Detailed studies on seed oil of Salicornia SOS–7 cultivated at the egyptian border of Red Sea

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RESUMEN

Estudios detallados de aceite de semilla de Salicornia SOS–7 cultivada en la orilla egipcia del Mar Rojo.

Se han dilucidado ocho perfiles lipídicos de aceite de semilla de Salicornia usando HPLC como principal herramienta de análisis junto con la CGL capilar. El aceite completo es rico en linoleico (66.5%) con bajo contenido en ácido linolénico (1.4%). De los 22 triglicéridos (TG) determinados, los tres mayoritarios que contenían 3 y 2 cadenas de linoleico se detectaron por HPLC. El contenido en tocoferoles totales (720 ppm) se determinó directamente en el aceite por HPLC y se encontró al alfa–T (49.1%) y al gamma–T (48.2%) como predominantes. El perfil de esteroles completo, determinado por CGL, incluyó colesterol (1.0%), brasicasterol (1.4%), campesterol (2.4%), 5–estigmasterol (8.7%), beta-sitosterol (23.0%), espinasterol (17.0%), isofucosterol (0.8%), 7–estigmastenol (42.0%) y 7–avenasterol (3.7%).

Además, los perfiles de esteroles de los esteroles libres (sin acilar) (FS), esteroles acilados (AS), esterilglicósidos libres (FSG) y esterilglicósidos acilados (ASG) se determinaron por HPLC como sus antroilnitrilos. FS y AS se determinaron como sus esteril–9–antroilnitrilos (S–9–AN) mientras que FSG y ASG se analizaron como sus esterilglicosidil–1–antroilnitrilos (SG–1–AN) a niveles de nanogramos.

Comparando el aceite de Salicornia SOS–7 con los aceites de semillas de agua dulce tradicionales, el de Salicornia mostró principalmente una constitución lipídica característica.

PALABRAS-CLAVE: Aceite de semilla de Salicornia SOS–7 — Egipto — Lípido (composición).

SUMMARY

Detailed studies on seed oil of Salicornia SOS–7 cultivated at the egyptian border of Red Sea

Eight lipid patterns of Salicornia seed oil have been elucidated using HPLC as the main tool of analysis in conjunction with capillary GLC. The whole oil is rich in linoleic (66.5%) with lower amount of linolenic acid (1.4%). Of the 22 triglycerides (TG) determined, three major TG containing 3 and 2 linoleic acyls were detected by HPLC. The total tocopherols content (720 ppm) was determined directly in the oil by HPLC and it was found that alpha–T (49.1%) and gamma–T (48.2%) are predominating. The whole sterol profile, as determined by GLC, includes cholesterol (1.0%), brassicasterol (1.4%), campesterol (2.4%), 5–stigmasterol (8.7%), beta-sitosterol (23.0%), spinasterol (17.0%), isofucosterol (0.8%), 7–stigmastenol (42.0%) and 7–avenasterol (3.7%). Furthermore, sterols patterns of the free (nonacylated) sterols (FS), acylated sterols (AS), free sterylglycosides (FSG) and acylated sterylglycosides (ASG) were determined by HPLC as their anthroylnitriles. FS and AS were determined as their steryl–9–anthroylnitriles (S–9–AN) whereas FSG and ASG were analysed as their sterylglycosidyl–1–anthroylnitriles (SG–1–AN) at nanogram level.

Comparing Salicornia SOS–7 oil with traditional freshwater seed oils, Salicornia exhibits mostly a unique lipids constitution.

KEY-WORDS: Egypt — Lipid (composition) — SOS-7 Salicornia seed oil.

1. INTRODUCTION

A halophyte plant Salicornia, coded as SOS–7 had been cultivated in 5-hectare field trials at the Egyptian border of Red Sea (1,2). Full-strength seawater was used for irrigation and reasonably higher yields of seeds and biomass were obtained. The seeds contained 25–26% oil which is greater than oil contents of cottonseed and soybean. It seems feasible that this halophyte can help in reducing the deficit in edible oils that Egypt faces.

Studies on the chemical constitution were conducted (3,4), however there is still a lack of data on such new oil. Some of the lipid classes have not been hitherto investigated, whereas most of the published data are not definitive. Furthermore, studies on the oil of a similar Salicornia species, cultivated in field trials in coastal environment in Mexico, was restricted to oil characteristics and fatty acid composition (5).

The present work is undertaken to carry out a detailed study on the oil constituents of SOS–7 oil using HPLC as the main tool of analysis in conjunction with capillary GLC. Different specific HPLC methods are employed for elucidating not only the patterns of oil triglycerides and tocopherols but also the profiles of free and acylated sterols and sterylglycosides of the four sterol lipid classes. It is worthy to mention that the latter are present as minor components in vegetable oils (6,7,8). Furthermore, capillary GLC is employed for determining fatty acid and whole sterol composition of SOS–7 oil.

2. EXPERIMENTAL

A representative sample of seeds (400g) was extracted with chloroform–methanol (2:1 by volume) in a waring Blendor. The extract was dried over anhydrous sodium sulphate and the solvent was removed from the filtrate using a rotary evaporator under reduced pressure at 40°C.

The oil characteristics, namely iodine and saponification values as well as the unsaponifiable content of the oil were determined according to A.O.C.S. methods (9).

The lipid patterns namely, fatty acids (FA), triglycerides (TG), tocopherols (T), whole oil sterols and sterol of the sterol lipid classes, namely free sterols (FS), acylated sterols (AS), sterylglycosides (SG) and acylated sterylglycosides (ASG), were determined according to the methods mentioned below.

All the HPLC and GLC data recorded in the tables are the mean of two successive single determinations. The different lipids profiles of the SOS–7 oil were compared to the corresponding lipids patterns of some traditional seed oils that have been elucidated in this laboratory (10).

Fatty acid pattern

The oil is converted into methyl esters via transesterification with 5 per cent methanolic hydrogen chloride (11). Transesterification reaction was monitored with the help of TLC using silica gel G plates and n-hexane: diethyl ether: acetic acid (80 : 20 : 1 by volume) as a developing solvent.

Hewlett Packard – HP 5890 – A gas chromatograph was employed for the analysis of the mixed methyl esters under the following operating conditions:
Column, DB–23 (0.32 mm x 30m); temperature programming, 150-230°C, 3°C/min.; injector, 230°C; detector, FID at 240°C; carrier gas, Helium at flow rate of 1.3 ml/min. and split ratio, 100:1.

Calibration was made using standard fatty acid methyl esters. The results were recorded by an electronic integrator as peak area per cent.

Triglyceride profile

HPLC instrument (Toyo-Soda-CCPM) was employed for the determination of TG profile of the SOS–7 oil. A 10 µl solution of oil in chloroform (300 mg/ml) was injected onto the column, ODS capcel Pak, C_{18} (4.4 x 100 mm). Gradient elution with acetonitrile: dichloromethane (starting from 90:10 to 35:65 v/v) in 150 minute was conducted. FID detector (with moving band, Tracor 945) was attached to the instrument.

The carbon number assignment for the separated peaks was determined using HPLC chromatogram of soybean oil taken as reference containing 29 TG starting with trilinolein and terminating with tristearin (10).

The elution sequence was the same as that reported by El-Hamdy and Perkins (12). The following coding was used for fatty acyls: X = linolenic, L = linoleic, O = oleic, S = stearic and P = palmitic.

The results were automatically printed as peak area per cent by recording integrator.

Tocopherol pattern

Direct determination of tocopherols (T) in oil was accomplished using Toyo-Soda-CCPm HPLC instrument. An oil sample of 10 gram was solubilizied in n–hexane to make 10% solution and 10 µl was injected onto the silica column (YMC–A–012, 6.0 x 150 mm). Isocratic elution was conducted using n–hexane: isopropyl alcohol (100:0.5, by volume) as a mobile phase, at a flow rate of 1–2 ml/min. Hitachi–650–10S fluorescence detector was used. Spectral absorption was set at excitation and emmission wave lengths of 295 and 325 nm respectively. The conditions were optimized to elute delta–T after 10 minutes. The results were automatically recorder as peak area percentages by electronic integrator. From the peak area and the corresponding weight of each individual T in the standard mixture, the weight of each individual T in the oil (ppm) can be calculated.

Whole sterol profile

The unsaponifiable portion of the oil prepared (9), was subjected to preparative TLC on silica gel G plates (0.5 mm thickness) using chloroform/diethyl ether/acetic acid (95/4/1 by volume) as a developing solvent. The sterol zone was located with the help of standard beta–sitosterol applied alongside the sample prior to development. The scrapped zone was thoroughly extracted with chloroform and the solvent was distilled off from the filtered solution.

Hewlett Packard–HP 5890A gas chromatograph, was employed for analysis using the following operating conditions: Column, DB–17 (0.32 mm x 15 m, 0.25 µm coating) at 250°C; detector, FID at 260°C; injection 250°C; carrier gas, Helium (8.6 ml/min) and split ratio, 35:1. Standard sterols mixture containing known weights of available standard sterols, was used for identification and quantitation. (Camellia seed oil was taken as reference for spinasterol).

Sterol patterns of FS and AS

The subsequent FS and AS isolations, their derivatization into 9–anthroylnitriles (S–9–AN) and HPLC determination, were followed according to Wanaka and Murui (7).

FS and AS were isolated from the oil by preparative TLC using n–hexane/ether/formic acid (70/30/1 v/v) as developing solvent. The FS (R_f = 0.16) and AS (R_f = 0.63) were scrapped off and extracted with chloroform. Only AS were deacylated via mild alkaline hydrolysis (0.5 N KOH in isopropyl alcohol). The obtained FS as well as the original FS were separately derivatized into their sterol S–9–AN derivatives according to the same method mentioned above.

HPLC of S–9–AN was conducted under the following conditions: Reversed phase column, ODS–1250Y, Senshu Pak (4.6 x 250 mm); detector, fluorescence; excitation and emission wave lengths set at 360 and 460 nm; isocratic elution using acetonitrile/dichloromethane (75/25, v/v) at flow rate 1 ml/min.

Sterylglycoside profile of FSG and ASG

The procedure of separation from the oil, derivatization into sterylglycoside 1–anthroylnitrile (SG–1–AN) and HPLC analysis were carried out according to Murui *et al.* (8).

The FSG and ASG were separated from the oil by injecting the oil solution into silica gel cartridge (Sep–Pak, Waters) and were eluted with chloroform: methanol (1:1, v/v). The mixed compounds were subsequently fractionated into pure FSG and ASG with the help of preparative TLC. Chloroform/methanol/ formic acid (90/15/1, v/v) was used as a developing solvent. Only ASG was subjected to alkaline hydrolysis (0.5N KOH in isopropanol) to obtain FSG. The original and obtained FSG were derivatized, into SG–1–AN according to the same method mentioned above.

HPLC instrument, equipped with UV detector (Toso, UV 8000) and ODS Wakosil–5, C_{18} (6.4 mm x 250 mm) was used for SG–1-AN analysis. Gradient elution with acetonitrile/dichloromethane (from 50/50 to 68/32 v/v) was used. Absorption was measured at 254 nm. The method is sensitive to 0.5 nanogram of SG.

3. RESULTS AND DISCUSSION

The oil content of the halophyte seeds amounted to 26–27%, which is exceedingly higher than that of cottonseed and soybean.

It was found that the oil has an iodine value of 135 indicating that it is highly unsaturated oil than cottonseed and soybean oils. The saponfication value of 186 shows that the oil is similar to most of the conventional seed oils.

Fatty acid pattern

The fatty acid composition of SOS–7 oil is recorded in Table I. From these results, it was noticed that the halophyte oil is rich in linoleic (66.9%) but contains lower linolenic acid level (1.4%). Cottonseed, sunflower and soybean contain comparatively low linoleic but only soybean contain higher linolenic acid amounting to 6.8% (10). The oleic acid amounting to 17.5% in SOS–7 (16.5% n–9 and 1.0% n–7 isomer) is generally lower than those of cottonseed (19.9%) and soybean (23.1%) but very markedly lower than that of sunflower (46.0%).

It seems that the lower linolenic content of SOS–7 oil improves the oil stability which is comparable to that of cottonseed oil (3).

Triglyceride pattern

HPLC analysis of the halophyte oil TG molecular species (Table II) shows the presence of 22 TG's containing palmitic, stearic, oleic, linoleic and linolenic acyl groups designated as P, S, O, L and X respectively. TG's with lower ECN eluted a head of those with higher ECN and the elution sequence within each TG category having the same ECN (critical pairs) started with the highest number of double bonds and terminated with the lowest unsaturation (12). Designations of TG's do not imply the positional acyl distribution in the TG molecules, but a mixture of TG isomers. Major TG's are those containing three and two linolyl acyls, namely LLL (34.2%) LLO (21.2%) and LLP (15.3%) having 6,5 and 4 double bonds respectively.

It is noticed that XLL is not detected in cottonseed, soybean and sunflower oils, however it is present in SOS–7 oil at a level of 2.3%. In addition, the oil contains 34.2% of LLL which is higher than that present in the three conventional oils. LLP and LLO of the halophyte oil are somewhat similar to those present in soybean oil. OOO is found in reasonable amounts in cottonseed (1.7%) and soybean (2.8%) oils, and in lower amount in the halophyte oil, however it is present at a level of 12.9% in sunflower.

Table I. **Fatty Acid Pattern of the Halophyte Oil as Determined by GLC**

Fatty Acid Composition (Wt. %)										
14:0	16:0	18:0	18:1 (n-9)	18:1 (n-7)	18:2	18:3	20:0	20:1	22:0	24:0
0.3	9.0	3.4	16.5	1.0	66.9	1.4	0.5	0.4	0.4	0.2

Table II. **Composition of Triglyceride Molecular Species of Halophyte Seed Oil as Determined by HPLC**

Tg Type	Ecn	Weight%	Tg Type	Ecn	Weight%
XXX	36	-	XOS	46	-
XXL	38	-	LPP	46	0.4
XLL	40	2.3	XSP	46	-
XXO	40	-	OOO	48	0.8
XXP	40	-	LOS	48	0.6
LLL	42	34.2	OOP	48	1.7
XLO	42	2.2	LSP	48	0.7
XLP	42	0.8	POP	48	0.4
XXS	42	-	XSS	48	-
LLO	44	21.2	PPP	48	0.5
XOO	44	-	OOS	50	0.2
LLP	44	15.3	LSS	50	0.2
XLS	44	-	POS	50	0.3
XOP	44	-	SPP	50	0.3
XPP	44	-	SOS	52	0.2
LOO	46	7.1	SSP	52	-
LLS	46	4.8	SOA	52	-
LOP	46	5.8	SSS	54	-

X: Linolenic, L: Linoleic, O: Oleic, S: Stearic, P: Palmitic, ECN: Equivalent carbon number.
Retention time: XXX, 30.6 and SSS, 102.7 minutes.

Table III. **Direct HPLC Determination of Tocopherols Pattern of the Halophyte Seed Oil**

Tocopherol (ppm)	Tocopherols Composition %			
	Alpha-T	Beta-T	Gamma-T	Delta-T
720	49.1	0.6	48.2	2.1

Retention Time of Standard Tocopherols: Alpha-T, 3.35; Beta-T, 5.65; Gamma-T, 6.0 and Delta-T, 10.4 minute

Table IV. **Whole Sterol* Pattern of the Halophyte Seed Oil Elucidated by Capillary GLC**

Total Sterol content (mg / 100 g)	Sterol Composition %								
	Chole-sterol	Brassica-sterol	Campe-sterol	Stigma-sterol	Sito-sterol	Spina-sterol	Isofuco-sterol	7-Stigma-stenol	7-Avena-sterol
500	1.0	1.4	2.4	8.7	23.0	17.0	0.8	42.0	3.7

* Isolated Pure by Preparative TLC from the Unsaponifiable Fraction of the Oil.
Relative Retention Time: of Standard Sterols; Cholesterol, 0.63; Brassicasterol, 0.69; Campesterol, 0.81; 5-Stigmasterol, 0.88; Sitosterol, 1.0; Spinasterol, 1.03; Isofucosterol, 1.12; 7-Stigmastenol, 1.18 and 7-Avenasterol, 1.32 (12 minute).

Generally, it can be concluded that halophyte seed oil exhibits a very characteristic TG pattern in comparison with those elucidated for cottonseed, soybean and sunflower oils (10).

Tocopherols pattern

It can be noticed that total T content of Salicornia oil amounts to 720 ppm (Table III) which is somewhat similar to that determined in cottonseed and sunflower oils, however soybean oil contains an appreciably higher level of tocopherol amounting to 1080 ppm (10).

The tocopherol profile of SOS–7 oil showed that alpha–T (49.1%) as well as gamma–T (48.2%) are the major components. Therefore, this tocopherol profile is similar to that of cottonseed oil. It is worthy to mention that sunflower and soybean tocopherols include alpha–T (95.8%) and gamma–T (64.4%) as major tocopherols respectively (10).

Whole sterols pattern

The sterol mixture isolated from unsaponifiable portion of the oil, was subjected directly to GLC analysis and good resolution was achieved. From the results obtained (Table IV), the sterol composition of Salicornia oil is characterized by having 7–stigmastenol (42.0% of the total weight of sterols) as the major sterol, however beta–sitosterol constitutes 23.0%. It is worthy to mention that beta–sitosterol is the main sterol of most of vegetable oils and it constitutes 83.2%, 56.2% and 62.0% of the weight of total sterols in cottonseed, soybean and sunflower oils respectively (10). Another characteristic sterol, namely spinasterol, was found at a level of 17.0% in Salicornia sterols, however it was not detected among the sterol constituents of the three above mentioned vegetable oils. On the other hand brassicasterol, being a characteristic sterol in rapeseed oil (10), was found as a sterol constituent of Salicornia oil amounting to 1.4%. Cholesterol constitutes 1.0% of the total weight of Salicornia sterols (500mg/100 g oil) which is considered as minor component. It was found that 5–stigmasterol: campesterol ratio is about 4:1 in Salicornia oil which is not the case with other oils (10).

Sterol patterns of FS and AS

From the results recorded in Table V, was found that the total contents of FS and AS fractions were 170 and 570 mg/100 gram oil respectively. The contents are generally higher than those found in cottonseed, soybean and sunflower oils (10).

Using HPLC–fluorescence for the quantitation of sterols as their S–9–AN, it was able to determine these compounds even at 80 picogram level. High resolution of the S–9–AN derivatives with the exception of those of campesterol and 5–stigmasterol, was achieved. The latter two derivatives had the same retention characteristics and therefore they are unseparable pair on HPLC. Stigmasterol is about 4–fold the amount of campesterol as determined by GLC.

It was found that sterol patterns of both FS and AS fractions were different as shown from the lower avenasterol as well as the higher campesterol/stigmasterol and sitosterol levels in FS fraction. It was noticed that the sterol patterns of the FS and AS fractions of Salicornia oil were very distinguishable from those in other conventional oils (7,10). Higher spinasterol and 7–stigmastenol contents were generally higher than those determined in other seed oils.

Sterylglycoside profiles of FSG and ASG

The contents of FSG and ASG isolated from the oil as well as the HPLC determination of their SG–1–AN are recorded in Table VI. It was found that ASG content was about 4–fold the quantity of FSG. The HPLC–UV

Table V. **HPLC of Sterols as their 9-Anthroylnitrile Derivatives (S-9-AN)**

Sterol Fraction	Content mg / 100 g	Sterol Composition (%)							
		7-Avena-sterol	Isofuco-sterol	Brassica-sterol	Chole-sterol	Spina-sterol	Campe-/ stigma-sterol	7-Stigma-stenol	Sito-sterol
Free	170	4.3	2.6	-	0.5	16.3	18.4	33.2	24.7
Acylated sterols	570	9.2	2.6	-	0.8	17.2	7.7	48.5	14.0

Relative Retention Time of Standard S-9-AN: 7-Avena-, 0.75; Isofuco-, 0.78; Cholest-, 0.83; Spina-, 0.87; Campe-, 0.90; 5-Stigma-, 0.90; 7-Stigma-, 0.9 and Beta-Sitosterol, 1.0 (12.0 minute)

Table VI. **HPLC of Sterylglycosides (SG) of Salicornia Oil as Their 1-Anthroylnitrile Derivatives (SG-1-AN)**

Sterylglycoside Fraction	Sterylglycoside Composition %								
	Content ppm	7-Avena-sterol	Isofuco-sterol	Brassica-sterol	Chole-sterol	Spina-sterol	Campe-/ stigma-sterol	7-Stigma-stenol	B-Sito-sterol
Free SG	92	2.7	4.3	0.6	1.1	6.0	32.2	12.9	40.2
Acylated SG	370	1.8	4.8	1.6	1.4	6.4	29.0	11.5	43.5

Relative Retention Time of Standard SG-1-AN: 7-Avena-, 0.75; Isofuco-, 0.76; Brassica-, 0.79; Chol.-, 0.84; Spina-, 0.88; 5-Stigma-, 0.91; Camp-, 0.91; 7-Stigma-, 0.97 and Sito-SG, 1.0 (13.0 minute)

detection proved to be very sensitive for the SG–1–AN and therefore minor components can be easily detected. In addition good resolution of the SG derivatives is noticed for nearly all the derivatives, except those including campesterol and stigmasterol moieties having the same retention characteristics (8).

The SG profiles of the FSG and ASG were generally different and it was of interest to detect brassica–SG and 7–Avena–SG at comparatively higher levels in ASG and FSG respectively. On the other side, the SG profiles in SOS–7 oil are markedly different from those of traditional oils (8, 10).

From the structural point of view, it was proposed by Stevanov and Popov (13) that SG in sunflower oil is steryl–ß–D (2 or 4–0–Fatty Acyl) glucopyranoside and the molar ratio of fatty acid: sterol is 1:1.

Generally, the present detailed studies on SOS–7 oil were effectively accomplished using HPLC as the main tool of analysis in conjunction with capillary GLC. Therefore 8 different lipid patterns, of Salicornia SOS–7 oil have been, for the first time, collectively elucidated to give more clear information on such new potential oil.

It is worthy to mention that the previous studies (3, 4) on SOS–7–oil were unable to detect five fatty acids, namely $C_{14:0}$, $C_{20:0}$, $C_{20:1}$, $C_{22:0}$ and $C_{24:0}$ as well as five sterols, namely cholesterol, brassicasterol, spinasterol, isofucosterol and 7–avenasterol. Furthermore, the oil glyceride structure previously computed on the basis of 1, 3–random distribution (4) was not in a good accord with that presently determined by HPLC. Meanwhile, tocopherol pattern and sterol profiles in the four sterol lipid classes have not been dealt with by any of the previous studies.

REFERENCES

1. Hassib, M.; Rashid, M. A. and Lachine, S. (1989).- "A Research Project on cultivation of halophyte SOS–7 in Egypt".- Sponsored by the Academy of Science, Cairo.
2. Halophyte Enterprises and GESCO Company (1989).- "Proposal for cultivating halophyte SOS–7 in Coastal Deserts in Egypt".
3. Nomaany, H. M.; El-Shami, S. and Zaher, F. A. (1990).- "Characteristics of SOS–7 halophyte oil and its oxidative stability compared to cottonseed oil".- Grasas y Aceites **41**, 154-157.
4. El-Shami, S. M. (1991).- "Glyceride structure and sterol composition of SOS–7 halophyte oil".- Grasas y Aceites **42**, 211-215.
5. Glenn, E. P.; Oleary, J. W., Watson, M. C., Thomson, T. L. and Kuehl, R. O. (1991).- "Salicornia bigelovii Tor: an oilseed halophyte for seawater irrigation".- Science **25**, 1065-1067.
6. Yoshida, H. and Kajimoto, G. (1979).- "Qualitative and quantitative changes in sterol lipids of cotyledons of germinating soybeans".- Agric. Biol. Chem. **43**, 705-712.
7. Wanaka, K. and Murui, T. (1992).- "Determination of sterols in edible oils and fats by HPLC with fluorescence labelling".- Yukagaku **40**, 306-311.
8. Murui, T., Wanaka, K. and Kuriyama, K. (1993).- "Determination of steryl glycosides by HPLC as their 1–anthroylnitrile derivatives".- Biosci. Biotech. Biochem. **57**, 614-617.
9. A.O.C.S.- "Tentative Methods of Analysis".- 2*nd* Edition, Chicago 1987.
10. El-Mallah, M. Hassan.- "Recent trends in lipid analysis of vegetable oils and fats: Part I. Cottonseed, sunflower and soybean oils".- (Unpublished data).
11. Christie, W. W.- "Lipid analysis: The preparation of volatile derivatives of lipids".- Chapter **4**, 85-102, Pergamon Press, 1973.
12. El-Hamady, A. H. and Perkins, E. G. (1981).- "High performance reversed phase chromatography of natural triglyceride mixtures: Critical pair separation".- J. Am. Oil Chemists'Soc. **58**, 867-872.
13. Stevanov, K. L. and Popov, A. D. (1976).- "Esterified sterol glycosides from sunflower seed lipids".- Comptes rendus de l'Academie Bulgare des Sciences **29**, 1463-1465.

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