Bioorganic & Medicinal Chemistry Letters 18 (2008) 3992–3994

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Hypaphorine, an indole alkaloid from *Erythrina velutina*, induced sleep on normal mice

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ARTICLE INFO

Article history:
Received 22 April 2008
Revised 30 May 2008
Accepted 3 June 2008
Available online 6 June 2008

Keywords:
Hypaphorine
Indole alkaloid
Sleep induced
Non-REM
Medicinal plant
Erythrina velutina

ABSTRACT

An indole alkaloid (hypaphorine (**1**)) was isolated from Brazilian medicinal plant, *Erythrina velutina* (Leguminosae). This compound was investigated for sleep promoting effects in mice, and the results showed that it significantly increased non-rapid eye movement (NREM) sleep time during the first hour after its administration. The NREM sleep time was enhanced by 33% in the experimental mice when compared to that of the controls. This study therefore confirmed its sleep promoting property.

Problems with sleeping in the general population are associated with a year to year increase in the aging rate, graveyard shift, and mental disorders including depression, neurosis, and dementia. The current clinical approach employs different varieties of benzodiazepine derivatives that are used as hypnotic agents for those having difficulties in falling asleep and maintaining sleep. However, these agents are often associated with adverse effects such as dependence, amnesia, nightmares, and prolonged sleepiness.[1] In addition, drug developments of the substitutes for benzodiazepines are required for discontinuation of chronic users.

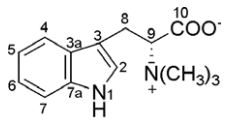
In our search for biologically active compounds from tropical plants,[2] we have studied the seeds of *Erythrina velutina*. This plant is commonly called 'Mulungu' in Brazil, and its bark is used for sedation, hypnogenesis, control of convulsions, and nervous coughs in the northern region in Brazil.[3] We study herein the alkaloidal constituents of the seed part of *E. velutina*. In this report, we describe the isolation and characterization of an indole derivative, hypaphorine (**1**) from Brazilian medicinal plant, *E. velutina* Willd (Leguminosae),[4] and the evaluation of its potency as a potential hypnotic agent.

The seeds of *E. velutina* (954 g) were crushed and then extracted with MeOH (19.0 g). The MeOH-soluble materials were successively partitioned between petroleum ether, EtOAc, and 3% aqueous tartaric acid. Water-soluble materials were adjusted to pH 10 with Na_2CO_3 and successively partitioned between $CHCl_3$ (6.07 g) and *n*-BuOH (3.48 g) to obtain the alkaloidal portions.

The $CHCl_3$-soluble materials (3.11 g) were subjected to silica gel column ($CHCl_3$-MeOH, 100:0 → 0:100)[5] and the last fraction was eluted with pure MeOH (349 mg) followed by a silica gel column [MeOH/$(CH_3)_2CO$, 99:1] to isolate hypaphorine (**1**, 44 mg)[6] (Fig. 1) and glycoerysodine (**2**, 5 mg).[7]

The data of hypaphorine (**1**) were analyzed with ^1H and ^{13}C NMR including 2D-techniques and FABMS spectra. Hypaphorine (**1**) was identified by comparison with the data in the literature, and was also determined as the 9*S* configuration from its optical rotation value.[8]

The hypnotic activity of hypaphorine (**1**) was evaluated by the electroencephalographic analysis of the sleep–wake cycle in freely behaving mice through the analysis of rapid eye movement (REM) sleep, non-REM sleep (NREM), wakefulness, and total sleeping times, respectively. Male mice (C57BL, 16–21-week, Crea Japan Inc., Tokyo, Japan) were obtained and kept under controlled conditions at 24 ± 1 °C with relative humidity of 56 ± 6%, and a light–



hypaphorine (**1**)

Figure 1. Chemical structure of hypaphorine (**1**).

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doi:10.1016/j.bmcl.2008.06.002

dark cycle of 12:12 h (light on 07:00 h). The animals were allowed to become acclimatized for 2 weeks. The surgical approach to attach the plate directly to the mouse brain for the simultaneous monitoring of cortical electroencephalogram (EEG) and neck electromyogram (EMG) electrodes and the feeding procedure in freely behaving mice were as described previously.[9] After the mice had recovered from the implantation of the plate attached to the EEG and EMG electrodes, the data on NREM, REM, wakefulness, and total sleeping time (NREM + REM) were collected from freely behaving mice. All experimental protocols were performed in accordance with the Guidelines for Animal Experimentation of Tokyo Medical and Dental University.

Table 1
The cross-over administration (ip) schedule of the extract of *E. velutina* and hypaphorine (**1**)

Group	A (n = 2)	B (n = 2)
1 d	Saline 0.5 mL	Extracts 1.6 mg/0.5 mL
2 d	Extracts 1.6 mg/0.5 mL[a]	Saline 0.5 mL
9 d	Saline 0.5 mL	Hypaphorine 1.6 mg/0.5 mL
10 d	Hypaphorine 1.6 mg/0.5 mL[b]	Saline 0.5 mL
	C (n = 4)	D (n = 4)
1 d	Saline 0.5 mL	Extracts 16 mg/0.5 mL
2 d	Extracts 16 mg/0.5 mL[a]	Saline 0.5 mL
9 d	Saline 0.5 mL	Hypaphorine 16 mg/0.5 mL[b]
10 d	Hypaphorine 16 mg/0.5 mL[b]	Saline 0.5 mL

[a] 30 mg/kg.
[b] 300 mg/kg.

The MeOH extracts of *E. velutina* (30 and 300 mg/kg), hypaphorine (**1**, 30, 300 mg/kg), and control (saline, 0.5 mL) were administered intraperitoneally (ip) to mice as shown in Table 1. The electrographic recordings for each data set were scored using the previously reported method.[7] The parameters of each data set for NREM, REM, wakefulness, and total sleeping time were analyzed with software (Kissei Comtec, Sleep Sign, Nagano, Japan), and the results verified visually according to the standard criteria. Discrimination of wakefulness (high EMG amplitude, low EEG amplitude), non-REM sleep [low EMG amplitude, high EEG amplitude with high power density in the delta band (0.5–4.0 Hz)] and REM sleep [silent low EMG amplitude, low EEG amplitude with high values in the theta band (4.0–8.0 Hz)] were performed. The analyzed sleep variables included the amount of non-REM sleep, REM sleep, and number and duration of each sleep parameter episode. These durations were determined for each hour in a 12-h period.

The dose selection was decided in reference to the data obtained from the prolonged sleep duration induced by sodium pentobarbital combined with the extracts of the leaves of *E. velutina*,[4] and the doses of hypaphorine (**1**) were decided in accordance with the contents its rate in the extracts. Administration was performed 30 min before the beginning of the dark period, and all recordings were continued till the lights on the period the next morning. In pairs of mice (A, B groups) and in groups of four of mice (C, D groups), the same substance and dose was administered at the

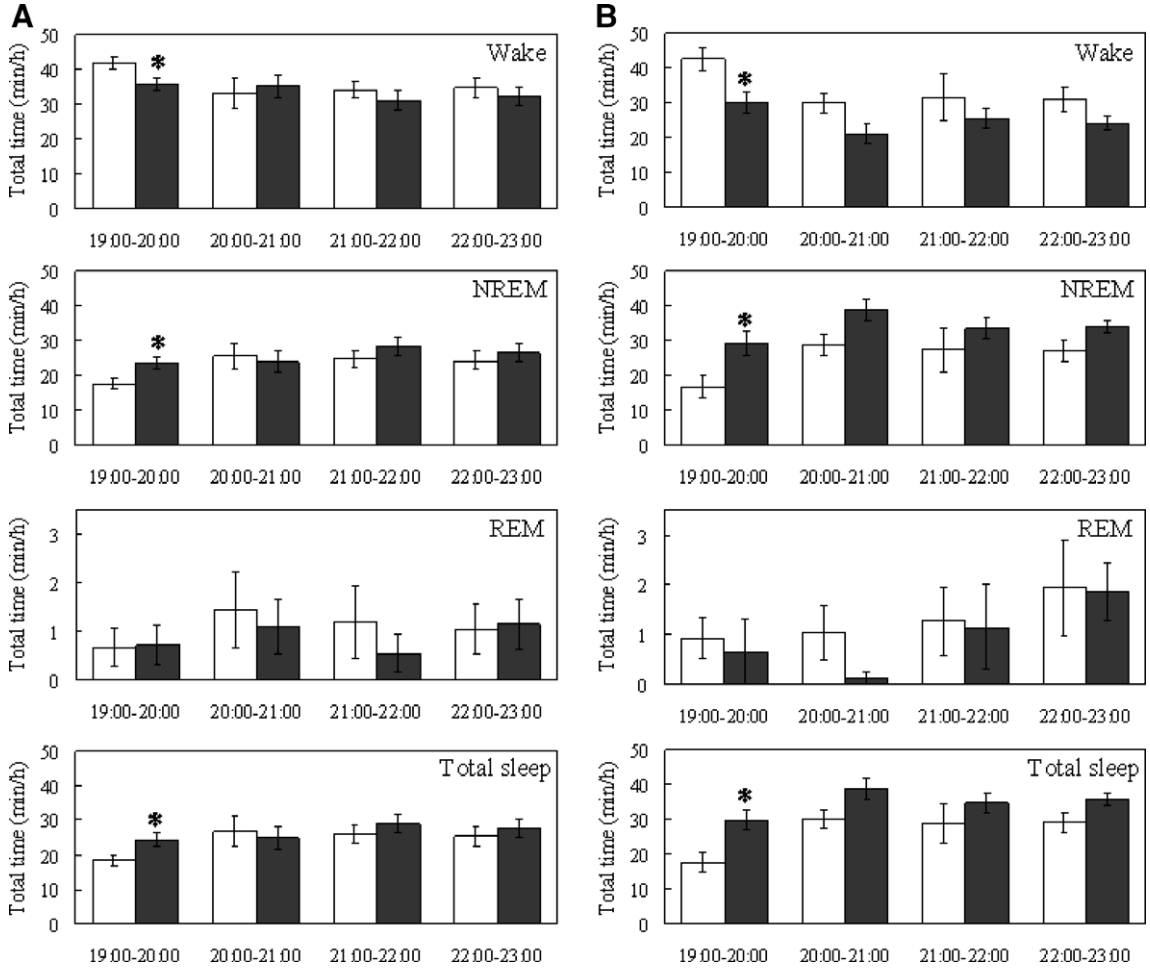

Figure 2. Effects of (A) hypaphorine (**1**, 300 mg/kg, ip n = 8), and (B) diphenhydramine hydrochloride (20 mg/kg, ip n = 5) for NREM, REM, wakefulness, and total sleep time. $^{*}p < 0.05$ versus control.

same time, respectively. The cross-over administrations were performed following the schedule as shown in Table 1.

The 30 and 300 mg/kg doses of the extracts of *E. velutina* and 30 mg/kg of hypaphorine (**1**) did not show any statistical significant effects (data not shown). However, administration of **1**, 300 mg/kg, showed that NREM and total sleep time were significantly ($p < 0.05$) increased in duration by 33% and 32%, respectively, during only the first hour of administration (19:00–20:00), when compared to the control group. On the other hand, the waking duration was significantly decreased by 16% ($p < 0.05$) when compared with the control group (Fig. 2A). Furthermore, the results showed that there was no significant difference in REM sleep duration between the mice who received the two doses of hypaphorine (**1**) and the control (saline) group. The data obtained continuously during the period of 3 h (21:00, 22:00, and 23:00) and the fourth hour (24:00) after administration of **1** did not show any significant effect. Therefore, the results only showed that hypaphorine (**1**) was only effective for 1 h after administration, and this has significant implication. It is therefore suggested that **1** might be a good candidate as a sleep inducing agent due to its short duration of action.

We also investigated the NREM sleep, REM sleep, wakefulness, and the total sleep time following administration of diphenhydramine hydrochloride (20 mg/kg, ip) as positive control.[10] The results are as shown in Figure 2B. NREM sleep time was significantly increased by 76% during the first hour period (19:00–20:00), after administration of the drug when compared to the control group. However, in the second period time (20:00–21:00), NREM sleep duration was also increased non-significantly. In a similar manner, NREM sleep duration was subsequently increased, but non-significantly, unlike the effect obtained with administration of hypaphorine (**1**) that was only effective for 1 h after administration without any residual effects.

This is the first report on the sleep inducing activity of hypaphorine (**1**). Furthermore, no adverse effects were seen in the experimental animals in our present study. The sleep inducing activity of this medicinal plant might be due to the effect of hypaphorine (**1**) since the bark of *E. velutina* also contains the same compound. Hypaphorine (**1**) is an L-tryptophan derivative, sited at the upper biogenetic cascade of nervous transmitter substances from L-tryptophan (**3**) to serotonin (5-HT) (**4**) and melatonin (**5**) (Fig. 3). The

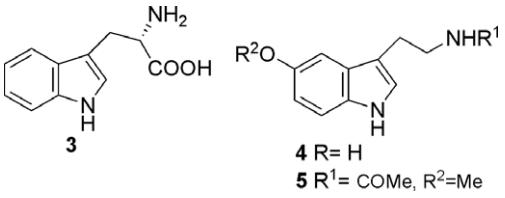

Figure 3. Chemical structures of L-tryptophan (**3**), serotonin (5-HT) (**4**), and melatonin (**5**).

study of the mechanism of the mode of action on the prolonged time of NREM represents very important data, as NREM is recognized as a state of deep sleep, the increased in NREM during observed in the present study could be related to fulfillment of sleep requirements. Hypaphorine (**1**) and its derivatives might be future candidates for moderate sleep inducing agents from natural resources. Elucidation of the neurocytological data and the mode of action of hypaphorine (**1**) are now in progress.

Acknowledgment

We thank Dr. G. Hashimoto (Centro de Pesquisas de História Natural, São Paulo, Brazil) for identification of *Erythrina velutina*.

References and notes

1. Vgontzas, A. N.; Kales, A.; Bixler, E. O. *Pharmacology* **1995**, *51*, 205.
2. Ohsaki, A.; Takashima, J.; Chiba, N.; Kawamura, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1109.
3. Dantas, M. C.; De Oliveira, F. S.; Bandeira, S. M.; Batista, J. S.; Silva, C. D.; Alves, P. B.; Antoniolli, A. R.; Marchioro, M. *J. Ethnopharmacol.* **2004**, *94*, 129.
4. Hashimoto, G. *Illustrated Cyclopedia of Brazilian Medicinal Plants*; ABOC-SHA: Kamakura, 1996. p. 679.
5. Various Erythrina alkaloids were isolated from *E. velutina*. Their structure and activities will appear in due course.
6. Kondo, K.; Nishi, J.; Ishibashi, M.; Kobayashi, J. *J. Nat. Prod.* **1994**, *57*, 1008; Hu, J.-F.; Shen, F.-J. *Gao. Xue. Hua. Xue.* **1995**, *16*, 1245.
7. Amer, M. E.; El-Marsy, E. S.; Shamma, M.; Ereyer, A. J. *J. Nat. Prod.* **1991**, *54*, 161.
8. $[\alpha]_D^{25} + 89.6$ (*c* 0.125, H_2O).
9. Akanmu, M. A.; Ukponmwan, O. E.; Katayama, Y.; Honda, K. *Neurosci. Res.* **2006**, *54*, 165; Akanmu, M. A.; Singkram, C.; Kagechika, H.; Honda, K. *Neurosci. Lett.* **2004**, *364*, 199; Akanmu, M. A.; Honda, K. *Brain Res.* **2005**, *1048*, 138; Honda, K.; Komoda, Y.; Inoué, S. *Brain Res.* **1994**, *636*, 253. and references cited therein.
10. Tokunaga, S.; Takeda, Y.; Shinoyama, K.; Hirase, M.; Kamei, C. *J. Pharmacol. Sci.* **2006**, *103*, 201.