

Review

Chemistry and Pharmacology of Analgesic Indole Alkaloids from the Rubiaceous Plant, *Mitragyna speciosa*

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The leaves of a tropical plant, *Mitragyna speciosa* KORTH (Rubiaceae), have been traditionally used as a substitute for opium. Phytochemical studies of the constituents of the plant growing in Thailand and Malaysia have led to the isolation of several 9-methoxy-Corynanthe-type monoterpene indole alkaloids, including new natural products. The structures of the new compounds were elucidated by spectroscopic and/or synthetic methods. The potent opioid agonistic activities of mitragynine, the major constituent of this plant, and its analogues were found in *in vitro* and *in vivo* experiments and the mechanisms underlying the analgesic activity were clarified. The essential structural features of mitragynines, which differ from those of morphine and are responsible for the analgesic activity, were elucidated by pharmacological evaluation of the natural and synthetic derivatives. Among the mitragynine derivatives, 7-hydroxymitragynine, a minor constituent of *M. speciosa*, was found to exhibit potent antinociceptive activity in mice.

Key words *Mitragyna*; alkaloid; indole; analgesic; opioid; structure–activity relationship

1. Introduction

Mitragyna speciosa KORTH (Rubiaceae), endemic to tropical Southeast Asia, is a species of particular medicinal importance.¹⁾ Known as “Kratom” in Thailand and “Biak-Biak” in Malaysia, the leaves have been traditionally used by natives for their opium-like effect and coca-like stimulant ability to combat fatigue and enhance tolerance to hard work under the scorching sun. It has been used also as a substitute for opium and for weaning addicts off morphine. However, the use of this plant has been banned in those countries because of its narcotic effect. Due to its unique medicinal properties, a number of chemical and pharmacological studies have been carried out over the last forty years. Several indole alkaloids have been found²⁾ and a preliminary study of the antinociceptive activity³⁾ of the major constituent has been reported. However, the principle and the mechanisms underlying the biological activities have not been completely elucidated at the time when we embarked on the chemical and pharmacological investigation of *M. speciosa*. In this review, recent findings from our study of *Mitragyna* alkaloids are discussed.

2. Chemical Constituents in the Leaves of *Mitragyna speciosa*

2.1. Thai Plant In the 1960s, the Chelsea group in the U.K. reported the isolation of several indole alkaloids from the leaves of *M. speciosa* from Thailand.^{4–6)} Almost ten years later, Shellard *et al.*, in their investigation of the alkaloidal constituents in various samples of *M. speciosa* from Thailand, isolated more than twenty kinds of Corynanthe-type alkaloids, including oxindole derivatives.^{7–9)} They pointed out that the variation in the constituents among dif-

ferent batches of leaves may be an indication of the presence of geographical variants of the species within Thailand. For chemical re-investigation, we chose the plant growing on the campus of the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Bangkok, in collaboration with Dr. Ponglux. From the young leaves of *M. speciosa*, mitragynine (**1**) was obtained as the major constituent (66.2% based on the crude base) together with its analogues, speciogynine (**2**, 6.6%), speciociliatine (**3**, 0.8%), and paynantheine (**4**, 8.6%). In addition, a new alkaloid, 7 α -hydroxy-7*H*-mitragynine (**5**), was isolated as a minor constituent (2.0%), the structure of which was elucidated by spectroscopic analysis and chemical transformation from mitragynine (**1**) (Fig. 1).¹⁰⁾ As is mentioned later, this new alkaloid was proven to be a key compound in this series of research.

2.2. Malaysian Plant The Chelsea group also started in the mid 60s the chemical investigation of Malaysian *M. speciosa*,¹¹⁾ which resulted in the isolation of several mitragynine-related indole alkaloids. Houghton and Said reported the isolation of a new alkaloid (**6**),²⁾ *i.e.*, a 3,4-dehydro derivative of mitragynine, from the fresh leaves of *M. speciosa* growing on the campus of Universiti Kebangsaan Malaysia. They also found new types of indole alkaloids, mitragynaline (**7**), corynantheidaline (**8**), mitragynalinic acid (**9**), and corynantheidalinic acid (**10**) (Fig. 2), in the very young leaves of the same plant.¹²⁾ Those alkaloids have an unusual skeleton, namely, a carbon function at the C14 position, compared with hitherto known monoterpene indole alkaloids. However, the structures of **7** and **8** were revised as described later (Section 2.3).

In collaboration with Dr. Said of Universiti Kebangsaan Malaysia, we also re-investigated the constituents in

Malaysian *M. speciosa*. From the methanolic extract of the mature leaves, five alkaloids identical with those in the Thai plant, *i.e.*, mitragynine (1), speciogynine (2), speciociliatine (3), paynantheine (4) and 7 α -hydroxymitragynine (5), were isolated. Although mitragynine (1) is the major constituent,

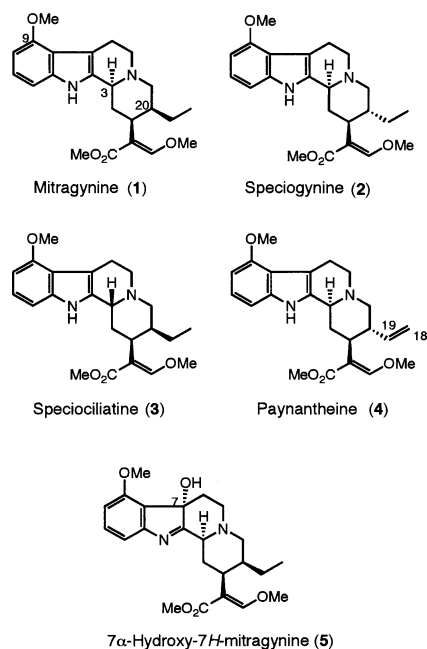


Fig. 1

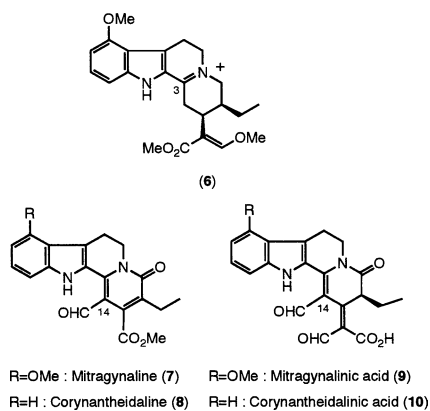


Fig. 2

accounting for approximately 12% of the total alkaloid extract, the yield is much less than that of the Thai plant (*ca.* 66%). In addition, mitragynaline and pinoresinol were obtained as minor constituents. Furthermore, three new indole alkaloids (11–13)¹³ were obtained (Fig. 3) and their structures were elucidated as follows.

The new compound named mitralactonal (11), which has a 9-methoxyindole nucleus, exhibited long-wavelength absorption at 496 nm in the UV spectrum, indicating a high degree of unsaturation in the molecule. The ¹³C-NMR and HMBC spectra disclosed the presence of six conjugated *sp*² carbons including an aldehyde carbon and an ester carbonyl carbon, besides the aromatic carbons due to the indole ring. Further HMBC analysis and structural comparison with common monoterpenoid indole alkaloids demonstrated the presence of C17-aldehyde and C22-ester functions, both of which were attached to the carbon at C16, and the conjugation was extended to the indole nucleus through the double bonds at the C15–16 and C3–14 positions. The NOE observed be-

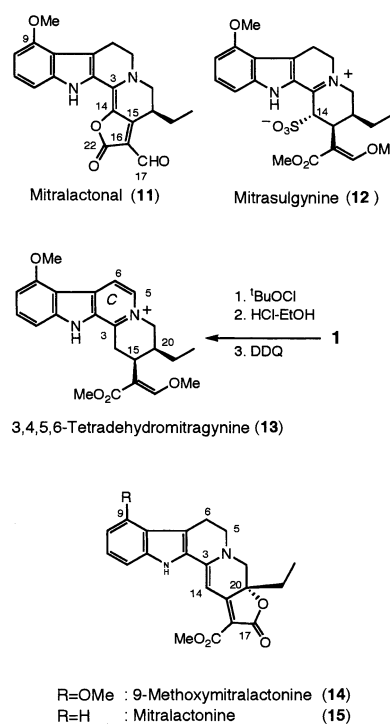


Fig. 3

Born in 1954 in Nagano, Japan, he received a Bachelor's degree in Pharmaceutical Science (1977) from Chiba University and a PhD (1982) from the same university under the supervision of Professor Shin-ichiro Sakai. After postdoctoral work (1982–1984) with Professor Ekkehard Winterfeldt (Institute of Organic Chemistry, Hannover University, Germany) with support from the Alexander von Humboldt Foundation, he joined the research group of Professor Tohru Koizumi at the Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, as a research associate. In 1986, he moved to the Faculty of Pharmaceutical Sciences, Chiba University, and was promoted to associate professor in 1994 and to full professor in the Chiba University Graduate School of Pharmaceutical Sciences in 2004. He has received the Sato Memorial Award (2000) and the Pharmaceutical Society of Japan Award for Divisional Scientific Promotions (2004). Currently, he is on the Board of Editors (vice-chairman) of *Natural Medicines*.



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tween the aldehyde proton and the protons at C19 and C20 proved the geometry at the C16 position. The molecular formula obtained from high-resolution mass spectra as well as ^{13}C -NMR data indicated the presence of a lactone function between the C14 and C22 positions. All the above data led to the determination of the molecular structure of the new alkaloid to be that shown in formula **11**.

The UV absorption that was shifted to the long-wavelength region (438, 359, 241 nm) compared with that of mitragynine (**1**), and a characteristic ^{13}C -NMR signal at δ 163.5 (C3) indicated the presence of a 9-methoxy-5,6-dihydro- β -carboline chromophore in the new alkaloid **12**. Two side chains, *i.e.*, an ethyl group at C20 and a methyl β -methoxyacrylate moiety, which are the fundamental structural units of Corynanthe-type indole alkaloids, were disclosed by ^1H - and ^{13}C -NMR spectra. A characteristic peak at δ 4.55 (1H, broad singlet) was unambiguously assigned to the proton at C14 by HH-COSY and 1D-HOHAHA spectra, and by the HMBC connectivities between both the C3 and C16 carbons. Those observations indicated the presence of a substituent at the C14 position. The high-resolution FAB-MS spectrum of **12** indicated a molecular formula corresponding to $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_7\text{S}$ and a fragmentation ion peak at 396 ascribable to the elimination of a sulfonate group ($\text{M}-\text{SO}_3$), signifying that **12** possessed an SO_3^- group at the C14 position and existed in the zwitterionic form between the N4 immonium and the sulfonium functions. All the above data suggested that the molecular structure of the new alkaloid, named mitrasulgynine, is that shown in formula **12**. To our knowledge, this is the first example of a monoterpene indole alkaloid possessing a sulfonate group in the molecule.

The new compound **13** was deduced to be 3,4,5,6-tetrahydromitragynine or its stereoisomer from spectroscopic analysis. To determine the structure including the stereochemistry at the C15 and C20 positions, chemical conversion of mitragynine (**1**) was carried out. Mitragynine (**1**) was treated with *tert*-butylhypochlorite in the presence of triethylamine to give 7-chloro-7*H*-mitragynine, which was then converted into the 3,4-dehydro derivative by treating with ethanolic HCl. Subsequent aromatization of the C-ring was accomplished by oxidation with DDQ to afford 3,4,5,6-tetrahydromitragynine (**13**). The semisynthetic compound was identified by comparison with natural compound **13** in terms of chromatographic behavior and spectral data. Thus, the structure including the absolute stereochemistry at the C15 and C20 positions of the new alkaloid **13** was established.

From the ethyl acetate extract of the young leaves of *M. speciosa*, nine Corynanthe-type indole alkaloids, namely, mitragynine (**1**), speciogynine (**2**), speciociliatine (**3**), paynantheine (**4**), 7 α -hydroxymitragynine (**5**), mitragynaline (**7**), corynantheidaline (**8**), corynantheidine, and isocorynoxine, were isolated. In addition, a porphine derivative, phaeophorbide a, was obtained. Furthermore, two new indole alkaloids, **14**¹⁴ and **15**,¹⁵ (Fig. 3) were obtained as minor constituents and their structures were elucidated by means of spectroscopic analysis as well as racemic and asymmetric total syntheses.

The ^1H - and ^{13}C -NMR spectra of **14** showed the presence of a 9-methoxyindole nucleus, an ethane bridge at C5–C6, an ethyl group at C20, and a methoxycarbonyl group, which are

the fundamental structural units of common Corynanthe-type monoterpene indole alkaloids. The UV spectrum exhibited long-wavelength absorption at 462 nm, indicating a long conjugation in the molecule. The ^{13}C -NMR and HMBC spectra disclosed the presence of six conjugated sp^2 carbons including an ester carbon and a lactone carbonyl carbon, besides the aromatic carbons due to the indole nucleus. The characteristic proton signal observed at δ 6.39 (1H, singlet) was unambiguously assigned to the proton at C14 by the HMQC spectrum, and this signal had HMBC connectivities between the C2, C3, C15, C16 and C20 carbons. The molecular formula ($\text{C}_{22}\text{H}_{22}\text{O}_5\text{N}_2$) obtained from high-resolution mass spectrum as well as the fact that the carbon signal at C20 resonated at δ 77.4 indicated the presence of a lactone function constructed between the oxygen atom at C20 and the carbonyl group at the C17 position. All the above findings as well as biogenetic consideration enabled us to determine the molecular structure of the new alkaloid to be that shown in formula **14**, which has a highly conjugated pentacyclic 9-methoxy-Corynanthe skeleton. In the ^{13}C -NMR spectrum, the sp^2 carbons at C14 and C16 resonated at unusually high positions, δ 87.08 and 94.91, respectively. This abnormal phenomenon as well as the novel chemical structure of **14** prompted us to synthesize the new alkaloid in a chiral manner in order to confirm the structure including the absolute configuration due to one chiral center at the C20 position. First, we prepared the chiral epoxy ketone **19**, which is an essential synthon for the construction of the functionalized tetracyclic compound **23** (Fig. 4). Synthesis of the optically pure **19** was achieved by a combination of Corey asymmetric reduction and Sharpless asymmetric epoxidation as follows. By reducing enone **16** with a chiral oxazaborolidine catalyst,¹⁶ an optically active alcohol (*R*)-(+)-**17** was obtained in 97% ee. Next, the allylic alcohol **17** (97% ee) was subjected to Sharpless asymmetric epoxidation under kinetic resolution conditions¹⁷ to give the (–)-epoxide **18** in >99% ee. The enantiomeric excess of **18** was determined by chiral HPLC analysis of the *p*-bromobenzoate derivatives and the absolute configuration of the quaternary center was found to be (*S*) by using the well-established enantioselectivity principle.¹⁸ The secondary carbinol in **18** was then converted into a ketone by Swern oxidation to give the (–)-epoxy ketone **19**. The thus obtained epoxide (–)-**19** and 5-methoxy-3,4-dihydro- β -carboline (**22**), which was prepared from 4-methoxytryptamine (**20**) through *N*-formylation and subsequent Bischler–Napieralski reaction, were condensed in heated MeOH to afford two diastereomeric tetracyclic compounds (**23a, b**) in 33% and 17% yields, respectively. The C3 configurations of the major and minor products were deduced by comparison of their CD spectra. The major isomer **23a** was subjected to Knoevenagel condensation with dimethyl malonate in refluxing toluene in the presence of AcONH_4 and AcOH to give directly the pentacyclic product **24** having a lactone residue in 51% yield. Interestingly, the same product **24** was obtained starting from the minor isomer **23b**, through the isomerization at C3 during the condensation under acidic conditions. Finally, a double bond was introduced to the C3–14 position in **24** by a two-step operation (*i.* *t*-BuOCl, Et_3N , *ii.* ethanolic HCl and then NaHCO_3). The synthetic compound **14** was identified by comparison with the natural product in terms of chromatographic behavior and spectral data. The observed optical ro-

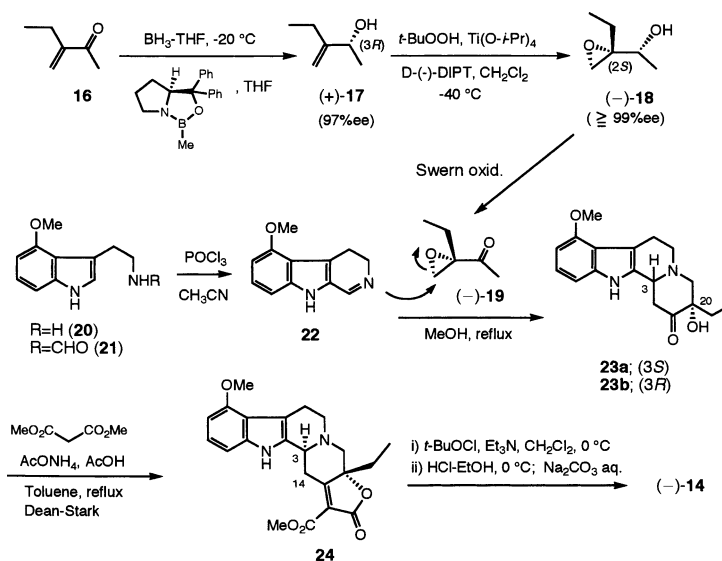
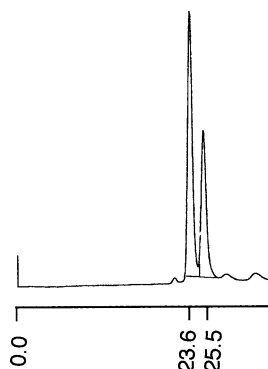


Fig. 4

Fig. 5. Chiral HPLC Analysis of Natural (-)-9-Methoxymitralactonine (**14**)

Conditions: Chiralcel OD; 20% EtOH/*n*-hexane; flow rate 0.5 ml/min; column temperature 30 °C.

tation of the synthetic compound was levorotatory, similar to that of the natural product; however, the specific rotation was very different $\{[\alpha]_D^{24} = -838^\circ (c=0.10, \text{CHCl}_3)\}$ from that of natural **14** $\{[\alpha]_D^{18} = -123^\circ (c=0.19, \text{CHCl}_3)\}$. Then, we synthesized racemic 9-methoxymitralactonine (**14**) starting from the achiral epoxy ketone **19** and analyzed the enantiomeric purities of both the synthetic (\pm)-**14** and (-)-**14** and the natural product by chiral column chromatography. As a result, we found that natural 9-methoxymitralactonine contained predominantly the (-)-enantiomer over the (+)-enantiomer in the ratio of 62 : 38 (Fig. 5).¹⁴

Concerning the structure elucidation of the new alkaloid, mitralactonine (**15**), the same approach as above was applied. As a result, we found that natural mitralactonine contained a slightly larger amount of the (-)-enantiomer than the (+)-enantiomer in the ratio of 54 : 46.¹⁵

2.3. Structure Revision of Mitragynaline and Corynantheidaline As mentioned above, mitragynaline and corynantheidaline were first isolated in 1991 and their novel structures were proposed as formulas **7** and **8**, respectively.¹² We have also obtained the same alkaloids by re-investigation of the young leaves of *M. speciosa* native to Malaysia, and have been keenly interested in these structurally unique alka-

loids from both synthetic and biological viewpoints. To establish an efficient and general synthetic route for the basic skeleton of these alkaloids, we initiated the synthesis of a simple alkaloid, nauclefidine (**28**).¹⁹ This compound was isolated from *Nauclea officinalis* and its UV spectrum was utilized for the structure elucidation of corynantheidaline and mitragynaline.¹² The pyridone ring possessing a C1 unit (formyl group) at the C14 position in formula **28** was prepared as follows (Fig. 6). The enamine **25**, prepared by the Bischler–Napieralski reaction of the amide,²⁰ was condensed with acryloyl chloride under two phase conditions to afford the dihydropyridone derivative (**26**) in 73% yield. The use of propioly chloride instead of acryloyl chloride under the same conditions gave the desired pyridone compound **27** in 52% yield. The ester group at C14 was converted into the aldehyde through DIBALH reduction (y. 80%) followed by oxidation of the resultant primary alcohol with activated MnO_2 (y. 88%). However, the synthetic compound **28** was not identical with natural nauclefidine in terms of mp, TLC behavior, and spectral data. Another structure **31** for nauclefidine, which would satisfy the spectroscopic data of the natural product, was suggested as a possible alternative. In particular, the unusual absorption maximum in the long-wavelength region (439 nm) in the UV spectrum of natural nauclefidine could be interpreted using structure **31** rather than **28**. Furthermore, from a biogenetic point of view, structure **31** seemed to be quite reasonable. Thus, this simple indole alkaloid could also be derived from strictosidine or related compounds, such as common monoterpenoid indole alkaloids.²¹ By fragmentation of the aglucone of strictosamide (**33**) or that of vincoside lactam (**34**), the C4 unit was eliminated and subsequent oxidation (aromatization) of the *D*-ring produced nauclefidine (**31**). To prove this structure, the total synthesis of **31** was carried out. The lactam ester **30** was prepared by Pictet–Spengler cyclization of tryptamine and the aldehyde **29**. A double bond was introduced to the C3–C14 position in **30** by oxidation with *t*-BuOCl followed by treatment with NaOMe in MeOH to obtain the dihydropyridone derivative in 74% yield. The *D*-ring was further oxidized with activated MnO_2 to afford the pyridone derivative **32** in 81% yield.

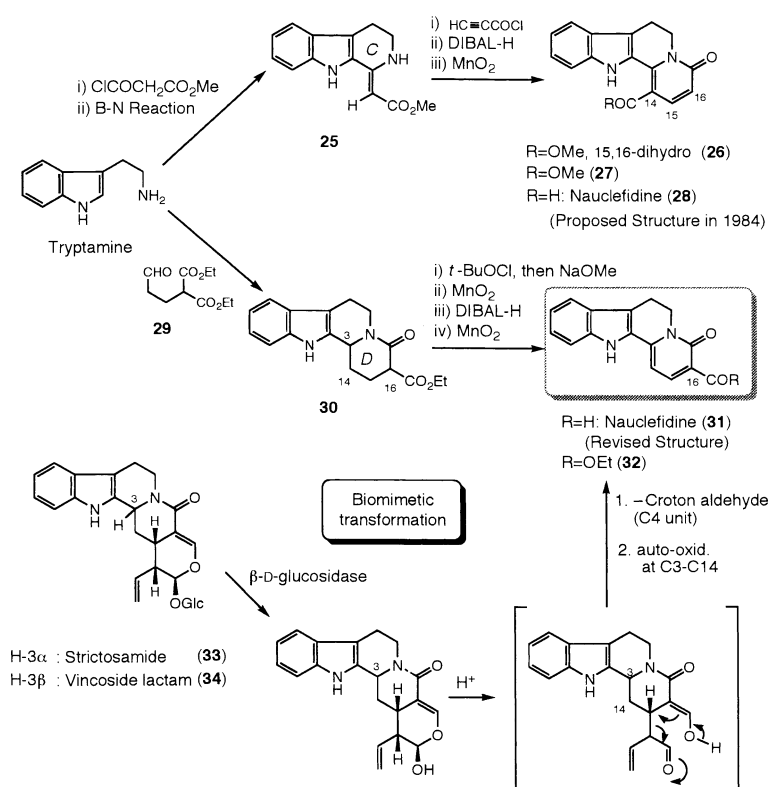
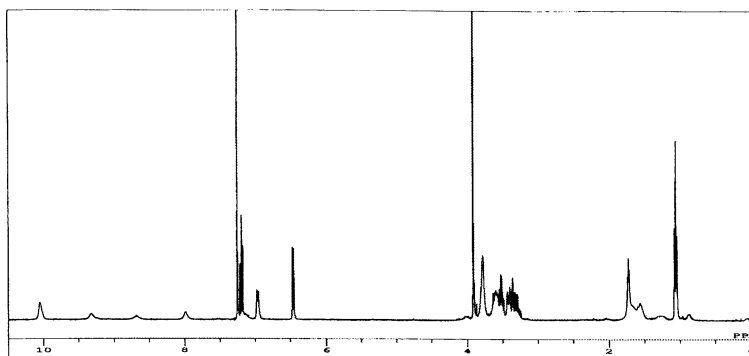


Fig. 6

Fig. 7. ^1H -NMR Spectrum of Mitragynaline (35) Measured at Ambient Temperature in CDCl_3

Finally, the ester group at the C16 position was transformed into the aldehyde *via* the reduction (DIBALH, y. 71%)-oxidation (MnO_2 , y. 89%) sequence. Direct comparison of the synthetic **31** with the natural product fully confirmed its identity. The structure of nauclefidine was determined to be 4,6,7,12-tetrahydro-4-oxo-indolo[2,3-*a*]quinolizine-3-carboxaldehyde.²²⁾ In keeping with the above biogenetic speculation, vincoside lactam aglucone (**34**) or strictosamide aglucone (**33**), corresponding to a plausible biogenetic precursor of **31**, was heated with 10% aqueous sulfuric acid in dioxane. Through the elimination of the crotonaldehyde unit and the subsequent auto-oxidation of the *D*-ring, nauclefidine (**31**) was produced, which was identical with the natural product in all respects. This result chemically supported our biogenetic hypothesis of nauclefidine.

Based on the above results concerning the structure revision of nauclefidine, we attempted to reconsider the structures of mitragynaline and corynantheidaline. However, these

alkaloids showed blunt signals in the ^1H - and ^{13}C -NMR spectra when measured at ambient temperature in CDCl_3 . As shown in Fig. 7, some widely swelled signals in the downfield region were observed in the ^1H -NMR spectrum of mitragynaline. In the ^{13}C -NMR spectrum, only sixteen signals including some tiny peaks were observed, although the presence of twenty-two carbons in the molecule was demonstrated by the high-resolution MS spectrum. When the solvent was changed to $\text{DMSO}-d_6$, two clear signals (δ 11.7, 9.8) appeared in the downfield region in the ^1H -NMR spectrum. Further, the ^{13}C signals were also sharpened generally, but only seven and thirteen signals were respectively observed in the sp^2 and sp^3 regions. On the other hand, by measuring the NMR spectra of mitragynaline at a low temperature (-50°C) in CDCl_3 , all the proton and carbon signals corresponding to the molecular formula appeared. The presence of a 9-methoxyindole nucleus, an ethane bridge at C5-C6, an ethyl group at C20, an sp^3 carbon at C21, and a

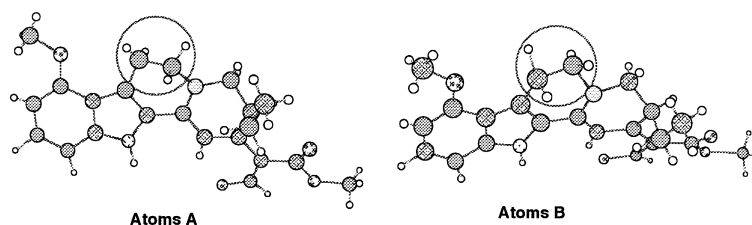


Fig. 8. X-Ray Crystallographic Analysis of Mitragynaline (35)
Two conformers, atoms A and B, exist in the ratio of 1 : 1 in the crystal.

methoxycarbonyl group, which are the fundamental structural units of common Corynanthe-type monoterpene indole alkaloids, was clarified. The ^{13}C -NMR and HMBC spectra at low temperature disclosed the presence of six conjugated sp^2 carbons including an ester carbon and an aldehyde carbonyl carbon, besides the aromatic carbons due to the indole nucleus. The UV spectrum exhibiting long-wavelength absorption at 485 nm also indicated a high degree of unsaturation in the molecule. The characteristic proton signal observed at δ 8.28 (1H, singlet), which was finally detected at low temperature, was unambiguously assigned to the proton at C14 by the HMQC spectrum, and this signal had HMBC connectivities between the C2, C3, C15, C16 and C20 carbons. The HMBC correlation between the aldehyde proton at δ 10.0 and the carbons at C15 and C16 indicated the location of the aldehyde residue at C17. All the above findings as well as biogenetic consideration enabled us to determine the molecular structure of mitragynaline to be that shown in formula 35. The geometry at C16 was confirmed by NOE observation between H14 and an aldehyde proton. Finally, the structure was determined by X-ray crystallographic analysis. Interestingly, the crystals composed of two conformers, A and B, in the ratio of 1 : 1 (Fig. 8), which might have been responsible for the blunt signals in the ^1H - and ^{13}C -NMR spectra of mitragynaline. When mitragynine (1) was treated with DDQ, we obtained unexpectedly mitragynaline (35) in 13% yield as one of the oxidation products (Fig. 9). The semi-synthetic compound was identified by comparison with the natural product in terms of chromatographic behavior and spectral data. The optical rotation of the semi-synthetic compound was levorotatory, establishing the absolute stereochemistry at the C20 position.²³⁾ Based on the spectroscopic comparison of mitragynaline (35) and corynantheidaline, the new structure 36 as corynantheidaline was proposed.

3. Analgesic Activity of *Mitragyna speciosa* and Its Constituents

In 1988, Jansen and Prast reviewed the literature published since 1907 on *M. speciosa*, including those on pharmacological properties and the clinical case reports of Kratom users.¹⁾ Macko *et al.* reported that mitragynine (1) exhibits antinociceptive and antitussive activities after oral (*o.p.*), subcutaneous (*s.c.*) or intraperitoneal (*i.p.*) administration in rodents.³⁾ However, there appeared to be some qualitative differences in the pharmacological properties between mitragynine and narcotic analgesics. For example, nalorphine, an opioid receptor antagonist, failed to antagonize the antinociceptive activity of orally administered mitragynine in the tail-flick test in rats. Their findings suggest that non-opioid sys-

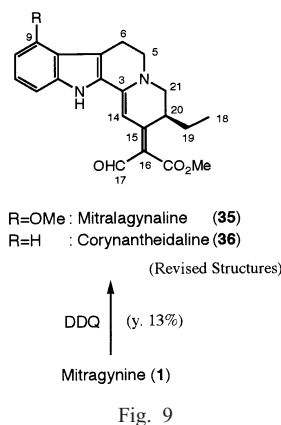


Fig. 9

tems are involved in the activity of mitragynine (1), the major constituent of the herb, in spite of the opium-like use of Kratom. In collaboration with Drs. Watanabe and Horie in Chiba University, we evaluated the opioid agonistic activity of the crude MeOH extract of *M. speciosa* from Thailand by measuring twitch contraction induced by electrical stimulation in guinea pig ileum preparation. The crude extract (1—300 $\mu\text{g}/\text{ml}$) inhibited the twitch contraction in a concentration-dependent manner, and this inhibitory effect was reversed by an opioid receptor antagonist, naloxone. This result indicated that the crude extract has an opioid agonistic effect.²⁴⁾ The crude extract was then partitioned into crude base, *n*-BuOH, and water-soluble fractions by means of activity-guided fractionation. We confirmed the antinociceptive activity of the crude base fraction in mice in the PBQ writhing, hot-plate, pressure and formalin test. The crude base fraction was found to also inhibit twitch contraction.^{24,25)} To clarify the active principle in the alkaloidal fraction, the crude base was separated by column chromatography as mentioned above (Section 2.1) to give five alkaloids including one new compound. Initially, several pharmacological evaluations of the major alkaloid, mitragynine (1), were performed to give the following significant results.^{26–28)} Mitragynine (1) showed antinociceptive activity in mice in the PBQ writhing test in a dose-dependent (3.0—100 mg/kg, *p.o.*) manner and in the formalin-induced pain response test in the dose range of 30—60 mg/kg, *p.o.* The effect of 1 on electrically stimulated contraction was next studied in a guinea pig ileum preparation. Mitragynine (1) (1 nM—3 μM) inhibited ileum contraction elicited by electrical stimulation, and its pD_2 value was 6.59 ± 0.13 ($n=5$); the inhibitory effect was reversed by the addition of naloxone. Its potency is one-fourth of that of morphine (37). Mitragynine did not show any effect on smooth muscle contraction induced by acetylcholine or histamine. The results suggest that mitragynine in-

hibits the electrically stimulated contraction of guinea pig ileum through opioid receptors. The affinities for μ -, δ -, and κ -opioid receptors were determined by displacement upon binding of [^3H]DAMGO, [^3H]DPDPE, and [^3H]U69593, respectively, to guinea pig brain membranes. The binding assay demonstrated that **1** has relatively high selectivity for μ -opioid receptors.²⁹⁾

In cooperation with Drs. Watanabe and Matsumoto in Toyama Medical and Pharmaceutical University, the mode of action of mitragynine (**1**) was studied with a different approach. Using the tail-pinch and hot-plate tests, the antinociceptive activity of **1** after i.p. and intracerebroventricular (i.c.v.) injection into mice was investigated.³⁰⁾ Mitragynine (5.0–30 mg/kg, i.p. and 1.0–10 μg /mouse, i.c.v.) exerted a dose-dependent antinociceptive activity that was maximal at 15–45 min after injection. The antinociceptive activity of i.p. administered mitragynine was completely abolished by both s.c. and i.c.v. administered naloxone. The activity of i.c.v. administered mitragynine was also antagonized by i.c.v. administered naloxone. These results indicate that mitragynine itself can induce antinociceptive activity by acting in the brain, and that the supraspinal opioid systems are at least partly involved in the antinociceptive activity in mice. Next, the roles of central monoaminergic systems in the antinociceptive activity of mitragynine (**1**) were investigated.³¹⁾ Supraspinally administered mitragynine exerted antinociceptive activity in the tail-pinch and hot-plate tests. Both descending noradrenergic and serotonergic systems were involved in the antinociceptive activity of mitragynine on mechanical noxious stimulation, whereas the descending noradrenergic system contributed predominantly to the activity of mitragynine on thermal noxious stimulation. Moreover, a study of the effect of mitragynine (**1**) on cAMP formation in NG108-15 cells possessing δ -opioid receptors and $\alpha 2\text{B}$ -adrenoceptors has suggested that mitragynine (**1**) acts directly on opioid receptors but not on $\alpha 2$ -adrenoceptors, to show antinociceptive activity.³²⁾ An investigation of the effects of mitragynine (**1**) on the head-twitch response induced by 5-methoxy-*N,N*-dimethyltryptamine in mice indicated that the stimulation of postsynaptic $\alpha 2$ -adrenoceptor, the blockade of 5-HT_{2A} receptors, or both, are involved in the suppression of 5-HT_{2A} receptor mediated head-twitch response by mitragynine.³³⁾ In addition, it was suggested that antinociception caused by i.c.v. administration of mitragynine is dominantly mediated by μ - and δ -opioid receptor subtypes, and that the selectivity of mitragynine for the supraspinal opioid receptor subtypes differs from that of morphine in mice.³⁴⁾ Recently, Tsuchiya *et al.* reported that mitragynine (**1**), similar to morphine, inhibits 2-deoxy-D-glucose-stimulated gastric acid secretion in urethane-anesthetized rats through opioid receptors.³⁵⁾

The results of pharmacological evaluations of minor constituents, speciogynine (**2**), speciociliatine (**3**), paynantheine (**4**), and 7-hydroxymitragynine (**5**), are discussed in sections 5 and 6.

4. Synthesis of Mitragynine and Its Derivatives for SAR Study

The above findings of the potent pharmacological effects of mitragynine (**1**), particularly its analgesic activity *via* opioid receptors, prompted us to embark on the synthesis of

novel lead compounds based on the mitragynine skeleton, which may be utilized for the development of practical analgesics and as a new type of probe for the study of opioid receptor systems.

4.1. Asymmetric Total Synthesis of Mitragynine In order to supply a large quantity of mitragynine (**1**) for structure–activity relationship (SAR) study, we first planned the development of an efficient route for the asymmetric total synthesis of mitragynine (**1**). We initiated the total synthesis from the preparation of the optically pure alcohol (*R*)-**40**. The racemic acetate **38**, which was prepared from commercially available 6-chloronicotinic acid, was subjected to enzymatic hydrolysis using Lipase SAM II under phosphate-buffered (pH 7.0) conditions to produce the secondary alcohol (+)-**40** (32% chemical yield, 100% ee) and the acetate (–)-**38** (38% chemical yield, 100% ee). Alternatively, by reduction of the ketone derivative **39** using a chiral oxazaborolidine catalyst,¹⁶⁾ an optically active alcohol (+)-**40** (93% ee) was obtained in 80% yield, which was then esterified with (*R*)-*O*-methylmandelic acid in order to prepare the optically pure alcohol **40**. The resulting diastereomeric mixture was separated by column chromatography and then the diastereomers were respectively hydrolyzed to give the enantiomerically pure alcohols **40**. The optically pure alcohol (–)-**40**, which was obtained by the hydrolysis of (–)-**38** or the chiral reduction route, could be converted into its enantiomer (+)-**40** using the sequential Mitsunobu reaction/alkaline hydrolysis. The other part, 4-methoxytryptophylbromide (**41**), was prepared from 4-hydroxyindole *via* a five-step operation (i. *O*-methylation, ii. reaction with oxalyl chloride, iii. ethanolytic reduction with LiAlH₄, and v. bromination with PBr₃). The thus obtained bromide **41** and the optically pure pyridine derivative (*R*)-**40** were condensed in heated benzene in the presence of a catalytic amount of sodium iodide. The pyridinium salt **42** was then reduced with sodium borohydride to yield two diastereomers (**43**, **44**) in 33% and 27% yields, respectively. Although two isomers were formed in the reduction step, we anticipated that the configuration at C3 could be settled in the desired form in the subsequent reactions. Then, in order to attach an acetic acid residue to the C15 position, each allylic alcohol (**43**, **44**) was subjected to Claisen rearrangement.^{36–39)} By heating with trimethyl orthoacetate in the presence of a catalytic amount of benzoic acid in *o*-xylene, **43** and **44** produced acetates **45** and **46**, respectively, as the sole product. The absolute configurations at C3 in **45** and **46** were clearly determined from the CD spectra.⁴⁰⁾ Furthermore, the stereochemistry at C15 and C19 could be elucidated by ¹H- and ¹³C-NMR analyses. Compound **45** had the appropriate absolute configuration at the C3 and C15 positions for further transformation into mitragynine, whereas the other isomer **46** had the opposite configuration at C3, which could be inverted into **45** by an oxidation-reduction sequence *via* a 3,4-dehydroimmonium salt. In this manner, we could convergently prepare the optically pure Corynanthe-type compound **45**. Next, according to the conventional method (LDA, HCO₂Me), a formyl group was introduced to C16 in **45**, which was then converted to the dimethyl acetal derivative. Treatment of the acetal with KO^tBu gave the methyl enol ether in good yield. Finally, by stereoselective reduction of the double bond at the C19–20 positions over PtO₂ under H₂ atmosphere, the target compound, mitragynine

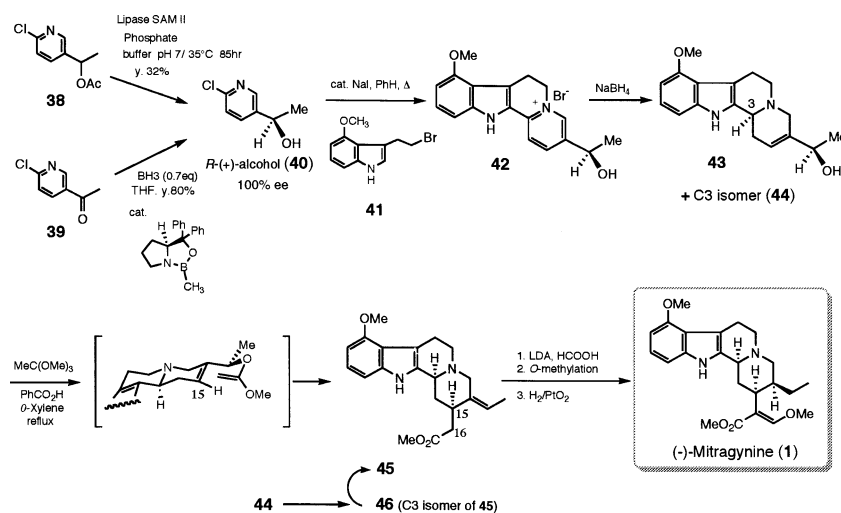


Fig. 10

(**1**), having the natural absolute configuration was obtained (Fig. 10).⁴¹⁾

4.2. Preparation of Mitragynine Derivatives In investigating SAR, we initially directed our attention to the presence of a methoxyl group at the C9 position on the indole ring in mitragynine (**1**), because it was a structural characteristic of *Mitragyna* alkaloids, compared with common Corynanthe-type indole alkaloids isolated from other plant genera.⁴²⁾ As is described in section 5 in detail, corynantheidine (**47**), which corresponds to 9-demethoxymitragynine, was devoid of opioid agonistic activity in guinea pig ileum preparation. This finding indicated that the methoxy group at C9 in **1** is essential for eliciting the analgesic activity. Based on this result, we carried out the chemical modification of the C9 function in mitragynine (**1**). As shown in Fig. 11, the methyl ether at C9 was selectively cleaved with EtSH and AlCl₃ in CH₂Cl₂ to give 9-hydroxycorynantheidine (**48**) in 90% yield. The thus obtained phenolic function was converted into ethyl, *i*-propyl and methoxymethyl ether derivatives (**49**, **50**, **51**). In addition, acetate **52** was prepared with a view to its relation to morphine and heroin. The N_b oxide derivative **53** was also prepared by treatment of **1** with 1 equiv of *m*-chloroperbenzoic acid. Modification of the β-methoxyacrylate residue in **1** was also carried out to obtain three compounds (**54**, **55**, **56**).

In 1974, Zarembo *et al.* reported that mitragynine pseudoindoxyl (**57**), which was obtained by the microbial transformation of **1** by the fungus *Helminthosporium* sp., exhibited in the D'Amour–Smith test an almost tenfold higher analgesic activity than mitragynine.⁴³⁾ Based on this information, we prepared some oxidative derivatives of the indole nucleus in **1**, as shown in Fig. 12. Treatment of **1** with lead tetraacetate (Pb(OAc)₄)^{44,45)} gave the 7-acetoxyindolenine derivative **58** in moderate yield. By alkaline hydrolysis of **58**, 7-hydroxymitragynine (**5**), a minor constituent in the leaves of *M. speciosa*, was obtained in 95% yield. Treatment of **5** with sodium methoxide in methanol gave the pseudoindoxy derivative **57**.⁴⁶⁾ Mitragynine (**1**) afforded 7-methoxy or 7-ethoxy indolenine derivative (**59** or **60**) by treatment with iodobenzene diacetate in MeOH⁴⁷⁾ or EtOH, respectively. Treatment of mitragynine (**1**) with NaH in DMF in air gave the 4-quinolone⁴⁸⁾ derivative **61** in 49% yield.

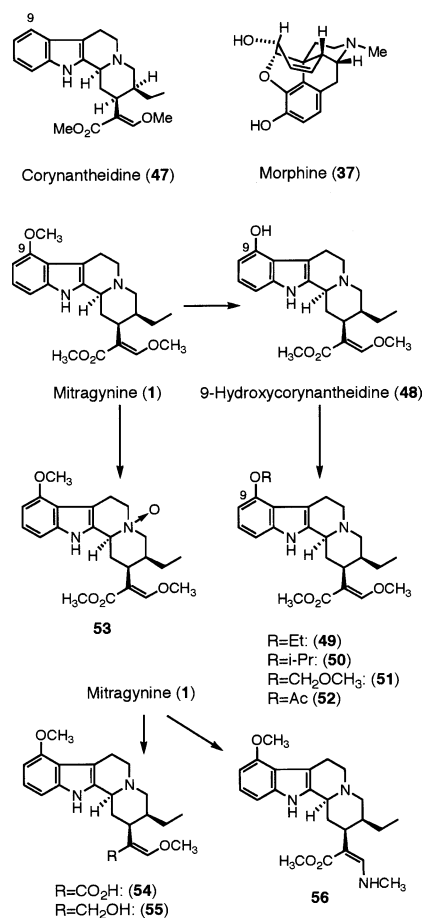


Fig. 11

At this stage, we re-investigated the oxidation of **1** with Pb(OAc)₄ or hypervalent iodine reagents in order to develop an efficient method for the preparation of 7-hydroxymitragynine (**5**), because this compound was proved to be a highly potent analgesic and was required for further biological evaluation (*vide infra*). In general, 7-acetoxyindolenine derivatives are prepared from the corresponding indoles by oxidation with Pb(OAc)₄.^{44,45)} When yohimbine was treated with Pb(OAc)₄, the 7-acetoxyindolenine derivative was obtained

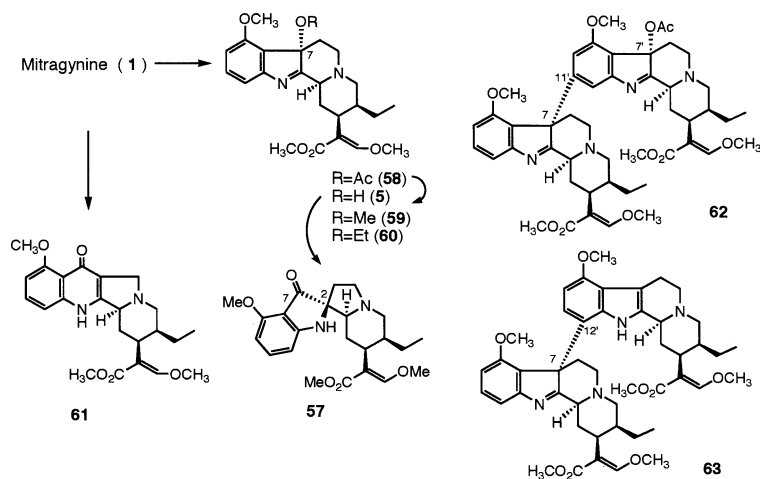


Fig. 12

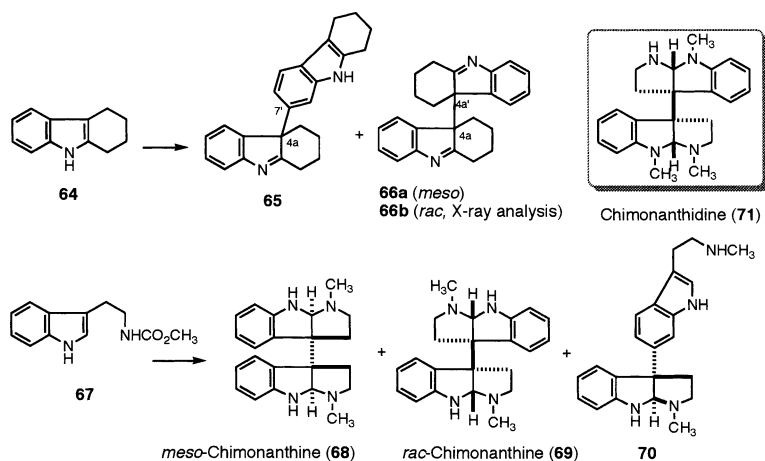


Fig. 13

in quantitative yield. However, in the case of mitragynine (**1**), the yield of the desired indolenine **58** was 50% at most, and the presence of by-products was shown on thin-layer chromatography. By careful purification of the reaction residue, we isolated **58** together with an unusual dimeric compound **62** in 3% yield (Fig. 12).⁴⁹ In order to prepare 7-hydroxymitragynine (**5**) more conveniently, mitragynine (**1**) was exposed to a hypervalent iodine(III) reagent, PIFA, in aqueous CH_3CN , expecting the introduction of a hydroxyl group at C7, to give the desired product **5** in 50% yield.⁵⁰ Investigation of the minor products in the residue led to the isolation of a novel dimeric compound **63** in 6% yield (Fig. 12). The structure of **63** was determined by spectroscopic analysis, in particular, by measuring the NMR spectra at -50°C . To examine the generality of the dimerization of indoles with hypervalent iodine(III), we chose a simple indole derivative, 1,2,3,4-tetrahydrocarbazole (**64**), as substrate (Fig. 13). Oxidation of **64** with 0.5 equiv of PIFA in dry CH_2Cl_2 gave a dimeric compound **65** having a C–C bond between the β position of an indolenine unit and the C7 position of an indole nucleus, in 77% yield, together with trace amounts of two different types of dimers (**66a**, **b**). Quite interestingly, these dimeric compounds, which have a linkage between the β – β' positions of two indolenine units, were obtained as major products in 42% total yield, when phenyliodine(III) diacetate

(PIDA) was used as a reagent instead of PIFA. These two dimeric compounds, *i.e.*, *meso* (**66a**, less polar) and *racemic* (**66b**, more polar), could be separated by SiO_2 chromatography, and the structure of **66b** was determined by X-ray analysis. Using this new synthetic procedure, we attained the total synthesis of chimonanthines,⁵⁰ which are dimeric pyrrolidinoindoline alkaloids isolated from plants belonging to Calycanthaceae, Idiospermaceae, and Rubiaceae, as well as from dendrobatid frog. The potent antinociceptive activity of dimeric or polymeric pyrrolidinoindoline alkaloids that interact with opioid receptors was recently reported by Elisabet-sky–Verotta's group.^{51,52} The syntheses of these alkaloids have been accomplished so far by using the direct coupling reaction of tryptamine derivatives under various conditions^{53–57} as well as by employing a multistep procedure *via* an elegant strategy.^{58–60} Initially, we treated N_b -carbo-methoxytryptamine (**67**) with PIDA under the conditions developed above; however, this resulted mainly in the recovery of the starting material. After several attempts under various conditions, we succeeded in the preparation of the desired dimeric compounds in good yield (Fig. 13). Thus, compound **67** was treated with 0.5 equiv of PIFA in $\text{CF}_3\text{CH}_2\text{OH}$ at -30°C and the resultant residual mixture of carbamates, without purification, was reduced with Red-Al in toluene to afford *meso*-chimonanthine (**68**, 30% yield) and *rac*-chimo-

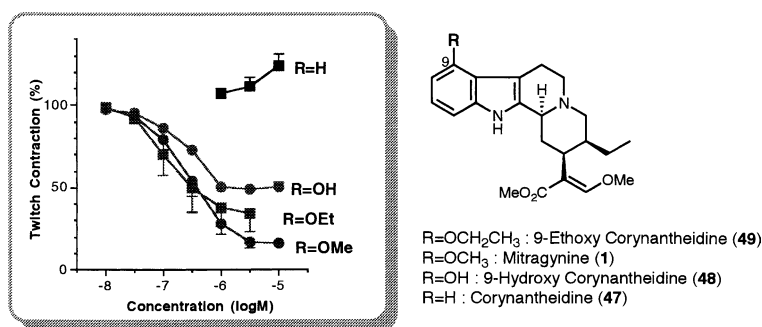


Fig. 14. Effect of the Substituents at C9 in Mitragynine on Twitch Contraction Induced by Electrical Stimulation in the Guinea-Pig Ileum

nanthine (**69**, 13% yield), together with a dimeric compound (**70**, 23% yield) and N_b -methyltryptamine (9% yield). This newly developed procedure was applied to the structure elucidation of a new alkaloid, chimonanthidine (**71**), recently isolated from *Calycanthus praecox* in our laboratory.⁶¹

5. Pharmacological Evaluation of Mitragynine Analogues for Opioid Receptors and SAR Study

The opioid agonistic activities of the natural analogue of mitragynine and semi-synthetic compounds derived from mitragynine (**1**) were evaluated by measuring twitch contraction induced by electrical stimulation in guinea pig ileum preparation. Similarly to that above, the affinities for μ -, δ -, and κ -opioid receptors were determined by displacement upon binding of [³H]DAMGO, [³H]DPDPE, and [³H]U69593, respectively, to guinea pig brain membranes. The pharmacological effects of mitragynine (**1**) itself have been described in section 3.

The 9-demethoxy analogue of mitragynine, *i.e.*, corynantheidine (**47**), did not show any opioid agonistic activity at all (Fig. 14), but reversed the morphine-inhibited twitch contraction in guinea pig ileum.²⁹ Its antagonistic effect was concentration-dependent. Corynantheidine (**47**) did not affect the muscarinic receptor antagonist atropine- or the Ca^{2+} channel blocker verapamil-inhibited twitch contraction. These results suggest that corynantheidine (**47**) inhibits the effect of morphine (**37**) *via* functional antagonism of opioid receptors. The receptor binding assay proved that corynantheidine (**47**) selectively binds to μ -opioid receptors. Taken together, corynantheidine (**47**) was found to have an antagonistic effect on μ -opioid receptors. The 9-demethyl analogue of mitragynine, 9-hydroxycorynantheidine (**48**), also inhibited the electrically induced twitch contraction in guinea pig ileum, but its maximum percentage inhibition was lower than that of mitragynine (Fig. 14). The receptor binding assay clarified that 9-hydroxycorynantheidine (**48**) binds to μ -opioid receptors. Taken together, the results suggest that 9-hydroxycorynantheidine (**48**) is a partial agonist of opioid receptors. It is interesting that a transformation of the substituent at C9, *i.e.*, from OMe to OH or to H, led to a shift of activity from a full agonist *via* a partial agonist to an antagonist of opioid receptors. Thus, it was found that the functional group at C9 of mitragynine-related compounds manages the relative inhibitory activity, which means the intrinsic activity on opioid receptors. The introduction of an acetoxy group at C9 on the indole ring (compound **52**) led to marked reduction of both intrinsic activity and potency compared

with those of mitragynine (**1**). Compounds **49** and **50**, having the elongation of the methyl group of 9-methoxy ether, induced naloxone-insensitive inhibition of twitch contraction (Table 1), suggesting an inhibitory effect *via* mechanisms distinct from those of the stimulation of opioid receptors. Compound **51** did not show any opioid agonistic activities. The results demonstrate that the intrinsic activities of the compounds on opioid receptors are determined by the functional groups at the C9 position, and that a methoxy group at the C9 position is the most suitable functional group for pharmacophore binding to opioid receptors.

Speciociliatine (**3**), a minor constituent of this plant, is the C3 stereoisomer of mitragynine, and assumes a folded *cis*-quinolizidine conformation in the *C/D*-ring junction, as depicted in Fig. 15. The potency of this compound to opioid receptors was 13-fold lower than that of mitragynine (**1**) in ileal preparation (Table 1), indicating that the flat *trans*-quinolizidine form was a more efficient conformation for exhibiting the activity than the folded *cis*-form. This compound inhibited the twitch contraction in a naloxone-insensitive manner. Speciogynine (**2**) and paynantheine (**4**) inhibited the twitch contraction in a naloxone-insensitive manner, and inhibited the contraction induced by direct stimulation of muscarine receptors on ileal smooth muscle. Therefore, both alkaloids act on ileal smooth muscle, leading to the inhibition of the electrically induced twitch in guinea pig ileum preparation. The compounds (**54**–**56**) modifying the β -methoxyacrylate moiety in **1** exhibited very weak or no opioid agonistic activity, indicating that this function is essential for binding to the opioid receptors. The N_b oxide derivative (**53**) showed no opioid agonistic activity in guinea pig ileum preparation. The N_b -lone electron pair was also found to be essential for opioid agonistic activity. These results suggest that the N_b -lone electron pair of this series of compounds constitutes the pharmacophore binding to opioid receptors.

7-Hydroxymitragynine (**5**), a minor constituent of *M. speciosa*, was found to exhibit high potency to opioid receptors: approximately 13- and 46-fold higher than morphine (**37**) and mitragynine (**1**), respectively (Fig. 16). The intrinsic activity of 7-hydroxymitragynine (**5**) suggests its full agonistic effect on opioid receptors. The introduction of a hydroxyl group at the C7 position led to a higher potency compared with mitragynine (**1**). 7-Hydroxymitragynine (**5**) tends to show selectivity for μ -opioid receptors, and its relative affinity for κ -opioid receptors is higher than that of mitragynine (**1**). The potent antinociceptive activities of this compound in mice are described in section 6. In turn, the 7-acetoxy deriva-

Table 1. Opioid Agonistic Activities of Mitragynine-Related Compounds and Morphine in Electrically-Stimulated Guinea-Pig Ileum Preparation

Compounds	<i>n</i>	pD ₂ Value	Relative potency	Maximum inhibition (%)	Relative inhibitory activity
37 (Morphine)	5	7.17±0.05	100%	87.2±1.8	100%
1 (Mitragynine)	5	6.59±0.13**	26%	83.1±3.7	95%
57	6	8.71±0.07***	3467%	83.5±3.3	96%
37	5	—	—	-18.1±8.6***	-21%
48	5	6.78±0.23	41%	49.4±3.1***	57%
49 ^{a)}	5	NS	NS	NS	NS
50 ^{a)}	5	NS	NS	NS	NS
51 ^{b)}	5	NE	NE	NE	NE
52	5	5.39±0.12	2%	33.2±8.8***	38%
53	5	—	—	-123.5±39.2***	-142%
58	6	6.50±0.16**	21%	13.4±12.7***	15%
5	5	8.20±0.14***	1071%	86.3±4.8	99%
59	5	6.45±0.04***	19%	60.9±7.2**	70%
60	5	5.29±0.12***	1%	22.9±1.1***	26%
61	5	6.70±0.04***	34%	74.1±5.6	85%
3	5	5.40±0.07***	2%	85.9±2.7	101%

Relative potency is expressed as a percentage of the pD₂ value of the compound against that of morphine. Maximum inhibition (%), which is elicited by the compound when the response reached a plateau, was calculated by regarding electrically-induced contraction as 100%. The concentration range of tested compounds was from 100 pM to 30 μM. Relative inhibitory activity, which means intrinsic activity on opioid receptors, is expressed as a percentage of the maximum inhibition by compounds against that by morphine. Each value represents the mean±S.E.M. of the results obtained from five to six animals (*n*). ***p*<0.01, ****p*<0.001, significantly different from the morphine group. *a*) In the case of the naloxone-insensitive inhibition, the effect was regarded as “non-specific (NS)”. *b*) In the case that significant inhibition was not obtained at 30 μM of the compound, the effect was regarded as “no effect (NE)”.

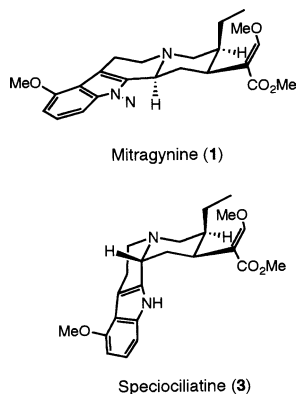


Fig. 15. Stereostructure of Mitragynine (1) and Speciociliatine (3)

tive (58) has lower intrinsic activity than mitragynine (1), although its potency is nearly equal to that of mitragynine (1) (Table 1). The introduction of a methoxy or an ethoxy group at the C7 position (compounds 59, 60) led to a marked reduction in both intrinsic activity and potency to opioid receptors (Table 1). It is conceivable that the hydroxyl group at the C7 position in the mitragynine skeleton is necessary for the increased potency to opioid receptors. 4-Quinolone derivative (61) retained almost the same opioid agonistic activity as that of mitragynine (1).

Mitragynine pseudoindoxyl (57) (100 pM–30 nM) inhibited the electrically stimulated ileum contraction in a concentration-dependent manner,⁶²⁾ and its pD₂ value was 8.71±0.07 (Table 1). This potency was approximately 100- and 20-fold higher than those of mitragynine (1) and morphine (37), respectively (Fig. 16). On the other hand, it had no effect on the contraction elicited by direct stimulation of the cholinergic receptors on smooth muscle. Moreover, mitragynine pseudoindoxyl (1 nM–1 μM) also inhibited the electrically stimulated vas deferens contraction in a concentration-dependent manner, affording a pD₂ value of

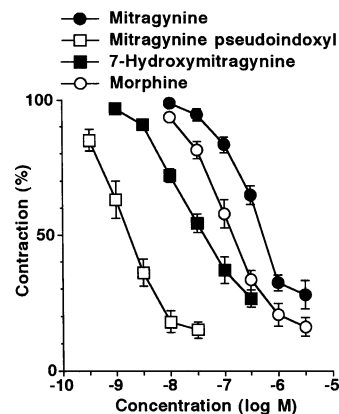


Fig. 16. Concentration Response Curves for Inhibitory Effect of Mitragynine (1), Mitragynine Pseudoindoxyl (57), 7-Hydroxymitragynine (5), and Morphine (37) on Twitch Contraction Induced by Electrical Stimulation in the Guinea-Pig Ileum

7.40±0.11, which was 35-fold higher than that of morphine (pD₂=5.85±0.08). The inhibitory effects of mitragynine pseudoindoxyl (57) on electrically stimulated ileum and vas deferens contractions were completely reversed by naloxone and naltrindole, respectively. In the receptor binding assay, mitragynine pseudoindoxyl (57) showed similar affinities to DAMGO and DPDPE at μ- and δ-opioid receptors, respectively. However, it showed negligible affinity at κ-opioid receptor.

6. Antinociceptive Activity of Mitragynine Pseudoindoxyl and 7-Hydroxymitragynine in Mice

Among the mitragynine derivatives described above, mitragynine pseudoindoxyl (57) and 7-hydroxymitragynine (5) emerged as the compounds with potential analgesic effects in the guinea pig ileum test (Fig. 16). In this regard, the antinociceptive activities of these compounds were evaluated in mice.

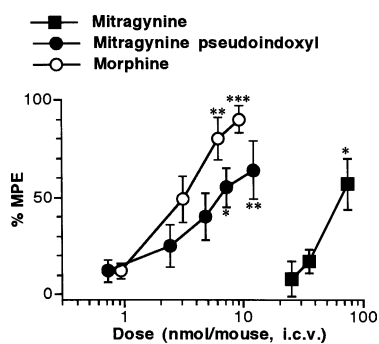


Fig. 17. Dose-Response Curves for Analgesic Activities Induced by Intracerebroventricular Administration of Mitragynine (1), Mitragynine Pseudoindoxyl (57) and Morphine (37) in Tail-Flick Test in Mice

Tail flick latencies are presented as a percentage of maximum possible effect (% MPE).

Mitragynine pseudoindoxyl (57), administered by i.c.v. injection, showed antinociceptive activity in the tail-flick test in mice. Its activity reached a maximum at approximately 15–45 min after the injection. The activity of mitragynine pseudoindoxyl (57) is lower than that of morphine (Fig. 17). In addition, the activity of mitragynine (1) is lower than that of mitragynine pseudoindoxyl (57) (Fig. 17). The analgesic effects of these compounds are dose-dependent. The EC_{50} value estimated for mitragynine pseudoindoxyl (57) was 6.51 nmol/mouse (95% confidence limit, 3.78–11.20 nmol/mouse), whereas that of morphine (37) was 3.20 nmol/mouse (95% confidence limit, 2.11–4.88 nmol/mouse). The EC_{50} value estimated for mitragynine (1) was 60.22 nmol/mouse (95% confidence limit, 39.10–92.75 nmol/mouse). The antinociceptive activities of mitragynine (1), mitragynine pseudoindoxyl (57) and morphine (37) were completely inhibited by naloxone at 2 mg/kg, s.c. Therefore, these compounds induce analgesic activity *via* opioid receptors. Mitragynine pseudoindoxyl (57) exhibited lower analgesic activity than morphine, in spite of the very high opioid agonistic activity in the isolated guinea pig ileum test. It is speculated that the low analgesic activity of mitragynine pseudoindoxyl (57) results from the instability of the compound in the brain.

Administration of 7-hydroxymitragynine (5) (2.5–10 mg/kg, s.c.) induced dose-dependent antinociceptive activities in tail-flick and hot-plate tests in mice.⁶³ Its activity was higher than that of morphine (Fig. 18) in both tests. It is noteworthy that 7-hydroxymitragynine (5) possesses potent antinociceptive activity after oral administration (5–10 mg/kg) in the tail-flick (Fig. 19) and hot-plate tests. By contrast, orally administered morphine (20 mg/kg) did not show antinociceptive activity in either test. These results suggest that 7-hydroxymitragynine (5) may be well absorbed when administered orally. It is well known that morphine administered orally is rapidly metabolized in the liver and excreted in urine. Consequently, a large amount of morphine is administered orally when it is clinically used as an analgesic. However, the repeated administration of morphine leads to induction of tolerance, and high doses of morphine may elicit undesirable side effects.

In conclusion, by chemical and pharmacological investigation on the indole alkaloids from the rubiaceae plant, *M. speciosa*, we found a promising lead molecule, 7-hydroxymi-

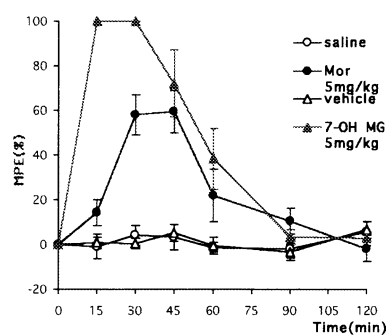


Fig. 18. Antinociceptive Effects of 7-Hydroxymitragynine (7-OH MG, 5) and Morphine (Mor, 37) in the Tail-Flick Test in Mice (s.c.)

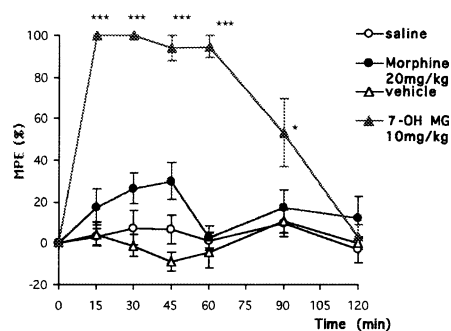


Fig. 19. Antinociceptive Effects of 7-Hydroxymitragynine (7-OH MG, 5) and Morphine (Mor, 37) in the Tail-Flick Test in Mice (p.o.)

tragnine (5), for the development of new types of analgesics having structures different from that of morphine. Moreover, considering the opioid agonistic activities and the unique analgesic properties described above, it appears that 7-hydroxymitragynine (5) may be the active principle of the herb used in traditional medicine. Further chemical and pharmacological studies on *Mitragyna alkaloids* are in progress in our laboratories.

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