ture at pH ≈ 4.5–5, where (A,B,C) = constants of polynomial of the second degree and T = absolute temperature. Gibbs energy change can be estimated according the equation

$$\Delta G^\circ = \gamma\, RT \ln (CMC) \quad (1)$$

where R = gas constant and γ = degree of counterion binding. If $\gamma = 1$ (the anti-ions are completely ionized), if $\gamma = 2$ (the all of anti-ions are binding to micelles). The enthalpy of micellization is defined by the equation

$$\Delta H^\circ = -\gamma\, RT^2 \left[\partial \ln (CMC)/\partial T\right] \quad (2)$$

and the entropy contribution of micellization can by calculated as follows:

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T \quad (3)$$

ΔG°, ΔH°, ΔS° values are listed in the Table.

Based on the presented results it can be concluded that

- ΔG° values are negative and slightly decline with temperature,
- Depreciations of standard molar enthalpy ΔH° are more significant at more negative values. It means that micelization process becomes more exothermic at increasing temperature,
- ΔS° values are positive and decline at increasing temperature.

Experimental

The local anaesthetic heptacainium chloride was synthesised according to Čižmárik and Borovanský (1975).

Methanol, ethanol and n-propanol used in the present work were obtained from Merck. The absorbance of the solutions were measured at various temperatures using a spectrophotometer HP 8452. Diode Array (Hewlett Packard, BRD). The pH of the medium was measured with a pH meter (Portamess 943 pH, Elekronische Messgeräte GmbH Co., Berlin). The temperature was controlled by a Thermostat (Veb ML W Prüfgerate-Werk Medingen/Sity/Freital (BRD). The critical micellar concentrations were determined by a method of Ščukin et al. (1990).

This study was supported by the VEGA grant 1/1186/04

References

Akhter MS, Al-Alawi SM (2000) Influence of alcohols on the critical micelle concentration of non-aqueous micellar solutions. Coll Surf 164: 247–255.

Čižmárik J, Borovanský A (1975) Synthesis u.v. and i.r. spectra of 2-piperidinoethyl esters of alkyloxyphenylcarbamic acids. Chem Pap 29: 119–123.

Evans DF, Wightmann PJ (1982) Micelle formation above 100 °C. J Coll Inter Sci 86: 515–524.

Ščukin ED, Percov AV, Amelinová EA (1990) Colloidal Chemistry (in Czech),1st ed., Praha Academie, p. 303–305.

School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, China

Antidepressant activity of sarsasapogenin from *Anemarrhena asphodeloides* Bunge (Liliaceae)

L. X. Ren, Y. F. Luo, X. Li, Y. L. Wu

Received June 15, 2006, accepted August 19, 2006

Dr. Yingliang Wu, School of Pharmacy, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang, 110016, China
yingliangwu_sypu@yahoo.com.cn

Pharmazie 62: 78–79 (2007)
doi: 10.1691/ph.2007.1.6630

The aim of this study was to investigate the effects of sarsasapogenin from *Anemarrhena asphodeloides* Bunge (Liliaceae) on two experimental models of depression in rats. After a two-week treatment, sarsasapogenin markedly shortened the immobility time in the forced swimming test and decreased the number of escape deficits in the learned helplessness paradigm, however, locomotor activity was not affected.

Anemarrhena asphodeloides Bunge (Liliaceae) is a medicinal herb that has long been used as a constituent of traditional Chinese prescriptions aiming at treatment of senile dementia, epilepsy, depression and mental stress. Sarsasapogenin, 5β,20α,22α,25*S*-spirostan-3β-ol, is a major active component of *Anemarrhena asphodeloides*, which exhibits a variety of pharmacological effects such as the promotion of neurogenesis activity, antioxidative action, and improving cognitive impairment (Wang et al. 2004; Meng et al. 1999; Hu et al. 2005). However, no information is available about the antidepressant activity of sarsasapogenin. Thus, in the present study, we assessed the potential antidepressant effects of sarsasapogenin in two experimental models of depression in rats.

In the forced swimming test (FST), sarsasapogenin at the doses of 5, 15 and 45 mg/kg reduced, in a dose-dependent manner, the immobility time in the FST, resulting in a 9.3%, 20.9% and 29.5% immobility reduction compared with the control group, respectively (Table 1). The activity was comparable to the reference drug fluoxetine. The FST is the tool most widely used for assessing antidepressant activity preclinically. Most clinically active antidepressants

Table 1: The effects of sarsasapogenin on the immobility time in the forced swimming test and the number of line crossings in the open-field test in rats[a]

Treatment	Dose (mg/kg)	Immobility time (sec)	Number of line crossings
Control		169.3 ± 15.7	35.4 ± 8.24
Fluoxetine	15	110.6 ± 12.5[b]	37.0 ± 7.19
Sarsasapogenin	5	153.5 ± 13.0	36.2 ± 8.52
	15	134.0 ± 10.8[c]	34.9 ± 6.95
	45	119.4 ± 11.5[b]	32.8 ± 9.16

[a] Values are mean ± S.E.M. (n = 10)
[b] $P < 0.01$, [c] $P < 0.05$ vs. control (by ANOVA, Dunnett's t-test)

Table 2: The effects of sarsasapogenin on escape failure in the learned helplessness paradigm in rats[a]

Treatment	Dose (mg/kg)	Number of escape failures		
		Day 1	Day 7	Day 14
Control		24.5 ± 2.01	23.0 ± 1.93	22.1 ± 1.52
Fluoxetine	15	22.5 ± 2.25	15.9 ± 1.86[b]	13.7 ± 1.60[b]
Sarsasapogenin	5	21.7 ± 1.95	20.0 ± 2.05	17.5 ± 1.69[c]
	15	24.5 ± 1.88	17.9 ± 1.94[c]	16.4 ± 1.50[c]
	45	22.7 ± 2.30	16.9 ± 1.75[c]	13.5 ± 1.77[b]

[a] Values are mean ± S.E.M. (n = 10)
[b] $P < 0.01$, [c] $P < 0.05$ vs. control (by ANOVA, Dunnett's t-test)

are effective in the FST, while neuroleptics and anxiolytics produce different effects (Porsolt et al. 1979). Antidepressants can also be distinguished from stimulants because stimulants cause marked motor stimulation (Borsini and Meli 1988). In our study, the antidepressant effect of sarsasapogenin cannot be attributed to an increase in motor activity because they did not induce hyperlocomotion in the open-field test (Table 1).

The learned helplessness paradigm (LH) is a depression model in which rodents exposed to inescapable and unpreditable electric shock, and subsequently develop coping deficits for aversive but escapable situations (Overmier and Seligman 1967). It represents a model with good similarity to the symptoms of depression, construct, and predictive validity in rats (Vollmayr and Henn 2001). Potential antidepressant e.g. fluoxetine significantly decreased escape failures and increased avoidance responses. In this study, administration of sarsasapogenin (5, 15, 45 mg/kg) for 14 days significantly reduced the behavioral deficits in LH rats as compared with the control group. Treatment with sarsasapogenin (15, 45 mg/kg) for 7 days also significantly declines the escape failure. However, acute treatment produced no overt change (Table 2). The results indicate that the antidepressant effects of sarsasapogenin were dose- and time-dependent.

In summary, the results of this study showed that subacute treatment with sarsasapogenin possessed antidepressant properties in animal models. Moreover, it has been reported that sarsasapogenin is a safe and potent drug that has been applied in clinic for Alzheimer's disease treatment in China. Therefore, this report could be of interest in the study of the potential therapeutic of sarsasapogenin on depression treatment.

Experimental

1. Chemicals

Sarsasapogenin with a purity of 98% was obtained as described previously (Hu et al. 2005). All drugs were suspended in 0.5% CMC-Na and administered orally once daily for 14 days.

2. Animals

Male Wistar rats, weighing 240–280 g, were used throughout the study. Rats were maintained under standard housing conditions in 12L : 12D light/dark cycle (light on 6:30) with free access to water and standard food.

3. Forced swimming test

Rats were divided into five equal groups: control, fluoxetine and sarsasapogenin (5, 15, 45 mg/kg) treatment groups. FST was similar to that described by Porsolt et al. (1978). Briefly, the rats were trained 24 h before the test and the test was performed for 5 min. Individual rats were forced to swim inside a glass-polycarbonate cylinder filled to a depth of 25 cm with water maintained at 24 °C. The absence of hind leg movement was record as immobility. Testing was performed 1 h after the final drug administration.

4. Open-field test

The open-field apparatus was a field, 70 cm in diameter, which was demarcated into 18 approximately equal areas. Each rat was placed in the center of the open-field apparatus, and the locomotor activity was assessed immediately before the FST. The number of times the animal crosses squares was recorded for 3 min.

5. Learned helplessness paradigm

Rats were divided into five equal groups: control, fluoxetine and sarsasapogenin (5, 15, 45 mg/kg) treatment groups. The LH test was similar to that described by Sairam et al. (2002). Briefly, each rat was placed in a Plexiglas chamber and exposed to 60 inescapable electric footshocks (intensity: 0.8 mA, duration: 15 s) at variable intervals of 20–90 s once a day for 3 days. The escape performances of the rats were tested in the shuttle-box at 1, 7 and 14 days after the third day of exposure to footshocks. The animals were individually placed in the shuttle-box, allowed to habituate to the environment for 5 min and then subjected to a 30 trials testing session at variable intervals of 8–22 s. In each trial, a tone signal was first presented for a maximum of 3 s with a light signal. If no avoidance response occurred, an electric shock (0.8 mA) was delivered to the rats through grid floor for a maximum of 3 s with the tone and light signal. The rats could escape the shock by moving to the other side of the box (escape response); the signals and the shock terminated on the response. If no escape response occurred, the shock and signals terminated automatically. A non-crossing response during the shock delivery was referred to as an escape failure.

6. Statistical analysis

The effects of sarsasapogenin were analyzed by one-way analysis of variance (ANOVA). When significant ($P < 0.05$ or 0.01) were found, post hoc comparisons were performed with Dunnett's t-test.

Acknowledgement: The authors wish to thank Dr. Yao Yao for providing the test sample of sarsasapogenin.

References

Borsini F, Meli A. (1988) Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology 94: 147–160.

Hu Y, Xia Z, Sun Q, Orsi A, Rees D (2005) A new approach to the pharmacological regulation of memory: Sarsasapogenin improves memory by elevating the low muscarinic acetylcholine receptor density in brains of memory-deficit rat models. Brain Res 1060: 26–39.

Meng ZY, Zhang JY, Xu SX, Sugahara K (1999) Steroidal saponins from *Anemarrhena asphodeloides* and their effects on superoxide generation. Planta Med 65: 661–663.

Overmier JB, Seligman ME (1967) Effects of inescapable shock upon subsequent escape and avoidance responding. J Comp Physiol Psychol 63: 28–33.

Porsolt RD, Anton G, Blavet N, Jalfre M (1978) Behavioral despair in rats: a new model sensitive to antidepressive treatments. Eur J Pharmacol 47: 379–391.

Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M (1979) Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. Eur J Pharmacol 57: 201–210.

Sairam K, Dorababu M, Goel RK, Bhattacharya SK (2002) Antidepressant activity of standardized extract of *Bacopa monniera* in experimental models of depression in rats. Phytomedicine 9: 207–211.

Vollmayr B, Henn FA (2001) Learned helplessness in the rat: improvements in validity and reliability. Brain Res Brain Res Protoc 8: 1–7.

Wang WD, Cheng ZJ, Xu JP (2004) Effect of saponins from *Anemarrhena asphodeloides* bunge on the cell proliferation in cultured adult hippocampus neural precursor cells *in vitro*. Jiang Xi Med 39: 96–98.