

## ISOLATION OF DIOSGENIN FROM *ASPARAGUS SPRENGERI*\*

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**Abstract**—An unsaturated  $C_{27}$  steroid sapogenin has been isolated from the roots of *Asparagus sprengeri* and identified as diosgenin. This compound was found in all other parts of the plant except the ripe seeds. Apart from the etiolated shoots of *A. officinalis*, however, it was not present in nine related species. The presence of ruscogenin was detected by TLC in *A. maritimus*.

STEROID-saponins are generally considered to be characteristic of the Liliaceae<sup>1</sup> from which twenty-one compounds of this type have been so far isolated, among which diosgenin was found in twelve genera.

Hegnauer has grouped the genus *Asparagus* together with *Ruscus* and *Semele* into the tribe Asparageae within the sub-family Asparagoideae.<sup>1</sup> Chemical examination of *Asparagus* and *Ruscus* species showed that saponins were present,<sup>1-7</sup> but sarsasapogenin (saturated 3-monool type) was the only steroid sapogenin isolated from *Asparagus*, and ruscogenin and neoruscogenin (unsaturated 1,3 diol types) from *Ruscus*. *Semele* species were not studied. *Asparagus sprengeri* has not been studied so far in detail, and the available evidence is rather inconsistent. Villar and Palasi<sup>1</sup> claim that the rhizomes contain saponin, and saponin has also been demonstrated in the leaves by Aurich.<sup>8</sup> Chetverikova, on the other hand, could not find any saponin in either part of the plant.<sup>2</sup>

In order to resolve this problem, different parts of ten *Asparagus* species have been examined using the method described earlier.<sup>9</sup> In the course of our examination, we have found three sapogenins in *A. sprengeri* which were not reported in previous work. The main component was isolated by preparative TLC and was identified as diosgenin on the basis of m.p., i.r. spectrum and comparison on TLC of the compound and its acetate with authentic materials (same  $R_f$  value in five solvent systems, same colour reactions).

On the basis of its TLC behaviour, the second component was found to be identical with the  $C_{25}$  epimer of diosgenin, yamogenin. The third genin was not identified. From its

\* Part IV in the series, Examination of Steroids of Plant Origin.

<sup>1</sup> R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Bd. II. Birkhäuser, Basel-Stuttgart (1963).

<sup>2</sup> L. S. CHETVERIKOVA, V. I. ZICHENKO and N. M. UTKIN, *Trudi VILAR* 202, Medgiz, Moskva (1959).

<sup>3</sup> O. S. MADAeva, N. A. SEROVA, L. S. CHETVERIKOVA, YU. N. SEJNKEr and V. I. KICEHNKO, *Trudi VILAR* 229, Medgiz, Moskva (1959).

<sup>4</sup> K. PEACH and M. V. TRACEY, *Modern Methods of Plant Analysis*, Springer Verlag, Berlin-Göttingen-Heidelberg, 191 (1955).

<sup>5</sup> F. F. ANZALDI, J. MARANON and S. ANCHETA, *Philippine J. Sci.* **85**, 305 (1956).

<sup>6</sup> H. KREITMAIR, *Pharmazie* **8**, 300 (1953).

<sup>7</sup> R. LAORGA and M. PINAR, *An. Real. Soc. Espan. Fys. y Quim. (Madrid)* **568**, 797 (1960).

<sup>8</sup> O. AURICH, C. OSSKE, K. PUFAHL, A. ROMEIKE, H. RÖNSCH, K. SCHREIBER and G. SEMBDNER, *Die Kulturpflanze* **13**, 621 (1965).

<sup>9</sup> D. VÁGUJFALVI, GY. HELD and P. TÉTÉNYI, *Arch. Pharm.* **299**, 812 (1966).

TLC behaviour, it is not smilagenin, sarsasapogenin, tigogenin, neotigogenin, ruscogenin or neoruscogenin.

Quantitative chromatographic examination showed that both the underground and aerial parts of *A. sprengeri* contain diosgenin in about the same quantity (0.08 per cent on a dry wt. basis). The diosgenin content of the ripe fruits was nearly half that in the unripe fruits, and the compound was not detectable in the ripe seeds.

TLC screening of other species showed that only the etiolated shoot of *A. officinalis* contains diosgenin and yamogenin. Sarsasapogenin is more common and was detected in eight of the ten species examined (Table 1). The two *Ruscus* species examined both contained

TABLE 1. OCCURRENCE OF STEROID SAPOGENINS IN ASPARAGEAE

Name of plant	Part of plant*	Sapogenin†			
		D	Y	S	R
<i>Asparagus sprengeri</i> Rgl.	L.	+	+	—	—
	R.	+	+	—	—
<i>A. officinalis</i> L.	Etiol. bud	+	+	+	—
	L.	—	—	+	—
<i>A. maritimus</i> Pall.	L.	—	—	+	—
	R.	—	—	+	+
<i>A. plumosus</i> Bak.	L.	—	—	+	—
<i>A. trichophyllus</i> Bnge	L.	—	—	+	—
	R.	—	—	+	—
<i>A. dahuricus</i> Fisch	L.	—	—	+	—
	R.	—	—	+	—
<i>A. pseudoscaber</i> L.	L.	—	—	+	—
	R.	—	—	+	—
<i>A. verticillatus</i> L.	R.	—	—	+	—
<i>A. schoberioides</i> Kunth.	R.	—	—	+	—
<i>A. falcatus</i> L.	R.	—	—	—	—
<i>Ruscus aculeatus</i> L.	L.	—	—	—	+
	R.	—	—	—	+
<i>R. hypoglossum</i> L.	L.	—	—	—	+
	R.	—	—	—	+

\* L, leaves; R, roots.

† D, Diosgenin; Y, yamogenin; S, sarsasapogenin; R, ruscogenin.

ruscogenin, but did not contain either diosgenin, sarsasapogenin, or yamogenin. On the other hand, ruscogenin was found in *A. maritimus*. Thus, besides *Ruscus* and *Tribulus*,<sup>10</sup> *Asparagus* can be considered as the third ruscogenin-containing genus. This fact supports the close relationship between *Asparagus* and *Ruscus*<sup>1</sup> and does not give any reason for the separation of family Ruscaceae.<sup>11</sup>

## EXPERIMENTAL

### Preparative TLC Examination

400 g of dry root of *Asparagus sprengeri*, freed from lipids with ether, was extracted with methanol. The extract was concentrated and the saponins precipitated with ether. After hydrolysis with 1 N H<sub>2</sub>SO<sub>4</sub> and

<sup>10</sup> J. M. BROWN and W. T. DE KOCK, *South African Ind. Chem.* **13**, 189 (1959).

<sup>11</sup> J. HUTCHINSON, *The Families of Flowering Plants*, Vol. II, 2nd edition, Clarendon Press, Oxford (1960).

ethanol 96% (1:1), the material was neutralized and the sapogenins extracted with  $\text{CHCl}_3$ . The samples of material, after removal of  $\text{CHCl}_3$ , were applied to plates coated with 2 mm Kieselgel G. (Solvent: petrol-benzene-ethyl acetate, 85:5:10.) The plates were developed in the solvent twice and the separated sapogenins eluted with  $\text{CHCl}_3$ -methanol (1:1), and further purified twice in  $\text{CH}_2\text{Cl}_2$ -ether (199:1).<sup>12</sup> The separated diosgenin was eluted, and after several recrystallizations, 60 mg of diosgenin was obtained. M.p. 199–200° (uncorrected). I.r. spectra was measured in nujol—with a Perkin-Elmer "Infracord". The acetate (acetic anhydride pyridine) was identical with diosgenin acetate.

*TLC Examination of Other Species (Table 1)*

1 g of dry material, freed from lipids, was extracted with 80% methanol. The dry extract was hydrolysed as above and the  $\text{CHCl}_3$ -extracted material examined on Kieselgel G. (Solvents:  $\text{CH}_2\text{Cl}_2$ -ether, 199:1 (twice);  $\text{CHCl}_3$ -methanol (19:1); petrol-benzene-ethyl acetate (85:5:10); toluene-glacial acetic acid-ethyl acetate (40:20:50); cyclohexane-ethyl acetate- $\text{H}_2\text{O}$  (60:40:0.1).) Spray reagents: 20%  $\text{CHCl}_3$  solution of  $\text{SbCl}_3$ ; 10 mg  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  + 10 ml conc.  $\text{H}_2\text{SO}_4$  acid + 10 ml glacial acetic acid; 1% vanillin in 50% phosphoric acid; acetic anhydride + conc.  $\text{H}_2\text{SO}_4$  + ethanol (1:1:10); 0.5% formaldehyde in conc.  $\text{H}_2\text{SO}_4$ .

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<sup>12</sup> R. D. BENNETT and E. HEFTMANN, *J. Chromatogr.* **21**, 488 (1966).