Stefan Nikolov & Čavdar Gusev

A chemotaxonomic comparison of the genera *Asparagus* and *Ruscus (Liliaceae)*

Abstract

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A review of steroidal sapogenins (SGs) found in *Asparagus* and *Ruscus* species is presented. Intergeneric differences in SG content and structure can be observed. For *Asparagus* monohydroxy-SGs are characteristic, whereas in *Ruscus* di- and trihydroxy-SGs are found. Some published data, which appear to contradict this general picture, called for a phytochemical reinvestigation of *A. verticillatus*, *A. maritimus*, and *R. ponticus*. The results do not confirm the presence of (dihydroxy-SG) ruscogenin in *Asparagus* or of (monohydroxy-SG) diosgenin in *Ruscus*, thus supporting the assumption that the two genera have no SGs in common.

Introduction

Different taxonomic views exist on the position of the genera *Asparagus* L. and *Ruscus* L. (Engler 1888, Hutchinson 1959, Tahtadžjan 1966, 1987, Huber 1969, Dahlgren & al. 1985). Steroidal saponins or sapogenins (SG) have been used as chemotaxonomic markers for the two genera (Hegnauer 1986). These compounds are common to both, but whether they show intergeneric structural differences is not yet fully understood. Such differences might be found in the oxygen content of SGs (Borin & Gottlieb 1993). The aim of the present study is to reassess the published reports on the presence of ruscogenin in *Asparagus maritimus* and *A. verticillatus*, and diosgenin in *Ruscus ponticus*, since these reports contradict the presence of consistent chemotaxonomic differences between the two genera on the basis of the structure of their sapogenins.

Material and methods

Plant material of the following provenances was used for our investigation (vouchers in SOM): *Asparagus maritimus:* Bulgaria, Danube plain west of the city Orjahovo, dry bushy places (SOM No. 273-Co); *A. verticillatus:* Bulgaria, Black Sea coast around

Table 1. Steroidal sapogenins in *Asparagus* L.

Compound	Structure	Species	References
Sarsasapogenin	25S-spirostan-3b-ol	*A. acutifolius* L.	Dávila & Panizo (1958)
		A. adscendens Roxb.	Rao (1952)
		A. africanus Lam.	Kar & Sen (1986)
		A. albus L.	Díaz & al. (1967b)
		A. cochinchinensis (Lour.) Merr.	Okanishi & al. (1975)
		A. cooperi Baker	Kar & Sen (1986)
		A. curillus Roxb.	Sharma & al. (1983)
		A. davuricus Link	Ilarionov & al. (1983)
		A. falcatus L.	Díaz & al. (1967b)
		A. filicinus D. Don	Ding & Yang (1990)
		A. gonocladus Baker	Rao (1952)
		A. maritimus (L.) Mill.	Held & al. (1969)
		A. officinalis L.	Gorjanu & al. (1967)
		A. persicus Baker	Tairov (1969)
		A. plumosus Baker	Held & al. (1969)
		A. pseudoscaber Grecescu	Held & al. (1969)
		A. racemosus Willd.	Rao (1952)
		A. schoberioides Kunth	Held & al. (1969)
		A. stipularis Forssk.	Dávila & Panizo (1958)
		A. tenuifolius Lam.	Zong (1975)
		A. trichophyllus Bunge	Held & al. (1969)
		A. verticillatus L.	Held & al. (1969)
Diosgenin	25R-spirostan-5-en-3b-ol	*A. adscendens*	Sharma & al. (1980)
		A. asparagoides (L.) Druce	Giordano & González (1967)
		A. densiflorus (Kunth) Jessop	Kar & Sen (1986)
		A. officinalis	Held & al. (1969)
		A. plumosus	Sati & Pant (1985)
		A. racemosus	Rao (1952)
		A. sprengeri Regel	Held & al. (1969)
		A. trichophyllus	Ilarionov & al. (1983)
Yamogenin	25S-spirostan-5-en-3b-ol	*A. officinalis*	Held & al. (1969)
		A. plumosus	Sati & Pant (1985)
		A. sprengeri	Held & al. (1969)
		A. tenuifolius	Zong (1975)
Hecogenin	25R-spirostan-3b-ol-12-on	*A. plumosus*	Díaz & al. (1967c)
		A. scoparius Lowe	Díaz & al. (1967b)
		A. umbellatus Royle	Díaz & al. (1967a)
Ruscogenin	25R-spirostan-5-en-1b,3b-diol	*A. maritimus*	Held & al. (1969)
		A. verticillatus	Ilarionov & al. (1983)
Penogenin	25R-spirostan-5-en-3b,17a-diol	*A. asparagoides*	Giordano & González (1967)
		A. tenuifolius	Zong (1975)
Smilagenin	25R-spirostan-3b-ol	*A. albus*	Laorga & Pinar (1960)
Tigogenin	25R-spirostan-5a-3b-ol	*A. umbellatus*	Díaz & al. (1967 b)
Hispidogenin	25R-spirostan-5a-12-ol-3-on	*A. scoparius*	Díaz & al. (1967b)
		A. umbellatus	Díaz & al. (1967a)
	25R-spirostan-3,5-dien	*A. tenuifolius*	Zong (1975)

Table 2. Steroidal sapogenins in *Ruscus* L .

Compound	Structure	Species	References
Ruscogenin	25R-spirostan-5-en-1b-3b-diol	*R. aculeatus* L.	Lapin & Sannie (1955)
		R. hyrcanus Woronow	Iskenderov (1968)
		R. hypoglossum L.	Panova & al. (1974)
		R. hypophyllum L.	Pčeidze & al. (1971)
		R. ponticus Woronow	Pčeidze & al. (1971)
25S-ruscogenin	25S-spirostan-5-en-1b,3b-diol	*R. aculeatus*	Panova & Nikolov (1979)
		R. hypophyllum	Pčeidze & al. (1971)
Neoruscogenin	25S-spirostan-5-en-1b,3b-diol-25(27)-en	*R. aculeatus*	Mandell & al. (1960)
		R. hypoglossum	Panova & al. (1974)
27-hydroxyruscogenin	25S-spirostan-5-en-1b,3b-27-triol	*R. aculeatus*	Panova & Nikolov (1979)
		R. hypoglossum	Panova & al. (1978)
Isoandrogenin A	25S-spirostan-5-en-1b,3b,25S-triol	*R. aculeatus*	Panova & Nikolov (1979)
		R. hypoglossum	Panova & al. (1978)
Isoandrogenin B	25R-spirostan-5-en-1b,3b-25R-triol	*R. aculeatus*	Panova & Nikolov (1979)
		R. hypoglossum	Panova & al. (1978)
Diosgenin	25R-spirostan-5-en-3b-ol	*R. ponticus*	Pčeidze & al. (1971)

Nesebăr, scrub (SOM No. 274-Co); *Ruscus ponticus:* cultivated at the Department of Pharmacognosy, Pharmaceutical Faculty, Sofija, from seeds obtained from the Nikita Botanical Gardens, Jalta, Crimea, Ukraine (SOM No. 275-Co).

40 g dry weight of underground parts of each species were ground, then hydrolysed separately under reflux, over a water bath, with 500 ml 5 % H_2SO_4. The hydrolysed plant material was separated by filtration, washed with water until neutral, dried and extracted thrice for 1 hour with petroleum ether, and once with petroleum ether-ethanol (90 : 10). The extracts were combined and concentrated to dryness.

0.5 g of each sapogenin mixture were separated by column chromatography on Kieselgel Merck (30 g) with the eluent cyclohexane : ethylacetate = 1 : 1 (S_1). From *Asparagus maritimus* 12 fractions were obtained, from *A. verticillatus,* 14 fractions, and from *Ruscus ponticus,* 18 fractions. The collected fractions were studied by thin layer chromatography (TLC) over Kieselgel G Merck plates in the systems S_1; $CHCl_3$: MeOH = 50 : 2 (S_2); and C_6H_6 : EtOAc = 6 : 3 (S_3). All fractions from *A. maritimus* and *A. verticillatus* were compared with authentic samples of ruscogenin, and all fractions of *R. ponticus,* with diosgenin authentic samples. The sapogenins were developed with p-dimethylaminobenzaldehyde reagent (Nikolov & al. 1976).

Results and discussion

The SGs so far reported to occur in species of *Asparagus* and *Ruscus,* with mention of their structural differences, are shown in Tables 1 and 2, respectively. Monohydroxy-SGs such as sarsasapogenin, diosgenin, smilagenin, yamogenin, and tigogenin, all of which

are 3-β-OH SGs, are characteristic of *Asparagus.* Other monohydroxy-SGs found in that genus, which have one additional keto-group in the steroidal skeleton, are hecogenin and hispidogenin. The 25-spirostan-3,5-dien, which is without functional O-group in the steroidal skeleton, is also a marker for *Asparagus,* exhibiting low oxygen content. Dihydroxy-SGs such as penogenin and ruscogenin are derivatives of these monohydroxy-SGs. Of these, ruscogenin, 25 S-ruscogenin, neoruscogenin, 27-hydroxyruscogenin, isoandrogenin A and isoandrogenin B are characteristic of the genus *Ruscus.*

From the analysis of the published data it would appear that the only SGs that are in common to both genera are ruscogenin and diosgenin. Ruscogenin, general in *Ruscus,* was reported as a result of TLC screening for *Asparagus maritimus* (Held & al. 1969) and *A. verticillatus* (Ilarionov & al. 1983). Diosgenin, widespread in *Asparagus,* was reported once for *Ruscus ponticus* (Pčeidze & al. 1971)

On the basis on the aforementioned data, a reinvestigation of the SG content was carried out for the three latter species. Following direct acid hydrolysis of underground parts of *Asparagus maritimus, A. verticillatus,* and *Ruscus ponticus,* different sapogenin mixtures were obtained and chromatographed on different columns for the separation of their sapogenin components. Clear fractions obtained from the respective columns were studied by TLC in the systems S_1, S_2 and S_3 and compared with authentic samples of ruscogenin and diosgenin. The results of this investigation show that ruscogenin is absent from *A. maritimus* and *A. verticillatus,* and diosgenin is absent from *R. ponticus.*

These results are of considerable interest, as they show that the two genera do not have any SGs in common but are consistently different with respect to the structure of the steroidal sapogenins present.

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Addresses of the authors:
Ass. Prof. Dr S. Nikolov, Faculty of Pharmacy, Medical University, Dunav Str. 2, BG-1000 Sofija, Bulgaria.
Č. Gusev, Institute of Botany, Bulgarian Academy of Sciences, Akad. G. Bončev Str. 23, BG-1113 Sofija, Bulgaria.