also a material known as "JOHZAN".

Actually, the target compounds to be isolated from the plant material were decided by the technique of "the chemical screening". Namely, the spots which showed a Dragendorff's reagent positive reaction on TLC were selected. As a result, it was established that *O. japonica* contained a multitude of quinoline alkaloids. We have newly isolated 14 quinoline alkaloids, in addition to the 10 known such alkaloids. This number (24) of quinoline alkaloids isolated from a single plant is second to that (31 alkaloids) of *Ptelea trifoliata*.

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REFERENCES

1 Part of this work was presented at the 41st Symposium on the Chemistry of Natural Products (Nagoya, 1999); Book of Symposium papers, p. 141.

2 T. Namba, "Coloured Illustrations of WAKAN-YAKU (The Crude Drugs in Japan, China and the Neighbouring Countries)", Vol. 1, Hoikusha Publishing Co. Ltd., Osaka, **1980**, p. 148.

3 S. Funayama, T. Kageyama, K. Murata, M. Adachi, and S. Nozoe, *Heterocycles*, 1993, **35**, 607.

4 S. Funayama, K. Murata, and S. Nozoe, *Phytochemistry*, 1994, **36**, 525.

5 S. Funayama, K. Murata, and S. Nozoe, *Nat. Med.*, 1996, **50**, 182.

6 S. Funayama, K. Murata, and S. Nozoe, *Phytochemistry*, 1996, **41**, 1231.

7 S. Funayama, K. Murata, and S. Nozoe, *Chem. Pharm. Bull.*, 1996, **44**, 1885.

8 S. Funayama, R. Tanaka, T. Noshita, T. Mori, Y. Kumekawa, T. Kashiwagura, and K. Murata, *Biol. Pharm. Bull.*, submitted for publcation.

9 H. Abe, T. Kawashima, I. Maruta, M. Kodama, and S. Ito, *Phytochemistry*, 1971, **10**, 3330.

10 M. Terasaka, K. Narahashi, and Y. Tomikawa, *Chem. Pharm. Bull.*, 1960, **8**, 1142.

11 M. Terasaka, *Chem. Pharm. Bull.*, 1960, **8**, 523.

12 R. M. Bowman, J. F. Collins, and M. F. Grundon, *J. Chem. Soc., Perkin Trans. 1*, **1973**, 626.

13 S. Funayama, T. Noshita, and K. Murata, unpublished results.

14 K. Szendrei, M. Petz, I. Novak, J. Reisch, H. E. Bailey, and V. L. Bailey, *Herba Hung.*, 1974, **13**, 49 (*Chem. Abstr.*, 1975, 83, 40169g).

15 Z. Rózsa, S. Szendrei, I. Novák, E. Minker, M. Koltai, and J. Reisch, *J. Chromatography*, 1974, **100**, 218.

16 C. Moulis, K. R. Wirasutisna, J. Gleye, P. Loiseau, E. Stanislas, and C. Moretti, *Phytochemistry*, 1983, **22**, 2095.

17 T.-S. Wu, C.-S. Kuoh, and H. Furukawa, *Phytochemistry*, 1983, **22**, 1493.

18 R. M. Bowman and M. F. Grundon, *J. Chem. Soc*., **1967**, 2368.

19 M. Terasaka, *Pharm. Bull*., 1954, **2**, 159.

20 W. J. Donnely and M. F. Grundon, *J. Chem. Soc*., *Perkin Trans. 1*, **1972**, 2116.

21 L. A. Mitscher, M. S. Bathala, G. W. Clark, and J. L. Beal, *J. Nat. Prod*., 1975, **38**, 109.

22 M. F. Grundon, "The Alkaloids : Chemistry and Physiology" Vol 17, ed. by R. H. F. Manske, Academic Press, New York, 1979, 105.

23 A. I. Gray and J. J. O'Sullivan, *Planta Medica*, 1980, **39**, 209.

24 C. D. Adams, D. R. Taylor, and J. M. Warner, *Phytochemistry*, 1973, **12**, 1359.

25 R. G. Cooke and H. F. Haynes, *Aust. J. Chem.*, 1954, **7**, 273.

26 T. Yajima, N. Kato, and K. Munakata, Agric. Biol. Chem., 1977, 41, 1263.

27 T. Obata, Yakugaku Zasshi, 1939, 59, 145.

28 Y. Asahina, T. Ohta, and M. Inubuse, Yakugaku Zasshi, 1930, 50, 1117.

29 H. -H. Key and M. Luckner, Phytochemistry, 1970, 9, 2199.

30 I. Monkovic, I. D. Spenser, and A.O. Plunkett, Can. J. Chem., 1967, 45, 1935.

31 A. O. Colonna and E. G. Gros, Chem. Commun., 1970, 674.

32 K. Murata, S. Fushiya, T. Ohta, Y. Oshima, Y. Wataya, K.-S. Kim, and S. Funayama, Proceedings of the 117th Annual Meeting of the Pharmaceutical Society of Japan, 1997, vol. 2, p. 140.

33 K. Seya, I. Miki, K. Murata, H. Junke, S. Motomura, T. Araki, Y. Itoyama, and Y. Oshima, J. Pharm. Pharmacol., 1998, 50, 803.