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25S - RUSCOGENIN AND DIOSGENIN FROM THE LEAVES OF SANSEVIERIA CYLINDRICA, BOJER

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Chromatographic investigation of the aglycones and sapogenins of the hydroysed powder of Sansevieria cylindrica leaves revealed the presence of 13 components, three of them were chromatographically identical to B-sitosterol, diosgenin and ruscogenin in various TLC systems. Preparative TLC and column chromatographic techniques were used for the isolation of the three components. Their identity was confirmed by TLC, gas chromatography, IR, UV, NMR and mass spectral data. The compound corresponding to ruscogenin was stereochemically confirmed as 25S-ruscogenin by IR and NMR data.

Sansevieria cylindrica, Bojer is a herbaceous perennial plant belonging to the family Agavaceae, a family characterised by its steroidal sapogenin and saponin constituents. Steroidal sapogenins serve as important starting material for the chemical synthesis and industrial production of steroidal hormones[1-5]. Many sapogenins were isolated from various species belonging to different genera. Agave[1,3,6-7], Furcraea[8-9],

Yucca[6,8,10-13], Hesperaloe[6-7], Samuela[1,8], Nolina[1,6-7] and Deacaena[1,6]. One species of the genus Sansevieria i.e. S. trifasciata was reported to yield steroidal sapogenins[14-15]. Saponins were isolated intact from a number of Furcraea[16-17] and Yucca[18-20] species.

S. cylindrica has long been introduced as an ornamental plant in Egypt. No information on the sapogenins of this plant could be traced in the literature, while S. trifasciata was preported to contain ruscogenin, 25S-ruscogenin, neoruscogenin, sansevierogenin and abamogenin[14-15]. This motivated the interest in the study of the sapogenin of S. cylindrica Bojer .

RESULTS AND DISCUSSIONS

TLC of the crude chloroformic extract of the hydrolysed powder , containing the various sapogenin and other aglycones* revealed the presence of 13 components (Fig. 1). Three of the major components were identical with each of B-sitosterol, diosgenin and ruscogenin in R_f & spot solour, when using vanillin/H_2SO_4 as the spray reagent. These were isolated by preparative TLC as well as by column chromatography, and were designated substances A,B and C respectively.

Substance A, corresponding to B-sitosterol, was recrystallized from ethanol to give white crystals, mp 187°C. Its mixed mp with authentic material was undepressed and it showed a superimposable IR spectrum with that of reference B-sitosterol. Gas chromatographic investigation proved the substance to be composed of B-sitosterol as the only component.

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* This is the genin fraction, contaning aglycones of various typess of glycoside.

This was found interesting, since the sterol band of the unsaponifiable fraction was found to contain B-sitosterol (90%) and stigmasterol (10%). Thus S. cylindrica leaves contain B-sitosterol and stigmasterol as such in the lipid fraction as well as glycosidic B-sitosterol.

Substance B, corresponding to diosgenin, was recrystallized from ethanol as needle crystals, mp 201-203°C. Mixed mp with authentic diosgenin was undepressed. The IR spectrum of the isolated compound showed the four spiroketal bands at 980, 920, 900 & 870 cm^{-1}; the ratio of the intensities of the 920 cm^{-1} to the 900 cm^{-1} bands is indication of 25-configuration, confirming identity as diosgenin. Further the IR spectra of isolated and authentic compound were superimposable. The mass spectrum(Fig.1), showed M^{*} at m/e 414, and the fragmentation pattern of the isolated substance was in accordance with that expected for diosgenin [21].

Substance C, corresponding to the ruscogenin band, was recrystallized from ethyl acetate as colourless needles, with mp 196-200°C. The IR spectrum of substance C showed the four characteristic spiroketal bands at 980, 920, 900 and 870 cm^{-1}, with the relative intensities of the 920 cm^{-1} and the 900 cm^{-1} bands indicative of the 25B-configuration. The IR spectrum is otherwise superimposable on that of authentic ruscogenin*. NMR (Fig.2) spectrum proved the presence of a vinylic proton at position 6, indicative of a Δ^{5}(-).

Other chemical shift data are in accordance with those reported for 25S-ruscogenin[14]; they confirm 25B-CH_3, dihydroxy and Δ^{5}(-) as indicated from the chemical shift of the

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* Kindly supplied by Dr. Taha El-Alfy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

$-CH_3$ at position 10 . These data are in good correspondance with the reported data for 25S-ruscogenin. The mass spectrum (Fig. 3) showed an M^+ at m/e 430; further fragmentation pattern in the mass spectrum confirms the assigned structure as 25S-ruscogenin[21].

In conclusion, S.cylindrica, Bojer contains 13 aglycones, the major three of which were isolated and identified as B-sitosterol, diosgenin and 25S-ruscogenin. B-sitosterol exists also in the free form, together with stigmasterol in the lipid fraction.

EXPERIMENTAL

Plant material:

The cylinderical leaves were separated from the herb, sliced into thin slices and dried in a circulating hot-air oven at 50°C; it was then reduced to a fine powder.

Reafents & Chemicals:

All solutions were prepared from analytical grade chemical. Chromatographic reference solutions were separately prepared by dissolving 2 mg of either B-sitosterol, diosgenin or ruscogenin in 0.5 ml of chloroform.

Extraction of the aglycone and sapogenin fraction:

The powdered leaves were defatted with petroleum ether (40-60°C), then exhausted with chloroform. The dried marc was refluxed with 10% HCl for 4 hours (to hydrolyse the glycosides and saponins). The mixture was filtered and the hydrolysed powder was washed with distilled water to neutrality, then dried at 40°C. The dried hydrolysed powder was,

reexhausted with $CHCl_3$ the chloroformic extract was evaporated to dryness under vacuo and the residue was subjected to chromatographic investigation.

Thin-layer chromatography of the aglycone and sapogenin fraction

The chloroformic solution of the extract was subjected to TLC on various solvent systems; these are : Si gel G/ benzene- ethyl acetate (9:1) as well as (4:1) and Si gel G/ $CHCl_3$- MeOH (9:1). The best results were obtained with benzene-ethyl acetate (4:1) as the solvent system; 2% vanillin/sulfuric acid in ethanol and 10% H_2SO_4, followed by heating at 110°C for 10 min. were used as the locating agent.

Isolation of the major sapogenins by prepatative TLC:

The $CHCl_3$ solution of the crude genins (10%) solution was applied to thick layers of Si gel GF_{254} plates, along with reference diosgenin and ruscogenin. The plates were developed with benzene-ethyl acetate(4:1) and then dried and examined under UV to mark the sapogenin bands. The bands corresponding to each of diosgenin and ruscogenin, were separately scrapped off and each was extracted with $CHCl_3$. The solution of rach band was further purified by chromatographing each on a small silica gel column; elution was carried out using benzene containing increasing amounts of ethyl acetate (0-10%). Fractions were monitored by TLC; fraction corresponding to each of diosgenin and ruscogenin bands from their respective columns were separately pooled and subjected to crystallization from appropriate solvents (ethanol for dio-

sgenin & ethyl acetate for the ruscogenin component).

Isolation of B-sitosterol and the major sapogenins by column chromatography:

The major part of the crude genin extract was subjected to a column chromatographic separation on silica gel (Merck) using benzene containing increasing amounts of ethyl acetate (0-10%), using a stepwise gradient elution technique. Fractions were monitored by TLC, separately pooling the fractions of similar composition. Thus fractions corresponding to each of B-sitosterol, diosgenin and ruscogenin bands were pooled and each subjected to proper crystallizeation from appropriate solvents (see above; B-sitosterol was recrystallized from ethanol).

Each isolated substance was identified by a combination of physical (mp & mixed mp), chromatographic (TLC (all) & Gas chromatography (for B-sitosterol), and spectral data (primarily IR, NMR and mass spectra)*, as mentioned under" Results and Discussions".

Substance A.:

It was recrystallized from absolute ethanol to give colourless needles, mp 135 - 137°C; mixed mp with authentic B-sitosterol 135 - 137°C (undepressed); IR spectrum superimposable with that of authentic B-sitosterol; GLC of the isolated substance acetate (Prepared from substance A by reflux with acetic anhydride/pyridine) on a column (2 meter x 1/4 inch) of OV-17 on chromosorb W (100 1 120 mesh); helium was used as the carrier gas at a rate of 45 ml/min. and the development was isothermal at 270°C. The GLC results are shown in Fig. 1, and shows only one peak with retantion time of 27 min., corresponding to that of B-sitosterol.

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* GLC & Spectral measurements were performed at the "National Research Center, Service Unit, Dokki, Giza, Egypt.

Substance B.:

It was recrystallized from ethanol as colourless needles, mp 201-203°C; mixed mp with authentic diosgenin 201-203°C (undepressed); IR, γ OH 3400 cm^{-1}, γ (=) 3030, 2830 and 830 cm^{-1}, γ (spiroketal bands) 980, 920, 900 and 870 cm with the peak at 900 cm^{-1} more intense than that at 920 cm^{-1} (25 ∝ -); Ir spectrum was superimposable with that of authentic diosgenin; Mass spectrum, M^+ at m/e 414 (see (Figure 1) for details).

Substance C.:

It was recrystallized from ethyl acetate as colourless needles, mp 196 - 200°C; IR, OH 3500 & 3400 cm^{-1}, γ (=) 3030, 2830 & 830 cm^{-1} ,γ (spiroketal bands) 980, 920, 900& 870 cm^{-1} with the band at 920 cm^{-1} more intense than that at 900 cm^{-1} (25B-); NMR, δ (ppm), 0.78 (s, 3H, C_{13}-CH_3), 0.96 (d,3H, C_{20}-CH_3), 1.08 (d, 3H, C_{25}-CH_3), 1.10 (s, 3H, C_{10}-CH_3), 4.05, 3.87, 3.39 and 3.20 (m, 2H, C_{26}-H_2), 5.63 ppm (m, 1H, C_6-H); Mass spectrum, M^+ at m/e 430 (see Figure 3 for details).

Table 1: hR_f values and colour of spots(Vanillin/H_2SO_4 spray) genins of S. cylindrica leaves

Spot No.	*hR_f value*	*Colour*	*Authentic reference*
1	96	reddish brown	--
2	91	brown	--
3	83	yellow	--
4	78	yellow	--
5	70	violet	--
6	62	bluish violet	--
7	51	violet	B-sitosterol
8	45	yellow	diosgenin
9	32	yellow	--
10	23	grey	--
11	13	yellow	corresponds to ruscogenin
12	9.3	bluish violet	--
13	4.6	yellow	--

System: Si gel G/Benzene-ethyl acetate (4:1)

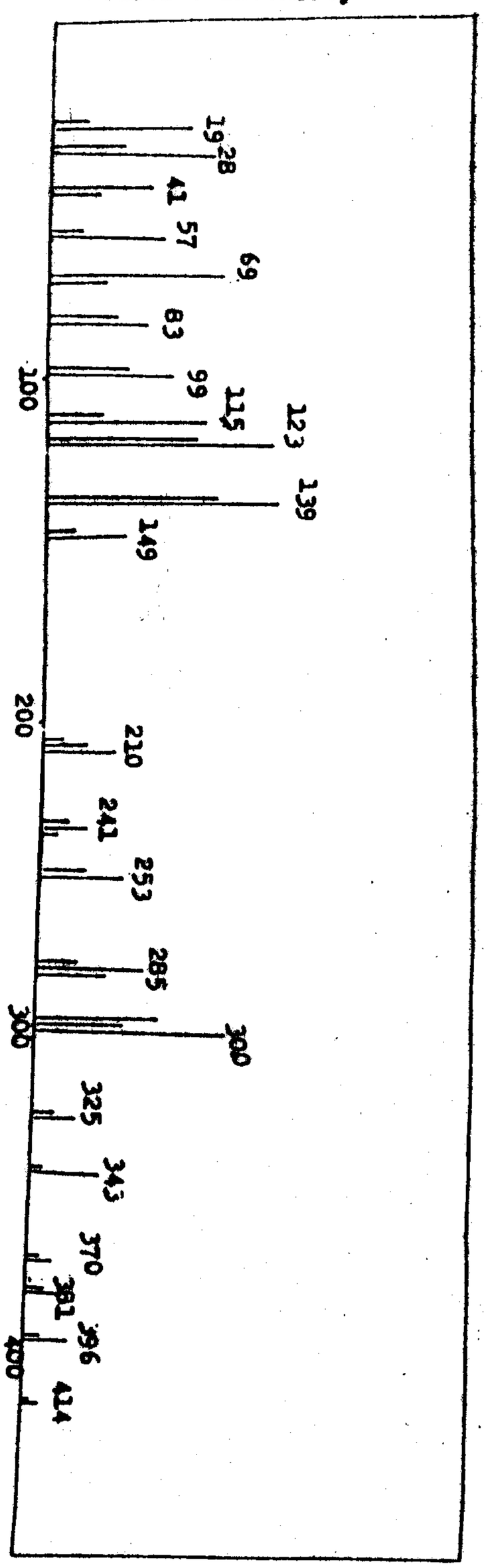

Figure 1 - Mass Spectrum of Substance B (Diosgenin)

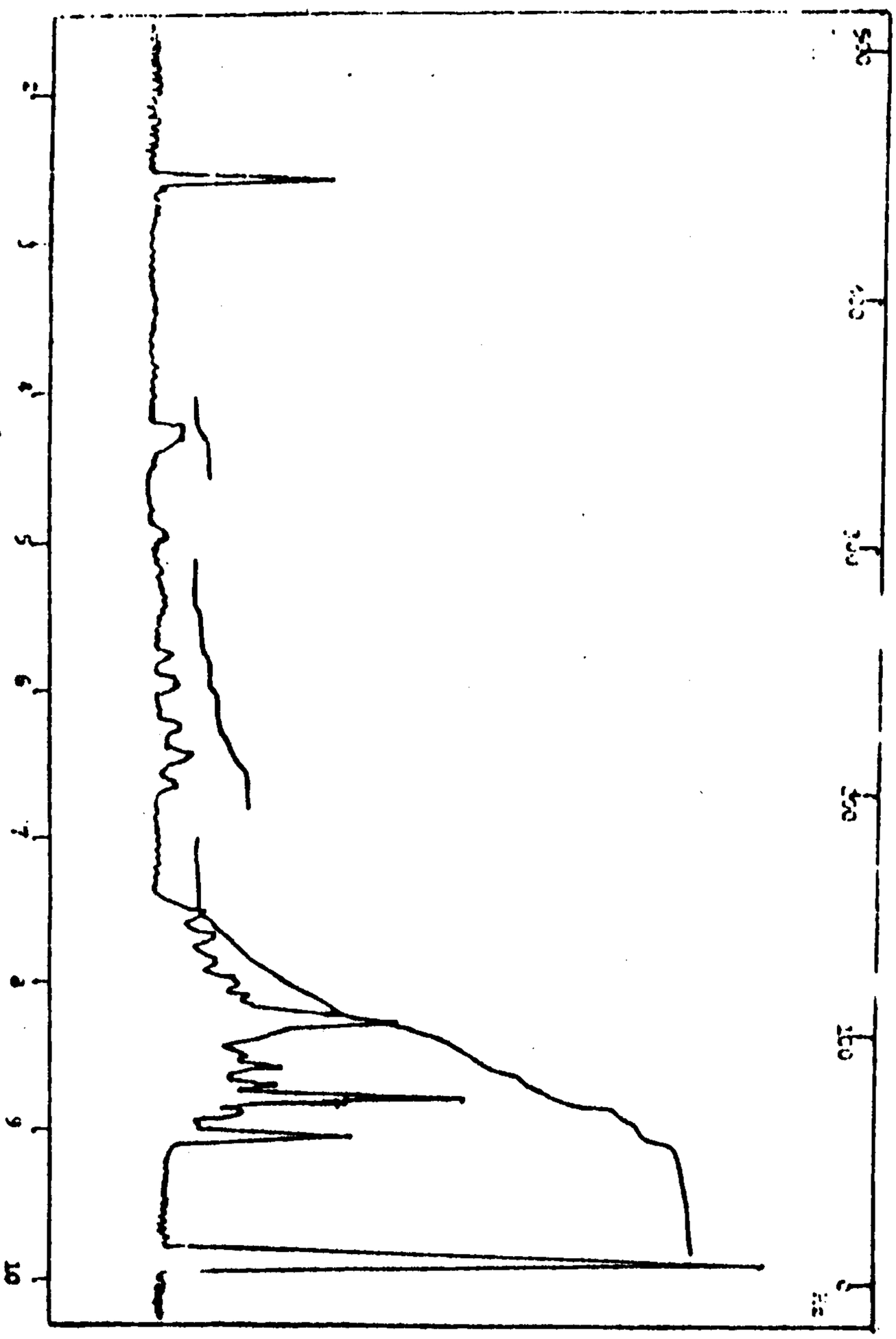

Figure 2 - NMR Spectrum of Substance C (25-S-ruscogenin)

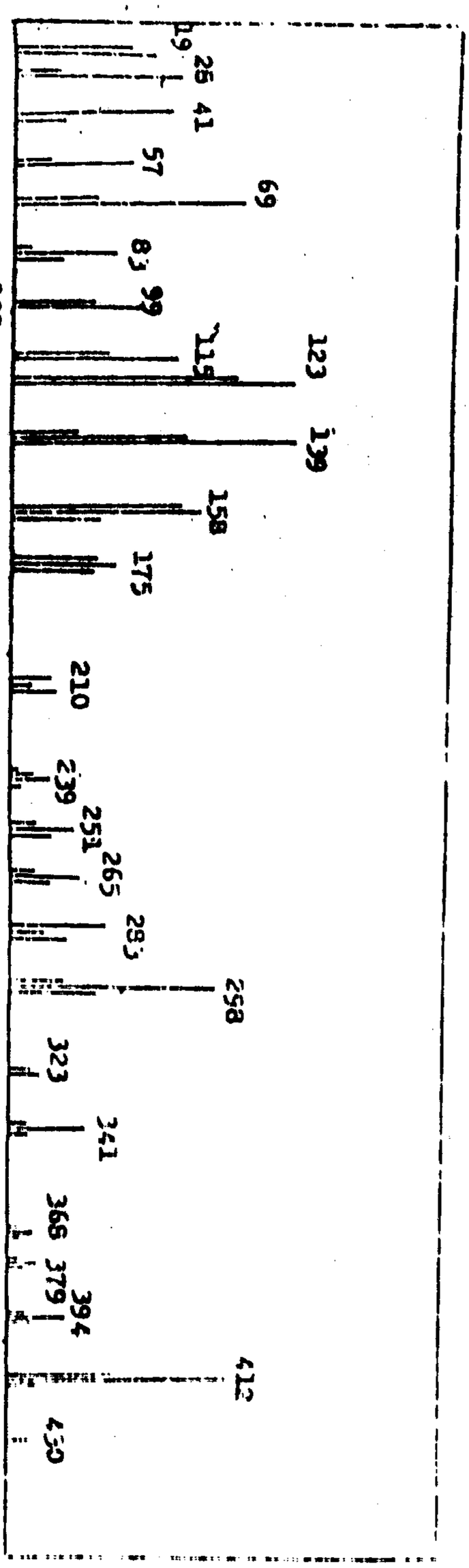

Figure 3 - Mass Spectrum of Substance C (25-S- nacogenin)

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الديوسجننين ، ٢٥ - س رسكوجتين

من

اوراق نبات السانسفيرا سيلندريكا (بوجر)

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اثبتت الدراسة الكرماتوجرافية لمادة الصابوجنين المحضرة من البودرة المحلله لاوراق نبات السانسفيرا سيلنديكا عن وجود ١٣ مركبا . ووجد ان ثلاثة من تلك المركبات متشابه مع البيتاسيتوسترول والديوسجنين ، الرسكوجين وتم فصلهم بواسطة كروماتوجرافيا العمود والطبقة الرقيقة . عملت دراسة كاملة على تلك المواد باستخدام كروماتوجرافيا الطبقة الرقيقة ، كروماتوجرافيا الغاز ، التحاليل الطيفية بالاشعة تحت الحمراء ، الفوق بنفسجة والرنين النووى المغناطيسى وكذلك طيف الوزن وبواسطة التحاليل الطيفية بالاشعة تحت الحمراء وكذلك الرنين النووى المغناطيسى ثم اثبات ان مادة الرسكوجنين هى ٢٥ - س لنفس المادة .

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