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Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis*

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Abstract

We have recently reported the presence of the anxiolytic flavone 6-methylapigenin (MA) and of the sedative and sleep-enhancing flavanone glycoside 2S (–) hesperidin (HN) in *Valeriana officinalis* and *Valeriana wallichii*. MA, in turn, was able to potentiate the sleep-inducing properties of HN.

The present paper reports the identification in *V. officinalis* of the flavone glycoside linarin (LN) and the discovery that it has, like HN, sedative and sleep-enhancing properties that are potentiated by simultaneous administration of valerenic acid (VA).

These effects should be taken into account when considering the pharmacological actions of valeriana extracts.

Keywords: Valeriana; Flavonoids; Linarin; Valerenic acid; CNS; Sedation; Sleep-enhancing properties; Potentiation

1. Introduction

The genus Valeriana belongs to the Valerianaceae and contains about 250 species. The three most important species in herbal medicine that are used as mild sedatives are *Valeriana officinalis L. s.l.*, *Valeriana wallichii DC.*, and *Valeriana edulis* Nutt. Ex Torr. and Gray ssp. *procera* (H.B.K.) F.G. Meyer.

The use of extracts of the valeriana roots and rhizomes to cause sedation and relieve sleep problems dates back to the 18th century (Madaus, 1976), but the exact composition of the preparations used was often not clear.

In the search for the active substances of valeriana, many compounds have been isolated and identified during the last 120 years, but it is as yet uncertain which of them are responsible for the recorded actions (Bos et al., 1996; Houghton, 1999).

The most popular compounds, in this connection, are the epoxy iridoids named valepotriates, their decomposition products, the baldrinals, and the nonvolatile terpenoids grouped as valerenic acid (VA) derivatives as well as some other members of the essential oil (Fig. 1) (Houghton, 1999).

However, several facts have cast doubts on the relevance of these compounds to explain valeriana extracts effects. The principal of them are as follows: (a) the central depressant action of valepotriates, valeranone, and of the essential oil of valeriana could not be demonstrated by a reduction of the glucose turnover in rat brain (Hölzl, 1997); (b) the sedative potency of these compounds is rather low (>30 mg/kg, in mice) (Hölzl, 1997); (c) the valepotriates rapidly decompose if water is present and the resulting baldrinals are chemically reactive and may form polymers (Bos et al., 1996), hence both valepotriates and baldrinals disappear rapidly from the extracts; and (d) the roots and rhizomes of different valeriana species show large differences with regards to their constituents: *V. officinalis* mainly contains VA and derivatives as well as valepotriates and other constituents, whereas *V. wallichii* and *V. edulis* do not contain VA and its derivatives (Houghton, 1999).

In vitro mutagenic effects have been described for the valepotriates and their decomposition products (Houghton, 1999). Hence, it would be prudent to prefer valeriana preparations, which are devoid of potentially hazardous valepotriates or baldrinals.

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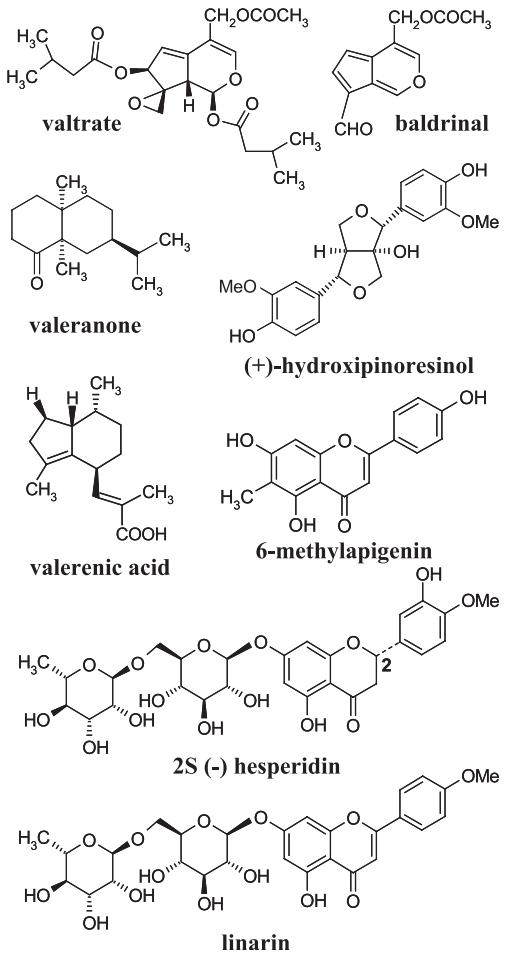

Fig. 1. Molecular structures of active compounds in valeriana including valtrate (as an example of valepotriates); baldrinal; valeranone; (+) hydroxipinoresinol, valerenic acid; 6-methylapigenin; hesperidin, and linarin.

A promising approach to detect new active substances in valeriana extracts consists in searching for the presence of ligands for the principal brain receptors predominantly associated to anxiolytic, sedative, and/or sleep-enhancing properties (Marder and Paladini, 2002). Unfortunately, studies of this kind have, in general, given inconclusive results quite probably due to the use of crude extracts in the assays (Hölzl and Godau, 1989; Mennini et al., 1993). Bodesheim and Hölzl (1997) found that the lignan (+) hydroxypinoresinol (Fig. 1), present in valeriana extracts, is a medium-low affinity ligand for the 5-HT receptor but its in vivo effects were not investigated.

In our laboratory, we have applied the "ligand-searching approach" using, as far as possible, purified extracts and were able to report the presence of 6-methylapigenin (MA) (Fig. 1) in *V. wallichii* and *V. officinalis* and to prove that it is a benzodiazepine binding site (BDZ-bs) ligand (Wasowski et al., 2002). We have also made the first report of the presence of 2S (−) hesperidin (HN) (Fig. 1) in *V. wallichii* and in *V. officinalis* and found that it has sedative and sleep-enhancing properties. MA, in turn, had anxiolytic activity and was able to potentiate the sleep-enhancing properties of HN (Marder et al., 2003).

The present paper describes the first identification of the flavonoid glycoside linarin (LN) (Fig. 1) in *V. officinalis*, the discovery of its sedative and sleep-enhancing properties in mice, and the potentiation of these effects by simultaneous administration with VA. The effective doses of these compounds are commeasurable with their concentrations in the plant extracts and with the doses used in folkloric medicine.

2. Methods

2.1. Plant material

V. officinalis L. (Valerianaceae) was obtained from a local commercial source. Its identification was done at the Botany Museum of the School of Pharmacy in Buenos Aires, where the voucher specimen 10392 BAF was deposited.

2.2. General procedures

Spectroscopic measurements were done as follows: NMR on a 300-MHz Bruker apparatus with the sample dissolved in DMSO-d_6; UV-Vis in a Shimadzu 160A spectrophotometer with methanolic solutions; and EIMS on a Shimadzu Mass-Spectrometer QP-5000 at 70 eV with direct probe inlet.

2.3. Subjects

Adult male Wistar rats weighing 250 g were used for biochemical experiments. Adult male Swiss mice weighing 25–30 g were used for pharmacological assays. Animals were housed in a controlled environment, with free access to food and water and maintained on a 12:12-h day/night cycle.

Housing, handling, and experimental procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Behavioral experiments were conducted from 10:00 a.m. to 2:00 p.m.

2.4. Biochemical experiments

Binding of ^{3}H-flunitrazepam (^{3}H-FNZ) (84.5 Ci/mmol; New England Nuclear, NEN) to BDZ-bs in washed crude synaptosomal membranes from rat cerebral cortex was carried out as described by Marder et al. (2003).

2.5. Behavioral experiments

2.5.1. Holeboard test

This assay was conducted in a walled acrylic arena of 60 × 60 cm square floor and 30 cm high walls, with four equally spaced holes in the floor, 2 cm in diameter each. The

holes housed an infrared light emitting diode. The interruption of the light beam by an exploring mouse during at least 100 ms triggers a counting device that records, in a computer, the number of head dips and the time head dipping. The mice were placed singly at the center of the board, facing away from the observer and the number and time of holes explored, as well as the number of rearings, in a 5-min session were recorded. After each trial, the apparatus was wiped clean to remove traces of the previous assay. A decrease in the number of head dips, the time spent head dipping, and/or the number of rearings reveal a sedative behavior (File and Pellow, 1985).

2.5.2. Sodium thiopental-induced sleeping time assay

A subhypnotic dose of sodium thiopental (35 mg/kg) was intraperitoneally injected to mice 20 min after a similar injection of vehicle or the drug. Sleeping time was determined as the interval between the loss and the recovery of the righting reflex (Ferrini et al., 1974).

2.6. Drugs or extract solutions and injection procedures

The drugs and extracts used to perform the pharmacological tests were as follows: dried fractions obtained from *V. officinalis* as described in Fig. 2; VA (Fig. 1), kindly provided by Dr. R. Bos; LN (Fig. 1) isolated by us from *V. officinalis* or the genuine drug obtained from Extrasynthese, Genay, France; and MA (Fig. 1) isolated by us from *V. wallichii* as described in Wasowski et al. (2002). These drugs were dissolved by the sequential addition of dimethylsulfoxide up to a final concentration of 10%, ethanol up to a final concentration of 10%, and saline to complete 100% volume. Sodium thiopental (Fada, Biochemie Gesellschaft, Kundl/Tirol, Austria) was dissolved in saline. The rodents were intraperitoneally injected 20 min before performing the tests. The volume of intraperitoneal injections was 0.15 ml/30 g of body weight. The potentiating effects were tested by coadministering VA and MA, LN and MA, LN and VA and LN, and MA plus VA as indicated in Table 2. In each session, a control group-receiving vehicle was tested in parallel with those animals receiving drug treatment.

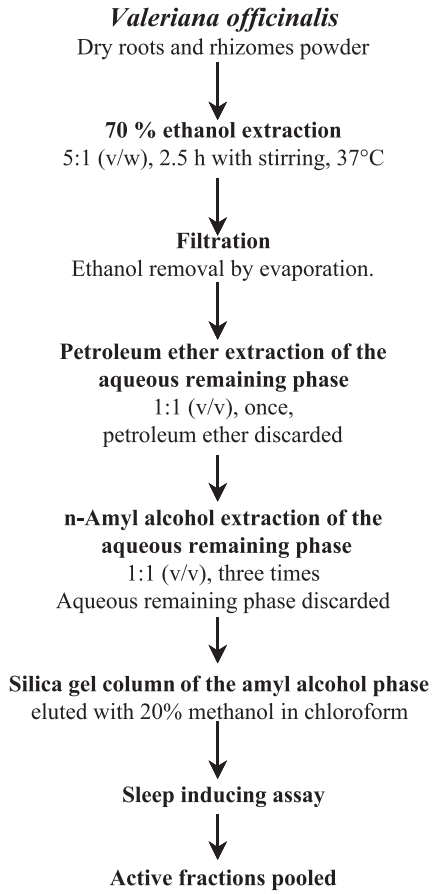

Fig. 2. Flow sheet of the *V. officinalis* fractionation scheme.

2.7. Statistical analyses

Data obtained from the holeboard test were subjected to one-way ANOVA. Post hoc comparisons between individual treatments and controls were made using Dunnett's multiple comparisons test. Dunn's multiple comparison test was used after Kruskal–Wallis test (nonparametric ANOVA) when sleeping times were compared. Significance was reported starting at the .05 level.

2.8. Isolation and identification of LN from ethanolic extracts of V. officinalis

Dry *V. officinalis* roots and rhizomes were submitted to the extraction and fractionation scheme shown in Fig. 2. Powdered dry roots and rhizomes (100 g) of *V. officinalis* were suspended in 500 ml of 70% ethanol and the mixture was kept 2.5 h at 37 °C, with stirring. The filtrate was concentrated to 1/3 of the original volume to eliminate most of the ethanol and extracted with an equal volume of petroleum ether, which was discarded. The aqueous phase slightly concentrated to eliminate the remained petroleum ether was extracted three times with an equal volume of amyl alcohol and the alcohol phase (AP) was evaporated to dryness. The solid residue was chromatographed in a silica gel column (4.5 × 20 cm), prepared from a suspension of silica H (Sigma, USA) in chloroform, which was eluted with 20% methanol in chloroform. The fractions showing the presence of compounds active in the sleep-enhancing assay were pooled. After partial evaporation of the solvent, the pool deposits a whitish precipitate that is collected by filtration and purified by recrystallization from ethanol. Yield: 0.4 mg per g of valeriana powder. This material permitted the identification of (7-[[6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-methoxyphenyl)-4H-benzopyran-4-one or acacetin-β-rutinoside or LN (Fig. 1). From the mother solution remaining after precipitation of LN, a pure sample of HN could also be isolated, as described by Marder et al. (2003).

LN was purified by crystallization from ethanol water and identified by UV, ^{1}H-NMR, and mass spectroscopy.

2.9. Identification of LN

All the spectra of valeriana LN and authentic LN (extrasyntheses) were identical: ^{1}H NMR δ_H 12.90 (1H, s, OH-5), 8.05 (2H, d, J = 8.81 Hz, H-2′ and H-6′), 7.14 (2H, d, J = 9.10 Hz, H-3′ and H-5′), 6.94 (1H, s, H-3), 6.78 (1H, d, J = 2.06 Hz, H-8), 6.44 (1H, d, J = 2.06 Hz, H-6), 5.05 (1H, d, J = 7.04 Hz, H-1 glucosyl), 4.44 (1H, d, J = 6.16 Hz, H-1 rhamnosyl), 3.86 (3H, s, OCH_3-4′), 3.09–3.46 (m, H-sugars), and 1.07 (3H, d, J = 6.16 Hz, CH_3-rhamnosyl); UV λ_{max} (methanol): 327.5, 269.0, and 210.0 nm.

2.10. Identification of acacetin in LN

To further characterize the aglycone moiety of the isolated compound, a sample of valeriana LN was hydrolyzed in boiling 1 M HCl for 1 h. The aglycone, acacetin, was extracted from the hydrolysate with ethyl ether. The spectroscopic analyses were as follows: ^{1}H-NMR δ_H 12.91 (1H, s, OH-5), 10.84 (1H, s, OH-7), 8.03 (2H, d, J = 8.80 Hz, H-2′ and H-6′), 7.10 (2H, d, J = 9.10 Hz, H-3′ and H-5′), 6.86 (1H, s, H-3), 6.49 (1H, d, J = 2.05 Hz, H-8), 6.18 (1H, d, J = 2.05 Hz, H-6), 3.84 (3H, s, CH_3-4′). EMS *m/z*: 284 (M^+), 269, 256, 241, and 152. UV λ_{max} (methanol): 328.5, 268.0, and 213.0 nm. These values were identical to those in the literature (Harborne, 1994).

A sample from the hydrolysate aqueous phase remaining after the ethyl ether extraction was submitted to thin layer chromatography on silica gel polyester sheets, with 254 nm fluorescent indicator (Sigma), developed with butanol/acetic acid/ethyl ether/water (9:6:3:1 v/v) as the solvent and stained using a general indicator for sugars (aniline/diphenylamine/acetone/phosphoric acid). This chromatography was performed alongside authentic sugar standards. The only sugars detected were glucose and rhamnose.

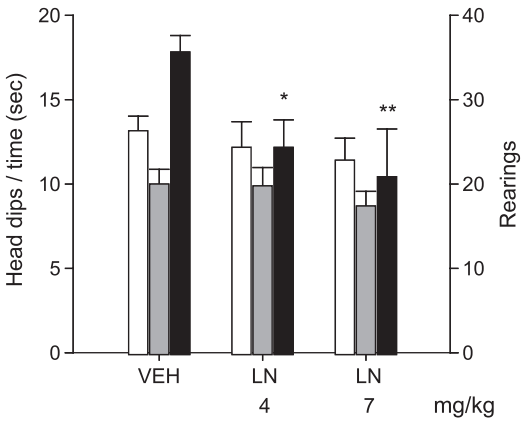

Fig. 3. Effects of LN on sedative behavior. Mean ± S.E.M. of number of head dips (open bars, left scale, in numbers) or time spent head dipping (grey bars, left scale, in seconds) and number of rearings (closed bars, right scale) registered in a 5-min session in the hole board test. * $P < .05$, ** $P < .01$, significantly different from vehicle; Dunnett's multiple comparison test after ANOVA. Number of animals per group ranged between 7 and 19.

Table 1
Effectiveness of VA, MA, LN, and AP on sodium thiopental-induced sleeping time in mice

Sample	Dose (mg/kg)	*n*	Sleeping time median, interquartile range (s)
VEH	–	18	0 (0/0)
VA	up to 15	15	0 (0/0)
MA	up to 10	13	0 (0/0)
LN	4	9	0 (0/0)
	7	7	1710 (1161/1800) * *
	14	11	900 (300/1800) *
AP	200[†]	10	585 (180/1275) *

Median (interquartile range) of sleeping time of mice measured in a sodium thiopental-induced sleep test after 20 min of an intraperitoneal injection of vehicle (VEH), linarin (LN 4, 7, and 14 mg/kg), valerenic acid (VA, up to 15 mg/kg), 6-methylapigenin (MA, up to 10 mg/kg), or the amyl alcohol phase (AP) (see Fig. 1). The sleeping time was measured as the time spent between disappearance and reappearance of righting reflex (see Methods); *n* = number of mice.

* $P < .05$, significantly different from vehicle; Dunn's multiple comparison test after Kruskal–Wallis test (nonparametric ANOVA).

** $P < .01$, significantly different from vehicle; Dunn's multiple comparison test after Kruskal–Wallis test (nonparametric ANOVA).

[†] 200 mg is the dry residue of the AP obtained from 4 g of *V. officinalis*.

3. Results

3.1. Biochemical experiments

LN at concentrations up to 100 μM did not displace ^{3}H-FNZ binding to BDZ-bs present in synaptosomal mem-

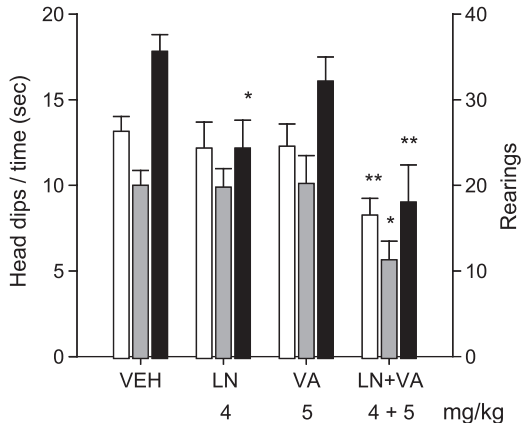

Fig. 4. Potentiation of LN sedative action by valerenic acid. Mean ± S.E.M. of number of head dips (open bars, left scale) or time spent head dipping in seconds (grey bars, left scale) and number of rearings (close bars, right scale) registered in a 5-min session in the hole board test performed 20 min after the intraperitoneal injection of vehicle (VEH), linarin (LN, 4 mg/kg), valerenic acid (VA, 5 mg/kg), or both drugs coinjected (LN 4 mg/kg + VA 5 mg/kg). * $P < .05$, ** $P < .01$, significantly different from vehicle; Dunnett's multiple comparison test after ANOVA. Number of animals per group ranged between 7 and 19.

Table 2
Effectiveness of coadministration of LN, VA, and/or MA on sodium thiopental-induced sleeping time in mice

Sample	Dose (mg/kg)	*n*	Sleeping time median, interquartile range (s)
VEH	–	18	0 (0/0)
VA	5		
+		6	0 (0/0)
MA	1		
LN	4		
+		6	0 (0/240)
MA	1		
LN	4		
+		15	960 (219/1650)**
VA	5		
LN	4		
+			
VA	5	9	300 (60/660)**
+			
MA	1		

Median (interquartile range) of sleeping time of mice measured in a sodium thiopental-induced sleep test after 20 min of an intraperitoneal injection of vehicle (VEH) or the coinjection of valerenic acid and 6-methylapigenin (VA 5 mg/kg + MA 1 mg/kg), linarin and 6-methylapigenin (LN 4 mg/kg + MA 1 mg/kg), linarin and valerenic acid (LN 4 mg/kg + VA 5 mg/kg), and linarin and valerenic acid plus 6-methylapigenin (LN 4 mg/kg + VA 5 mg/kg + MA 1 mg/kg). The sleeping time was measured as the time spent between disappearance and reappearance of righting reflex (see Methods); *n* = number of mice.

** $P < .01$, significantly different from vehicle; Dunn's multiple comparison test after Kruskal–Wallis test (nonparametric ANOVA).

branes of rat cerebral cortex. Its aglycone, acacetin, caused a reduction of approximately 30% in the binding only at a concentration of 100 μM.

3.2. Sedative and sleep-enhancing effects of LN

The sedative action of LN, determined in the holeboard test, is shown in Fig. 3. Doses of 4 and 7 mg/kg ip of LN significantly reduced the number of rearings performed in the holeboard test ($P < .05$ and .01, respectively). No significant differences were observed in the exploration of holes.

The effect of LN on sleep is shown in Table 1. LN, at doses of 7 or 14 mg/kg ip, significantly augmented the sleeping time induced by thiopental ($P < .01$ and .05, respectively).

The dry residue of the amyl AP obtained from 4 g of *V. officinalis* administered per kg of mice (200 mg dry residue/kg) increased significantly the sodium thiopental-induced sleeping time ($P < .05$; Table 1).

Moreover, in Table 1, it is also shown that MA (up to 10 mg/kg) and VA (up to 15 mg/kg) are devoid of sleep-enhancing capacity in mice.

3.3. Sleep-enhancing and sedative actions of LN coadministered with VA and/or 6-methyl apigenin

As shown in Fig. 3 and Table 1, an intraperitoneal injection of LN at a dose of 4 mg/kg had a moderate sedative effect but did not increase the sleeping time induced by sodium thiopental. On the other hand, an intraperitoneal administration of VA at a dose of 5 mg/kg was not sedative as measured in the holeboard test and did not increase the sodium thiopental-induced sleeping time (Fig. 4 and Table 1, respectively). However, the coadministration of both substances at these doses had sedative and sleep-enhancing effects as evidenced by the remarkable reduction in the exploration of holes ($P < .01$), the time mice spent head dipping ($P < .05$) and the number of their rearings ($P < .01$) as assayed in the holeboard test (Fig. 4), and also produced a striking increase in the sleeping time induced by sodium thiopental ($P < .01$; Table 2). In contrast, the coadministration of LN (4 mg/kg) and MA (1 mg/kg) or VA (5 mg/kg) and MA (1 mg/kg) showed no effect (Table 2).

When LN (4 mg/kg), VA (5 mg/kg), and MA (1 mg/kg) were coinjected, a significant increase in the sleeping time induced by sodium thiopental was observed ($P < .01$; Table 2). Nevertheless, this effect was not significantly different from the one observed when LN and VA were coadministered (Table 2).

4. Discussion

LN was first identified in *V. wallichii* by Thies (1968) in the form of its isovaleryl ester, but its pharmacological properties were not explored. More recently, it has been demonstrated that LN, isolated from the leaves of *Buddleia cordata*, exerts central analgesic properties and is responsible for the antipyretic activity of this plant (Martínez-Vázquez et al., 1996). The same authors later found that extracts of this species and LN itself exerted anti-inflammatory effects (Martínez-Vázquez et al., 1998).

The present paper reports the identification of LN in *V. officinalis* demonstrating its sedative and sleep-enhancing properties in mice, as shown in Fig. 3 and Table 1. Notwithstanding, LN and its aglycone acacetin are not ligands for the BDZ-bs in brain; hence, the mechanism of LN depressor actions is still unclear.

Hendriks et al. (1985) showed that VA had nonspecific central depressant effects following its intraperitoneal administration in mice. At doses above 100 mg/kg body weight, effects were found in a rotarod and in a traction tests. Higher doses were toxic. Spontaneous locomotor activity of mice was reduced by VA at a dose of 50 mg/kg, and a prolongation of the barbiturate-induced sleeping test was found as well (Hendriks et al., 1985). It was shown also that pure VA-antagonized picrotoxin induced convulsions in mice at 12.5 and 25 mg/kg ip (Hiller and Zetler, 1996). VA was assumed to be the most important active component in valeriana. This hypothesis is not supported today by the well-known fact, among others, that VA is only present in *V. officinalis* and not in other active species widely used like *V. wallichii* and *V. edulis*. We demonstrate here that

VA per se has no in vivo effects in mice at low doses (up to 15 mg/kg; Table 1).

The content of VA in the subterranean parts of various subspecies of *V. officinalis* ranges between 0.3 and 3 mg/g (Bos et al., 1996). Considering that the doses of various pharmaceutical forms of valeriana are usually equivalent to no more than 3 g of crude drug per day, the doses of VA administered in this way are not therapeutically significant unless we take into account the dramatically potentiating effect in sedation and sleep induction that is evident when VA and LN are acting together (Fig. 4 and Table 2).

We have already described the presence of HN and MA in *V. wallichii* and *V. officinalis* and demonstrated that MA was able to potentiate the sleep-enhancing properties of HN (Marder et al., 2003). In contrast, LN sleep-enhancing effect is not potentiated by its coinjection with a dose of MA that causes a clear anxiolytic effect (Table 2).

We propose that the sedative and hypnotic effects of *V. officinalis* extracts may be attributed to the presence of LN, HN, MA, and VA plus the potentiating effects produced by their combinations, as is shown in Table 2.

The results in this paper establish the existence in valeriana of flavonoid glycosides with sedative and sleep-enhancing properties and demonstrate the existence of potentiating effects in its extracts. The suspected presence of synergic effects in valeriana (Hobbs, 1989) has been substantiated by these findings and brought to the fore for future clarification of the mechanisms involved (Williamson, 2001). We consider also that the correct standardization of valeriana formulations, derived nutraceuticals, or other medicaments has now to be done in terms of the above mentioned compounds.

Acknowledgements

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References

Bodesheim U, Hölzl J. Isolierungm, Strukturaufklärung und Radioreceptorassays von Alkaloiden und Lignan aus *Valeriana officinalis* L Pharmazie 1997;52:386–91.

Bos R, Woerdenbag HJ, Hendriks H, Zwaving JH, De Smet PAGM, Tittel G, Wikström HV, Scheffer JJC. Analytical aspects of phytotherapeutic valerian preparations. Phytochem Anal 1996;7:143–51.

Ferrini R, Miragoli G, Taccardi B. Neuro-pharmacological studies on SB 5833, a new psychotherapeutic agent of the benzodiazepine class. Arzneim-Forsch (Drug Res.) 1974;24:2029–32.

File SE, Pellow S. The effects of triazolobenzodiazepines in two animal tests of anxiety and in the holeboard. Br J Pharmacol 1985;86:729–35.

Harborne JB. The Flavonoids. Advances in research since 1994. London: Chapman & Hall, 1994. p. 448–9.

Hendriks H, Bos R, Woerdenbag HJ, Koster AS. Central nervous depressant activity of valerenic acid in the mouse. Planta Med 1985;51:28–31.

Hiller KO, Zetler G. Neuropharmacological studies on ethanol extracts of *Valeriana officinalis* L.: behavioural and anticonvulsant properties. Phytother Res 1996;10:145–51.

Hobbs C. Valerian: a literature review. HerbalGram 1989;21(Fall):19–34.

Hölzl J. The pharmacology and therapeutics of Valeriana. In: Houghton PJ, editor. Valerian. The Netherlands: Harwood, 1997. p. 55–75.

Hölzl J, Godau P. Receptor binding studies with *V. officinalis* on benzodiazepine receptor. Planta Med 1989;55:642.

Houghton PJ. The scientific basis for the reputed activity of Valerian. J. Pharm Pharmacol 1999;51:505–12.

Madaus G. Lehrbuch der biologischen Heilmittel. Hidesheim: Georg Olms Verlag, 1976. p. 2770–7.

Marder M, Paladini AC. $GABA_A$-Receptor ligands of flavonoid structure. Curr Top Med Chem 2002;2:853–67.

Marder M, Viola H, Wasowski C, Fernández S, Medina JH, Paladini AC. 6-Methylapigenin and hesperidin: new valeriana flavonoids with activity on the CNS. Pharmacol Biochem Behav 2003;75:737–45.

Martínez-Vázquez M, Ramírez Apan TO, Aguilar H, Bye R. Analgesic and antipyretic activities of an aqueous extract and of the flavone linarin of *Buddelia cordata*. Planta Med 1996;62:137–40.

Martínez-Vázquez M, Ramírez Apan TO, Lastra AL, Bye R. A comparative study of the analgesic and anti-inflammatory activities of pectolinarin isolated from *Cirsium subcoriaceum* and linarin isolated from *Buddelia cordata*. Planta Med 1998;64:134–7.

Mennini T, Bernasconi P, Bombardelli E, Morazzoni P. In vitro study of the interaction of extracts and pure compounds from *Valeriana officinalis* roots with GABA, benzodiazepine and barbiturate receptors in rat brain. Fitoterapia 1993;64:291–300.

Thies PW. Linarinisovalerianate, ein bischer unbekannte Flavonoid aus V. wallichii D.C. Planta Med 1968;16:361–71.

Wasowski C, Marder M, Viola H, Medina JH, Paladini AC. Isolation and identification of 6-methylapigenin, a competitive ligand for the brain $GABA_A$ receptors, from *Valeriana wallichii* D.C. Planta Med 2002;68:934–6.

Williamson EM. Synergy and other interactions in phytomedicines. Phytomedicine 2001;8:401–9.