tion) anaesthetic activity in comparison to cocaine or procaine, according to the method of Vrba et al. [5].

3.2. Acute toxicity estimation

The letal effects of compound BM4 were determined after s.c. administration to mice. LD_{50} values were expressed as a range of the doses after which the animals stayed alive and those when the mortality in the group was 100% [6]. The results were recorded 24 h after administration of the compound.

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HPTLC densitometric determination of ruscogenins in dry extract of *Ruscus aculeatus* L.

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A constant increase in the application of *Ruscus aculeatus* L. dry extract as a biologically active substance has been observed due to the possibility to include it into different drug formulations. Drugs based on *Ruscus aculeatus* L. dry extract exhibit good antiinflammatory, vasotonic, antihemorroidal and antiulcer effect [1, 2].

The dry extract used in our studies was prepared via the hot extraction method with successive drying on a Nitro Atomizer dispersion drier [4]. The product is a light brown powder, possessing specific slight odor. It is soluble in water and ethanol. Since the extract is very hygroscopic Aerosil 200 was included into its samples at concentrations 13, 24 and 32% with respect to the extract amount [5].

Ruscus aculeatus L. dry extract with and without Aerosil 200 was standardized according to the percentage of residual moisture (Table) and the quantitative content of the biologically active substances. Our previous studies have shown densitometric HPTLC to be the most appropriate method for determining the ruscogenins in *Ruscus aculeatus* L. dry extract [4, 5]. The quantitative determination was preceded by studies on the optimization of the time and conditions of the acid hydrolysis: 3–3.5 h; 1 N HCl in the presence of *n*-butanol. The results for ruscogenin content in the dry extract before and after optimization of the method run with and without Aerosil are presented in the Table as mean values obtained from five experiments. The results were estimated and the quantity of ruscogenins was calculated from the peak area in the plot and from the concentration of a standard ruscogenin solution.

Table: Characteristics of samples, following European Pharmacopoeia

Sample	Residual water (%)	Ruscogenins (mg)
Extract*	1.97	0.4300
Extract	1.97	0.4881
Extract + 13% Aerosil	1.42	0.4836
Extract + 24% Aerosil	1.36	0.4920
Extract + 32% Aerosil	1.29	0.4995

* Sample hydrolysed with 5% H_2SO_4 without *n*-butanol

As seen from the Table the increase of Aerosil concentration leads to a smooth decrease in the percentage of residual moisture. This is an evidence that Aerosil could be used as a support for the dry *Ruscus* extract. Meanwhile the amount of ruscogenins remains almost unchanged in all samples. The only exception is the sample subjected to densitometry. It contains a smaller amount of ruscogenins. This is probably due to the fact that the acid hydrolysis of this extract sample was run with 5% sulphuric acid without *n*-butanol. On the other hand, the higher ruscogenins amounts in the other samples could be explained by the good solubility of aglycons in non polar organic solvents.

Experimental

1. Materials

Dry extract of *R. aculeatus* L. was obtained from overground and underground parts of plant material. Colloidal silice – Aerosil 200 (Degussa – Germany). Hydrochloric acid; sulphuric acid; *n*-butanol; methanol; ethylacetate; cyclohexan. All reagents were of analytical grade (Merck – Germany). chromatographic plate for HPTLC: Fertigplatten Kieselgel 60 10 × 20 cm (Merck – Germany); *p*-dimethylaminobenzaldehyde test.

2. Moisture determination

The measurements were made with a Moisture Analyzer Sartorius at 80 °C.

3. Assay of ruscogenin and neoruscogenin in dry extract

3.1. Acid hydrolysis

Approximately 100 mg of the extract was refluxed with 15 ml 1 N HCl for 3–3.5 h in a water bath at 95 ± 3 °C in the presence of 20 ml *n*-butanol. The *n*-butanol phase was washed several times with water and evaporated to dryness. The residue was dissolved in methanol in a 5 ml volumetric flask.

3.2. Densitometric high performance thin-layer chromatography

On a plate for HPTLC, with the help of a micropipette, 5 µl of the standard solution of ruscogenin (1 mg/ml) and of the solution of each sample were applied.
The plate was developed in a chamber saturated with the vapour of the system cyclohexane/ethylacetate (1 : 1) [3]. After the mobile phase reached the front line (8 cm), the plate was taken out, air-dried and sprayed with a solution of *p*-dimethylaminobenzaldehyde, after which it was dried at 110 °C for about 5 min and pink coloured spots appeared. The resulting coloured spots were submitted to densitometric chromatogram evaluation on CAMAG TLC SCANNER II at 520 nm.
The quantity of ruscogenins was calculated by determining the peak area of the sample solution with calibration curve obtained by chromatography of standards.

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Plasma protein binding properties of dimeric 4-aryl-1,4-dihydropyridines as novel non peptidic HIV-1 protease inhibitors

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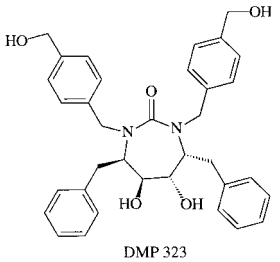
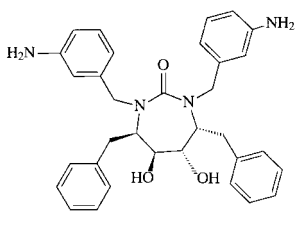
The plasma protein binding of drugs certainly influences their bioavailability. Protein binding of drugs with a small therapeutic range like the cardiac glycoside digitoxine is of great importance as such binding properties significantly lower the necessary blood levels [1, 2]. The bioavailability of drugs with poor intestinal absorption and, additionally, high plasma protein binding is often unsatisfying.
The peptidic HIV-1 protease inhibitors saquinavir and indinavir demand high doses due to their poor bioavailability that is partly caused by their high protein binding properties besides poor absorptions and extensive metabolism by the cytochrom P450 system [3].
Among the few non peptidic HIV-1 protease inhibitors, cyclic ureas like DMP 323 and DMP 450 with excellent anti-HIV activities in infected cell cultures failed in clinical trials because of unsatisfying bioavailabilities [4]. While DMP 323 showed poor absorption and extentsive oxidative metabolism, the therapeutic blood levels of DMP 450 were not achieved due to a high protein binding partly caused by interactions of the weakly basic anilino groups and α_1-acid glycoprotein.
Recently cage and *syn* dimeric 4-aryl-1,4-dihydropyridines have been introduced as novel non peptidic HIV-1 protease inhibitors with moderate activities [5–8]. With encouraging bioanalytical results of favourable poor metabolism and non-toxicity evaluated in Hep G2 cells those dimers hold promise as novel and perspective class of HIV-1 protease inhibitors [9, 10]. Nevertheless, their plasma protein binding properties had to be evaluated as determining factor of bioavailability.
For this study, the cage dimeric *N*-benzyl 4-phenyl-1,4-dihydropyridine (H 17) and the *syn*dimeric *N*-benzyl 4-phenyl-1,4-dihydropyridine (H 19) were selected.
Different concentrations of each compound were incubated with a solution of human serum albumin (4%) at 37 °C in a shaker. After separation of the protein bound share by centrifugation the drug concentrations were determined UV-spectroscopically. Comparing to the measured concentra-



DMP 323



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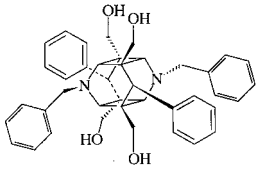
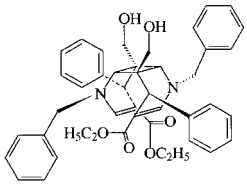


H17



H19