U.P.B. Sci. Bull., Series B, Vol. 73, Iss. 2, 2011 ISSN 1454-2331

IDENTIFICATION OF SUGARS FROM *SILENE ALBAE HERBA* USING GC–MS TECHNIQUE

George Dan MOGOŞANU[1], Alexandru Mihai GRUMEZESCU[2], Dan Eduard MIHAIESCU[3], Daniela ISTRATI[4], Diana Elena MOGOŞANU[5], Sandra ALICE BUTEICĂ[6]

În această lucrare se prezintă prima identificare a glucidelor din Silene albae herba. Extractul hidroalcoolic din Silene albae herba, uscat în prealabil la 40^{0}C, a fost dizolvat în acetonitri, s-a adaugat N,O-bis(trimetilsilil)-trifluoroacetamida (BSTFA), amestecul fiind apoi încălzit la 90^{0}C, timp de 12 ore, într-un flacon rezistent la presiune. Produşii de derivatizare au fost extrasi cu hexan si apoi analizati prin GC-MS. Complecsii glucidelor cu trimetilsilan, din extractul hidroalcoolic, au fost identificaţi pornind de la modelele de fragmentare ale glucidelor standard din spectrele de masă şi pe baza indicilor de retenţie Kováts. Au fost propuse mecanismepentru formarea ionilor moleculari ca urmare a procesului de fragmentare în timpul ionizării electronice.

In this paper, is reported the first identification of carbohydrates from Silene albae herba. The hydroalcoholic extract of Silene albae herba(previously dried at 40^{0}C) was dissolved in acetonitrile, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added, the mixture being then heated in a pressure-resistant vial at 90^{0}C for 12 hours. The derivatization products were extracted with hexane, and analyzed by GC–MS. Sugars from the hydroalcoholic extract were identified in the MS spectra on the basis of fragmentation patterns reference sugars from MS spectra and Kováts retention indices. The mechanisms for the formation of usual ions in electron-ionization fragmentation process have been proposed.

Keywords: *Silene albae herba*, hydroalcoholic extract, sugars, GC–MS technique

1. Introduction

---

[1] Lecturer, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, Romania, e-mail: mogosanu2006@yahoo.com

[2] Msc., Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: grumezescu@yahoo.com

[3] Lecturer, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: danmih@usamv.ro

[4] Associate Professor, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: d_istrati@yahoo.com

[5] Msc., Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania

[6] PhD. Student, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, Romania, e-mail: s_alice19@yahoo.com

Silene alba (Miller) E.H.L. Krause sin. *Melandrium album* (Miller) Garcke, White Campion, *Caryophyllaceae* family, originates in the major part of Europe, Western Asia and North Africa. It is an herbaceous annual, biennial or short-lived perennial plant growing in most open habitats, particularly wasteland and fields, preferring sunny areas that have rich and well-drained soils [1].

There are incomplete studies on the chemical composition of *S. alba* species with respect to its content in: flavonosides (apigenin- and luteolin-O,C-glycosides), phytoecdysteroids (ecdysone, 2-deoxy-20-hydroxyecdysone), triterpenoid saponins (gypsogenin and quillaic acid sapogenins), and polyphenolic acids [2–9].

2. Experimental

2.1. Plant material

The vegetal *S. alba* samples (aerial parts) were collected in the flowering period (April 2009) from the Botanical Garden of the University of Craiova, Dolj County, Romania. Voucher specimens (RC–1102009 code) are deposited in the Herbarium of the University of Medicine and Pharmacy of Craiova.

2.2. Preparation of hydroalcoholic extract (tincture)

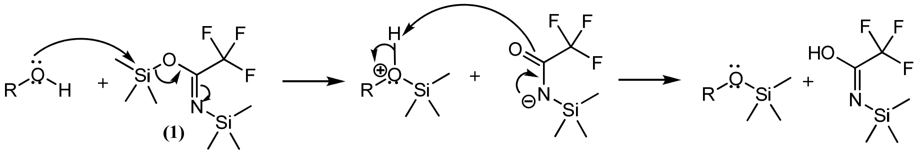
Accurately weighed samples of air-dried powdered aerial parts of *S. alba* species were macerated 10 days with 70% ethanol–water mixture at room temperature, according to the Xth edition of Romanian Pharmacopoeia [10]. The 20% hydroalcoholic extract (tincture) was filtered and then stored in dark bottles in the refrigerator.

2.3. Reagents and solvents

All of the analytical grade reagents (ethyl alcohol, BSTFA, hexane) were purchased from Merck (Darmstadt, Germany).

2.4. GC derivatization method

In order to develop a GC-MS method it was required to change the carbohydrates polarity. This was performed by functionalization of hydroxyl groups with silyl ether [11]. The general reaction for the formation of trialkylsilyl derivatives in this paper is shown in Scheme 1.



Scheme 1

N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was used as derivatization agent. The hydroalcoholic extract was dried at 40^0C and it was dissolved in acetonitrile, BSTFA was added, the mixture being heated in a pressure-resistant vial, at 90^0C, for 12 hours. The resulted derivatization products were extracted with hexane, and injected to GC–MS [12].

2 µL of silylated extracts were analyzed using a Varian 3400 gas chromatograph coupled with a Saturn II mass spectrometer (GC–MS). A DB5–MS capillary column (30 m × 0.25 mm I.D. x 0.25 µm) was used with helium as carrier gas. The chromatographic conditions were: injector temperature 280^0C, column temperature program: 50^0C/ 3 minutes, 5^0C/min. ramp to 300^0C/20 minutes. The samples were analyzed in the split mode (1:60 split rate).

2.5. Kováts retention index

Kováts retention index (I) is a concept used in gas chromatography to convert retention times into system-independent constants. The retention index of a certain chemical compound is its retention time normalized to the retention times of adjacently eluting *n*-alkanes. While retention time vary with column length, film thickness, diameter, carrier gas velocity and pressure, void time, the derived retention indices are quite independent of these parameters and allow comparing values measured by different analytical laboratories under varying conditions. Its applicability is restricted to organic compounds. The Kováts index is given by the equation:

$$I = 100 \times \left[n + (N-n)\frac{log(t'_{r(unknown)}) - log(t'_{r(n)})}{log(t'_{r(N)}) - log(t'_{r(n)})} \right] \tag{1}$$

In eq. (1), I = Kovats retention index, n = the number of carbon atoms in the smaller n-alkane, N = the number of carbon atoms in the larger n-alkane, t_r' = the adjusted retention time. [13].

3. Results and discussion

Thirty-one compounds from the 48 separated derivatized compounds were identified with mass spectra and confirmed by Kováts indices.

3.1. GC separation

Fig. 1 shows the GC–MS chromatogram of TMS–sugars from *Silene albae herba* hydroalcoholic extract.

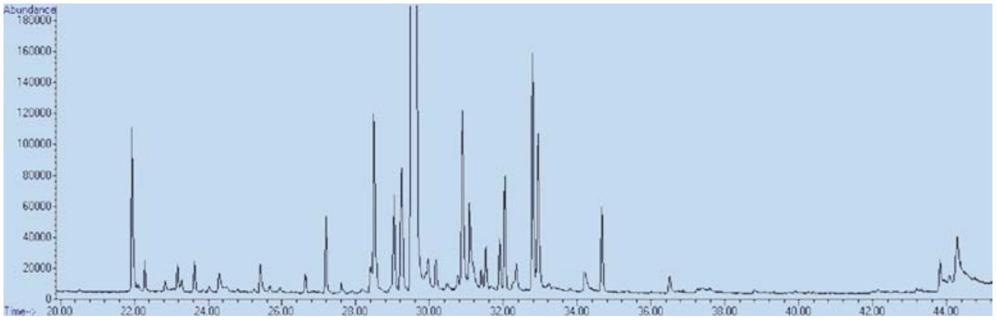


Fig. 1. GC–MS chromatogram of derivatized carbohydrates from *Silene albae herba* hydroalcoholic extract

TMS–sugars content (relative content in area percent) from *Silene albae herba* hydroalcoholic extract is presented in Table 1.

Table 1

Sugars content of *Silene albae herba* hydroalcoholic extract

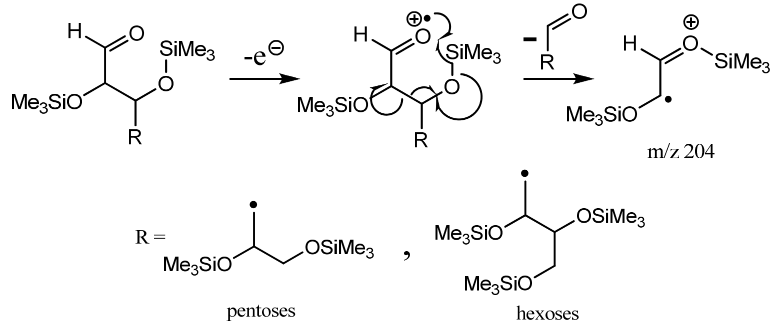
No.	**Sugar**	**Calculated K.I.**	**K.I.***	**R.T. [min.] α****	**R.T. [min.] β****	**Relative content [%]**
1.	D(-)-Xylulose	1603.05	1603	24.803	–	0.03
2.	D(-)-Lyxose	1638.08	1638	25.421	–	0.273
3.	L(+)-Arabinose	1644.21	1644	25.677	–	0.081
4.	L(+)-Rhamnose	1357.95	1658	25.959	–	0.062
5.	Galactosan	1680.88	1681	26.633	–	0.129
6.	Mannosan	1702.15	1702	27.195	–	0.5
7.	Levoglucosan	1724.02	1724	27.627	–	0.087
8.	Xylitol	1739.25	1739	27.906	–	0.026
9.	D(+)-Arabitol	1749.13	1749	28.174	–	0.038
10.	Ribitol	1756.04	1756	28.502	–	1.565
11.	D-Altrose	1820.09	1820	29.633	–	72.379
12.	D-Fructose	1839.11	1839	29.97	–	0.358
13.	D-Fructose	1846.98	1847	–	30.181	0.339
14.	6-Deoxy-D-glucose	1876.10	1876	30.763	–	0.155
15.	D-Allose	1881.98	1882	–	30.893	1.692
16.	D(+)-Talose	1887.95	1888	31.091	–	1.254
17.	D(+)-Galactose	1898.01	1898	31.394	–	0.169
18.	L(-)-Sorbose	1902.89	1903	31.522	–	0.339
19.	D-Fructose (pyrano)	1930.14	1930	–	31.895	0.391
20.	D(+)-Glucose	1932.09	1932	32.047	–	0.76
21.	D(+)-Galactose	1942.95	1943	–	32.257	0.063

No.	Sugar	Calculated K.I.	K.I.*	R.T. [min.] α**	R.T. [min.] β**	Relative content [%]
22.	D(+)-Talose	1947.96	1948	–	32.351	0.294
23.	D-Sorbitol	1981.03	1981	32.934	–	1.344
24.	D(+)-Glucose	2025.15	2025	–	34.662	0.621
25.	*myo*-Inositol	2130.08	2130	36.493	–	0.158
26.	Lactulose***	2697.11	2697	43.19	–	0.059
27.	D(+)-Sucrose	2712.05	2712	43.82	–	0.317
28.	Lactose	2713.01	2713	43.937	–	0.15
29.	D(+)-Maltose	2754.08	2754	43.937	–	0.28
30.	D(+)-Cellobiose	2757.95	2758	44.718	–	0.052
31.	D(+)-Turanose	2789.99	2790	44.753	–	0.154

* – Kováts Retention Index from literature [14]; ** – Retention Time (α, β – isomers); ***

3.2. MS fragmentation and proposed mechanisms for most abundant ions

McLafferty rearrangement of a trimethylsilyl group can be proposed accordingly to Scheme 2:



Scheme 2

The most abundant ions (*m/z*) of derivatized (TMS) sugars are presented in table 2.

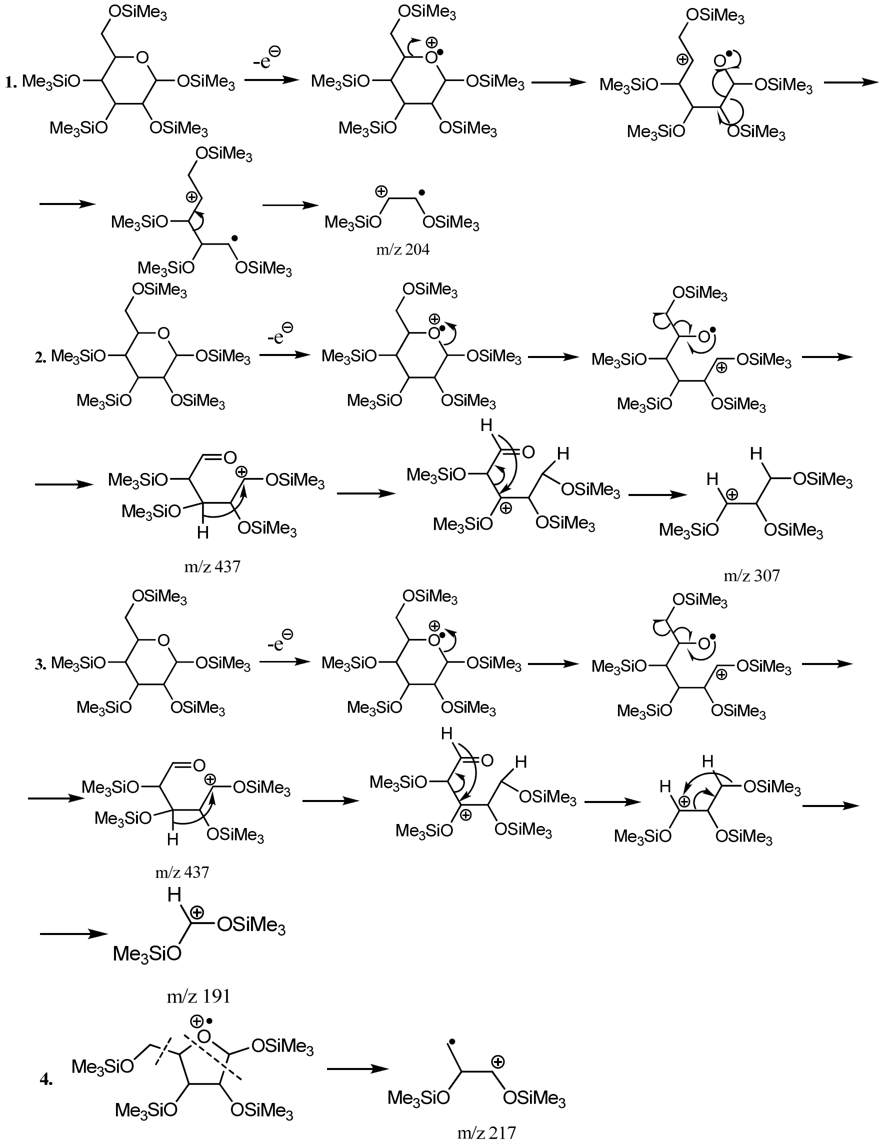
Table 2

The most abundant ions (*m/z*) of TMS–sugars

TMS–sugar	*m/z*	TMS–sugar	*m/z*
D(-)-Xylulose	*147, 218, 335*	Xylitol	*217, 307, 319*
L(+)-Arabinose	*191, 204, 217*	*myo*-Inositol	*147, 204,361*
D(-)-Lyxose	*191, 204, 217*	Lactulose	*147, 204, 361*
L(+)-Rhamnose	*147, 191, 204*	D-Sorbitol	*205, 307, 319*
Galactosan	*191, 204, 217*	D(+)-Sucrose	*204, 217, 361*
Mannosan	*204, 217, 333*	Lactose	*191, 204, 306*
D-Allose	*191, 204, 217*	D(+)-Maltose	*191, 204, 361*
D-Altrose	*191, 204, 217*	D(+)-Turanose	*147, 217, 361*
D(-)-Fructose	*147, 217, 437*	D(+)-Cellobiose	*204, 361*
D(+)-Galactose	*191, 204, 217*	6-Deoxy-D-Glucose	*174, 191, 204*
D(+)-Glucose	*191, 204, 217*	D(+)-Talose	*191, 204, 217*

TMS–sugar	***m/z***	**TMS–sugar**	***m/z***
D(+)-Mannose	*191, 204, 217*	Ribitol	*217, 307, 319*
L(-)-Sorbose	*147, 204, 437*	D(+)-Arabitol	*217, 307, 319*
Levoglucosan	*204, 217, 333*	–	–

From the pyranose derivatives (**1–3**) three characteristic abundant ions can be obtained, whereas one predominant ion can be formed from the furanose derivatives **(4)** as it can be seen in Scheme 3:



Scheme 3

Sugar alcohols, mono-, and disaccharides have been isolated (Fig. 2) from *Silene albae herba* hydroalcoholic extract.

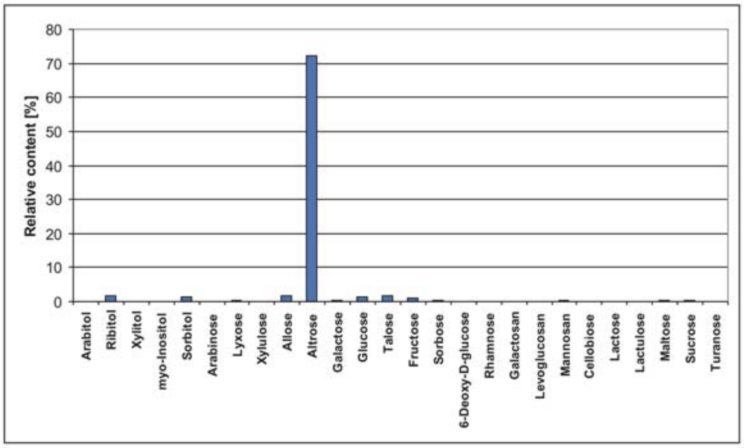


Fig. 2. The relative content [%] of sugar alcohols, mono-, and disaccharides from *Silene albae herba* hydroalcoholic extract

Analyzing the experimental results included in table 1 and Fig. 2 it can also be seen that altrose reaches the highest relative content (72.38%). The other sugars are found in low concentrations or even in traces. The sugar alcohols are represented by small amounts of pentitols (Arabitol, Ribitol, Xylitol) – 1.64% and hexitols (*myo*-Inositol, Sorbitol) – 1.51%. Aldopentoses (Arabinose, Lyxose) are in a relative low content (0.35%) being about 10 times higher than that of ketopentoses. Among the ketopentoses, only Xylulose (0.03%) was identified. From aldohexoses, Allose, Talose and Glucose have close values of the relative content (1.7%, 1.55%, and 1.38%, respectively), exception being Altrose. Galactose was found in low concentrations (0.23%). Ketohexoses (Fructose, Sorbose) were identified in small amounts (1.43%) compared to aldohexoses. 6-Deoxy-hexoses (6-Deoxy-D-glucose, Rhamnose) and 1,6-anhydro-hexoses (Galactosan, Levoglucosan, Mannosan) were identified in trace levels. The disaccharides (Cellobiose, Lactose, Lactulose, Maltose, Sucrose, Turanose) show all together a relative content much lower (1%) than that of monosaccharides (80%).

The BSTFA derivatization conditions (pressure, 90^0C, 12 hours) can also yield chemical degradation of some organic compounds. Trace levels of lactulose and other related compounds were assigned to this process taking into account the sugar degradation (Table 2). In order to insure the formation of lactulose in the derivatization reaction, five experiments were performed.

4. Conclusions

The sugar alcohols, mono-, and disaccharide content of *Silene albae herba* hydroalcoholic extract has been determined by GC–MS analysis, from a single solution by one injection with hexane as finally extraction solvent. Identification was based on MS spectra fragmentation patterns of authentic sugars and Kováts indices. Trace levels of other degradation compounds were also identified.

REFERENCES

[1] *V. Ciocârlan*, Flora ilustrată a României. *Pteridophyta et Spermatophyta* [The Illustrated Flora of Romania. *Pteridophyta et Spermatophyta*], Editura Ceres, Bucureşti, 2000, p. 224

[2] *R. Heinsbroek, J. van Brederode, G. van Nigtevecht, J. Maas, J. Kamsteeg, E. Besson, J. Chopin*, The 2”-O-glucosylation of vitexin and isovitexin in petals of *Silene alba* is catalysed by two different enzymes, Phytochemistry, 9, **19**, 1980, pp. 1935–1937

[3] *R. Hegnauer*, Chemotaxonomie der Pflanzen, Band 8, Birkhäuser Verlag, Basel–Boston–Berlin, 1989, pp. 215–220

[4] *M. Báthori*, Purification and characterization of plant ecdysteroids of *Silene* species, TrAC Trends in Analytical Chemistry, 6, **17**, 1998, pp. 372–383

[5] *Y. Meng, P. Whiting, L. Zibareva, G. Bertho, J.P. Girault, R. Lafont, L. Dinan*, Identification and quantitative analysis of the phytoecdysteroids in *Silene* species (*Caryophyllaceae*) by high-performance liquid chromatography: novel ecdysteroids from *S. pseudotites*, Journal of Chromatography A, 1–2, **935**, 2001, pp. 309–319

[6] *L. Zibareva, V. Volodin, Z. Saatov, T. Savchenko, P. Whiting, R. Lafont, L. Dinan*, Distribution of phytoecdysteroids in the *Caryophyllaceae*, Phytochemistry, 2, **64**, 2003, pp. 499–517

[7] *A. Simon, N. Tóth, G. Tóth, Z. Kele, J. Groska, M. Báthori*, Ecdysteroids from *Silene viridiflora*, Helvetica Chimica Acta, 4, **92**, 2009, pp. 753–761

[8] *Z. Jia, K. Koike, N.P. Sahu, T. Nikaido*, Triterpenoid saponins from *Caryophyllaceae* family, Studies in Natural Products Chemistry, 7, **26**, 2002, pp. 3–61

[9] *M. Larhsini, A. Marston, K. Hostettmann*, Triterpenoid saponins from the roots of *Silene cucubalus*, Fitoterapia, 3, **74**, 2003, pp. 237–241

[10] ***, Farmacopeea Română ediţia a X-a [Romanian Pharmacopoeia Xth edition], Editura Medicală, Bucureşti, 1993, pp. 921, 922

[11] *G. Epure, N. Grigoriu, L. Filipescu*, GC/MS studies on alcohol derivatization procedures applied to Lewisite 1 due to the increased instability of some ethers, University Politehnica of Bucharest, Scientific Bulletin Series B: Chemistry and Materials Science, 3, **72**, 2010, pp. 57–66

[12] *A.C. Ion, I. Ion*, Short remarque on the provenience of nanoparticles in environmental samples, University Politehnica of Bucharest, Scientific Bulletin Series B: Chemistry and Materials Science, 1, **71**, 2009, pp. 13–20

[13] *E. Kovats*, Gas-chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone, Helvetica Chimica Acta, 7, **41**, 1958, pp. 1915–1932

[14] *P.M. Medeiros, B.R.T. Simoneit*, Analysis of sugars in environmental samples by gas chromatography–mass spectrometry, Journal of Chromatography A, 2, **1141**, 2007, pp. 271–278.