Serotonergic Receptor Antagonist from *Nandina domestica* Thunberg

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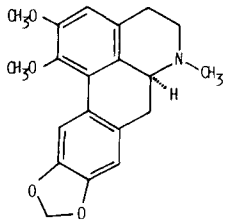
Abstract □ A crude methanolic extract of the fruit of *Nandina domestica* Thunberg strongly inhibits serotonin-induced contractions of the rabbit aorta. The extract of the fruit has been fractionated to afford nantenine (*O*-methyldomesticine) as the active agent.

Keyphrases □ *Nandina domestica* Thunberg—nantenine, extraction, serotonergic receptor antagonist, isolated rabbit aorta □ Nantenine—serotonergic receptor antagonist extracted from *Nandina domestica* Thunberg, isolated rabbit aorta □ Serotonergic receptor antagonist—nantenine, extracted from *Nandina domestica* Thunberg, isolated rabbit aorta

The fruit of *Nandina domestica* Thunberg (Berberidaceae) has been used to treat asthma, whooping cough, pharynx tumor, and uterine bleeding in Japan for many years (1). Chemical studies on the constituents of the plant have revealed that presence of numerous alkaloids in this species (1–18). Initially, it was found that the crude methanolic extract of the fruit selectively inhibited serotonin-induced contractions of isolated rabbit aorta without producing inhibitory effects on contractions induced by potassium chloride or histamine. In this paper, the isolation and the determination of the chemical structure of the antiserotonergic substance from the fruit are described.



Fruits of *Nandina domestica* Thunberg (4 kg)
↓ extracted with methanol
Methanolic extract (729 g) (+)
↓ partitioned between ethyl acetate and water

- Ethyl acetate-soluble portion (136 g) (−)
- Water-soluble portion (+) → extracted with *n*-butyl alcohol
  - *n*-Butyl alcohol-soluble portion (158 g) (+) → chromatographed on silica gel (chloroform-methanol)
    - Fraction I (37 g) (−)
    - Fraction II (44 g) (+) → chromatographed on silica gel (chloroform-methanol)
      - Fraction A (12.8 g) (+) → recrystallized (ethyl acetate) → nantenine (8.0 g) (+)
      - Fraction B (0.4 g) (−)
      - Fraction C (20.0 g) (−)
    - Fraction III (22 g) (−)
    - Fraction IV (26 g) (−)
  - Water-soluble portion (428 g) (−)

Scheme I—Procedure for the isolation of the serotonergic receptor antagonist from Nandina domestica *Thunberg. Key: (+) active; (−) inactive.*

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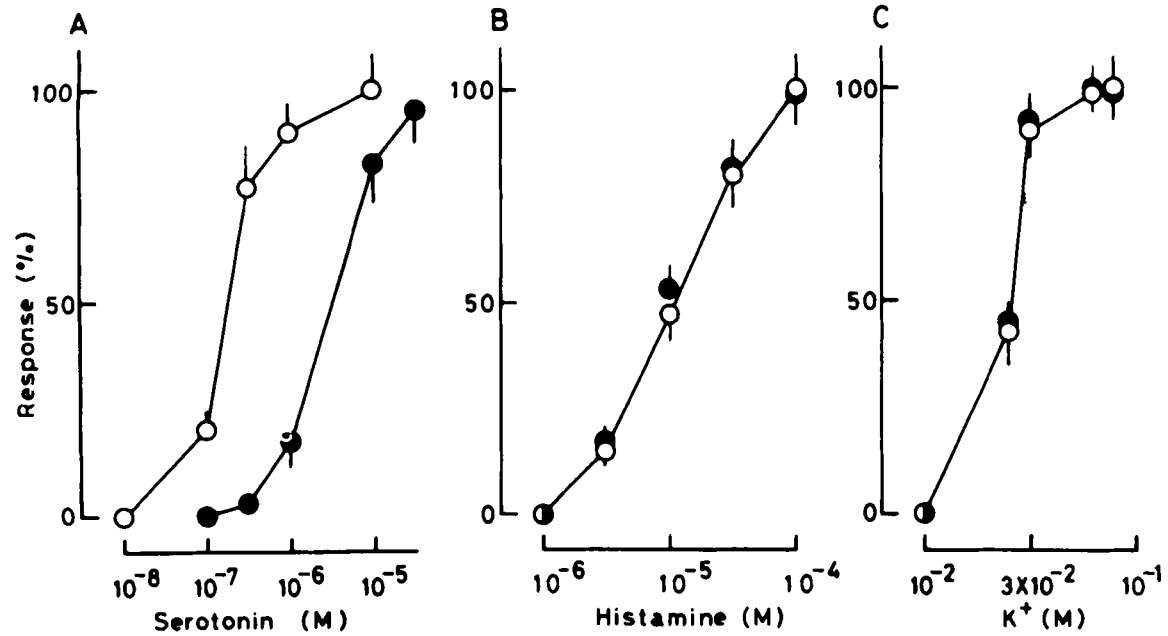

Figure 1—*Effect of nantenine on the dose-response curve for serotonin (A), histamine (B) and potassium chloride (K^+) (C) added 15 min after administration of nantenine to isolated rabbit aorta. The maximum response to serotonin (3×10^{-6} M), histamine (10^{-4} M), and K^+ (8×10^{-2} M) is expressed as 100%. Vertical lines indicate* SEM (n = 6). *Key: (○) control; (●) treatment with nantenine (3×10^{-6} M).*

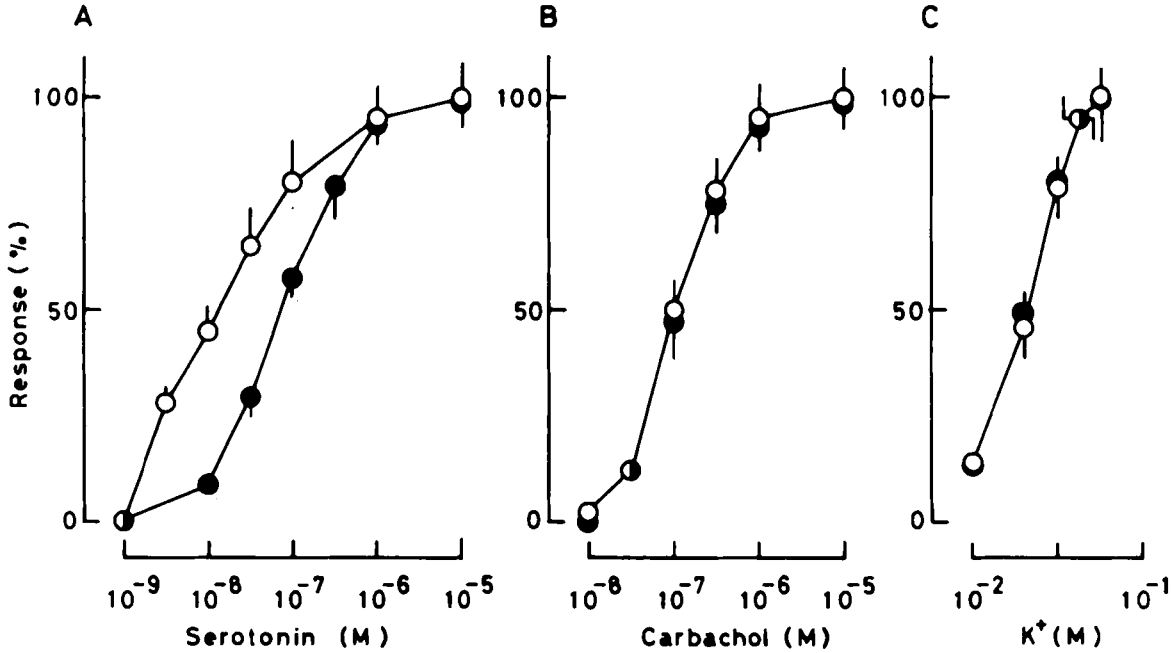

Figure 2—*Effect of nantenine on the dose-response curve for serotonin (A), carbachol (B), and potassium chloride (K^+) (C) added 15 min after administration of nantenine to isolated rat stomach. The maximum response to serotonin (10^{-5} M), carbachol (10^{-5} M), and K^+ (8×10^{-2} M) is expressed as 100%. Vertical lines indicate* SEM (n = 6). *Key: (○) control; (●) treatment with nantenine (3×10^{-6} M).*

EXPERIMENTAL[1]

Isolation—The dried, powdered fruit[2] (4 kg) of *N. domestica* were extracted with methanol (3 × 6 L) at room temperature yielding an extract (729 g), which was fractionated and monitored for inhibitory effects on the serotonin-induced contraction of rabbit aorta, as shown in Scheme I. The pharmacologically active *n*-butyl alcohol fraction was evaporated to dryness *in vacuo*, the residue (158 g) was chromatographed on silica gel[3] eluted with chloroform–methanol mixtures of increasing polarity, and fractionated successively. Each fraction was monitored by TLC[4] with chloroform–methanol–water (65:30:4) as the developing solvent and Dragendorf reagent as the detecting spray. The active second fraction (fraction II) was evaporated to dryness *in vacuo*, and the residue (44 g) was rechromatographed on silica gel using the chloroform–methanol solvent system. The active fraction (12.8 g) from the first eluate (fraction A) was recrystallized from ethyl acetate to give 8.0 g of nantenine as colorless needles ($C_{20}H_{21}NO_3$), mp 140–141°C [lit. (2) mp 139°C], $[\alpha]_D$ + 102.0° (*c* 1.0, $CHCl_3$) [lit. (2) $[\alpha]_D$ + 101.7°]; ^{1}H-NMR (pyridine-d_5): δ 2.43 (s, 3, N—CH_3), 3.72 (s, 3, C(1)—OCH_3), 3.76 (s, 3, C(2)—OCH_3), 6.00 (s, 2, O—CH_2—O), 6.66 (s, 1, C(3)—H), 6.87 (s, 1, C(8)—H), and 8.20 ppm (s, 1, C(11)—H); ^{13}C-NMR (pyridine-d_5): δ 29.66 (t, C-4), 35.52 (t, C-5), 44.03 (q, N—CH_3), 53.47 (t, C-7), 55.86 (d, C-6a), 60.06 (q, C(1)—OCH_3), 63.02 (q, C(2)—OCH_3), 101.47 (t, O CH_2—O), 108.95 (d, C-3), 109.22 (d, C-8), 111.80 (d, C-11), 126.21 (s, C-11b), 127.28

[1] Melting points were obtained on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotation was recorded on a Jasco DIP-180 digital polarimeter. UV spectra were obtained with a Hitachi 200-20 spectrophotometer. IR spectra were obtained on a Hitachi R-22 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Shimadzu LKB-9000B.

[2] Purchased from Nippon Hunmatsu Yakuhin, Ltd.

[3] Silica gel 60, Merck.

[4] TLC plates silica gel 60 F_{254} (0.25 mm), Merck.

(s, C-11c), 128.25 (s, C-7a), 129.44 (s, C-3a), 131.68 (s, C-11a), 145.24 (s, C-2), 147.01 (s, C-9*), 147.13 (s, C-10*), and 152.60 ppm (s, C-1). Assignments are interchangeable between carbons with an asterisk. The MS and UV spectra were identical with published spectra (11, 13).

Bioassay—Male albino rabbits (2–3 kg) were killed by cervical dislocation. The aortae were removed and cut into helical strips. The strips were mounted vertically in a 20-mL organ bath containing Krebs–Ringer bicarbonate solution (pH 7.4) of the following composition (in mmoles): sodium chloride, 120; potassium chloride, 4.8; calcium chloride, 1.2; magnesium sulfate, 1.3; monobasic potassium phosphate, 1.2; sodium bicarbonate, 25.2; and glucose, 5.8. The solution was bubbled with a gas mixture of oxygen–carbon dioxide (95:5, v/v) and maintained at 37°C. A resting tension of 1 g was applied to the strips and isometric contractions were recorded with a force displacement transducer.

Male Wistar rats (300–350 g) were killed by a blow on the neck and the stomach was removed. The fundus was cut longitudinally into strips (20 × 2 mm) as described by Offermeiser and Ariens (19). The strips were suspended in a 20-mL organ bath containing Krebs–Ringer bicarbonate solution. The solution was aerated with oxygen–carbon dioxide (95:5 v/v) and maintained at 37°C. A resting tension of 0.5 g was applied to the strips. Mechanical responses were recorded isotonically on a pen recorder through an isotonic transducer.

RESULTS AND DISCUSSION

A methanolic extract of the fruit of *N. domestica* almost completely inhibited the serotonin-induced contractions of isolated rabbit aorta and had no effect on contractions induced by potassium chloride or histamine. To isolate the active substance, fractionation of the methanolic extract of the fruits were performed (Scheme 1), accompanied by a bioassay using isolated rabbit aorta. Silica gel chromatography of the *n*-butyl alcohol-soluble portion of the methanolic extract afforded the active substance as colorless crystals (8.0 g, 0.2% dry weight of the fruit). The compound showed a positive Dragendorff test and its physicochemical properties (*i.e.*, melting point, specific optical rotation, UV absorption, and mass spectrum) agreed with those of nantenine (1, 5, 6, 11, 13), which was previously isolated as a major alkaloid of the same material. Furthermore, the ^{1}H- and ^{13}C-NMR spectra also supported the identity of the active substance as nantenine (see *Experimental*).

In isolated rabbit aorta, nantenine (3×10^{-6} M) produced a parallel, right shift of the dose–response curve for the contractile effect of serotonin, but had no effect on the dose–response curves for histamine and potassium chloride (Fig. 1), indicating competitive antagonism. Furthermore, in rat stomach strips, nantenine (3×10^{-6} M) shifted the dose–contractile response curve for serotonin to the right in a similar manner, but the dose–response curves for carbachol and potassium chloride were not affected by nantenine (Fig. 2). These results suggest that nantenine selectively inhibits the contractile response of these tissues to serotonin. On the basis of the present results, it is concluded that *N. domestica* Thunberg has a serotonergic receptor blocking action in the isolated rabbit aorta, and that the main active compound is nantenine.

REFERENCES

(1) T. Takase and H. Ohashi, *J. Pharm. Soc., Jpn.*, **535,** 742 (1926).
(2) Z. Kitasato, *J. Pharm. Soc. Jpn.*, **522,** 695 (1925).
(3) Z. Kitasato, *J. Pharm. Soc. Jpn.*, **523,** 791 (1925).
(4) Z. Kitasato, *J. Pharm. Soc. Jpn.*, **534,** 653 (1926).
(5) Z. Kitasato, *J. Pharm. Soc. Jpn.*, **536,** 843 (1926).
(6) H. Maniwa, R. Sakae, and I. Kan, *J. Pharm. Soc. Jpn.*, **536,** 833 (1926).
(7) H. Maniwa and R. Sakae, *J. Pharm. Soc. Jpn.*, **536,** 874 (1926).
(8) J. Ohta, *Yakugaku Zasshi*, **69,** 502 (1949).
(9) M. Tomita, Y. Inubushi, S. Ishii, and M. Yamagata, *Yakugaku Zasshi*, **71,** 381 (1951).
(10) M. Tomita and T. Kugo, *Yakugaku Zasshi*, **76,** 751 (1956).
(11) M. Tomita and T. Kitamura, *Yakugaku Zasshi*, **79,** 1092 (1959).
(12) M. Chikamatsu, M. Tomita, and M. Kotake, *Nippon Kagaku Zasshi*, **82,** 1708 (1961).
(13) M. Ohashi, J. M. Wilson, H. Budzikiewicz, W. A. Slusarchyk, and C. Djerassii, *J. Am. Chem. Soc.*, **85,** 2807 (1963).
(14) J. Kunitomo, K. Morimoto, S. Tanaka, and S. Hayata, *Yakugaku Zasshi*, **92,** 207 (1972).
(15) J. Kunitomo, M. Ju-ichi, Y. Yoshikawa, and H. Chikamatsu, *Yakugaku Zasshi*, **94,** 97 (1974).
(16) J. Kunitomo, M. Ju-ichi, Y. Yoshikawa, Y. Masada, K. Hashimoto, T. Inoue, and M. Fujioka, *Yakugaku Zasshi*, **94,** 1149 (1974).
(17) J. Kunitomo, J. Ju-ichi, Y. Ando, Y. Yoshikawa, S. Nakamura, and T. Shingu, *Yakugaku Zasshi*, **95,** 445 (1975).
(18) J. Kunitomo and Y. Murakami, *Shoyakugaku Zasshi*, **33,** 84 (1979).
(19) J. Offermeiser and E. J. Ariens, *Arch. Int. Pharmacodyn.*, **164,** 192 (1966).

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COMMUNICATIONS

Computation of Model-Independent Pharmacokinetic Parameters During Multiple Dosing

Keyphrases □ Pharmacokinetic parameters—model independent, multiple dosing

To the Editor:

In a recent article, Bauer and Gibaldi (1) reported an alternate, noncompartmental method to calculate pharmacokinetic parameters during multiple dosing. The method was based on reverse superposition from which a single-dose drug concentration–time curve was derived from data obtained at steady state. The following method would be a more general approach for computation of model-independent pharmacokinetic parameters during multiple dosing. The plasma drug concentration–time curve after the Nth dose (C_N) of a fixed dose of a drug at a given dosing interval of τ can be described by Eq. 1, when a drug obeys linear pharmacokinetics:

$$C_N = \sum_{i=1}^{n} A_i \frac{1 - \exp(-Nk_i\tau)}{1 - \exp(-k_i\tau)} \exp(-k_i t) = \sum_{i=1}^{n} I_i \exp(-k_i t) \quad \text{(Eq. 1)}$$

where A_i and I_i are the coefficients of the specific first-order rate constant, k_i, after a single dose and the Nth dose, respectively; t is the time after each drug administration. The total area under the plasma drug concentration–time curve (AUC) from the time after the Nth dose is given to time infinity, $\text{AUC}(\infty)_N$ can be obtained as follows:

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