FISHERIES AND MARINE SERVICE

Translation Series No. 3776

Identification and qualitative determination of sterols

by Th. Kartning and G. Mikula

Original title: Zur Identifizierung und Bestimmung von Sterolen

From: J. Chromatogr. 53: 537-543, 1970.

Translated by the Translation Bureau(VNN)
Multilingual Services Division
Department of the Secretary of State of Canada

Department of the Environment
Fisheries and Marine Service
Halifax Laboratory
Halifax, N.S.

1976

9 **pages typescript**

DEPARTMENT OF THE SECRETARY OF STATE
TRANSLATION BUREAU

MULTILINGUAL SERVICES DIVISION

CANADA

SECRÉTARIAT D'ÉTAT
BUREAU DES TRADUCTIONS

DIVISION DES SERVICES MULTILINGUES

F&m #3776

TRANSLATED FROM – TRADUCTION DE: German

INTO – EN: English

AUTHOR – AUTEUR: Th. Kartnig and G. Mikula

TITLE IN ENGLISH – TITRE ANGLAIS: Identification and quantitative determination of sterols

TITLE IN FOREIGN LANGUAGE (TRANSLITERATE FOREIGN CHARACTERS)
TITRE EN LANGUE ÉTRANGÈRE (TRANSCRIRE EN CARACTÈRES ROMAINS): Zur Identifizierung und Bestimmung von Sterolen

REFERENCE IN FOREIGN LANGUAGE (NAME OF BOOK OR PUBLICATION) IN FULL. TRANSLITERATE FOREIGN CHARACTERS.
RÉFÉRENCE EN LANGUE ÉTRANGÈRE (NOM DU LIVRE OU PUBLICATION), AU COMPLET, TRANSCRIRE EN CARACTÈRES ROMAINS.: Journal of Chromatography

REFERENCE IN ENGLISH – RÉFÉRENCE EN ANGLAIS: see above

PUBLISHER – ÉDITEUR	DATE OF PUBLICATION DATE DE PUBLICATION			PAGE NUMBERS IN ORIGINAL NUMÉROS DES PAGES DANS L'ORIGINAL
not shown	YEAR ANNÉE	VOLUME	ISSUE NO. NUMÉRO	537 – 543
PLACE OF PUBLICATION LIEU DE PUBLICATION				NUMBER OF TYPED PAGES NOMBRE DE PAGES DACTYLOGRAPHIÉES
not shown	1970	53	not shown	9

REQUESTING DEPARTMENT
MINISTÈRE-CLIENT: Department of the Environment

TRANSLATION BUREAU NO.
NOTRE DOSSIER N°: 1101420

BRANCH OR DIVISION
DIRECTION OU DIVISION: Fisheries and Marine Service
Halifax Laboratory

TRANSLATOR (INITIALS)
TRADUCTEUR (INITIALES): V.N.N.

PERSON REQUESTING
DEMANDÉ PAR: Dr. M. Kovacs

YOUR NUMBER
VOTRE DOSSIER N°:

DATE OF REQUEST
DATE DE LA DEMANDE: 23 June 1976

JUL 30 1976

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

SOS-200-10-6 (REV. 2/68)
7530-21-029-5333

CLIENT'S NO. Nº DU CLIENT	DEPARTMENT MINISTÈRE	DIVISION/BRANCH DIVISION/DIRECTION	CITY VILLE
	Environment Canada	Fisheries and Marine Service Halifax Laboratory	Halifax, N.S.
BUREAU NO. Nº DU BUREAU	**LANGUAGE LANGUE**	**TRANSLATOR (INITIALS) TRADUCTEUR (INITIALES)**	
1101420	German	V.N.N.	JUL 30 1976

"Zur Identifizierung und Bestimmung von Sterolen,"
Journal of Chromatography 53, 537 - 543, 1970

Identification and quantitative determination of sterols

by

Th. Kartnig and G. Mikula

From the Institut fuer Pharmakognosie
[Institute of Pharmacognosy]
Graz University,
Graz, Austria

(Received on 9 June 1970)

Summary - A method is described for the separation of free sterols by thin-layer chromatography using magnesium oxide as adsorbent and cyclohexane-diethyl ether-acetic acid as solvent system. When using other solvent systems, the naturally occurring palmitic and stearic acid esters of these sterols could also be separated on magnesium oxide. The quantitative estimation of sterols separated by precipitation with digitonin followed by thin-layer chromatography on magnesium oxide was carried out by ultraviolet spectrophotometric measurement in concentrated sulfuric acid. Application of this method is demonstrated by identification and quantitative determination of the sterols of three vegetable oils.

φ φ φ φ φ φ φ φ φ

Introduction

Investigation of plant and animal materials with respect to their sterol contents is not only of scientific interest, but also of practical importance. Since, in correspondence with the significance of sterol research, the number of pertinent publications is very large, we will not deal in detail with the literature, but simply refer the reader to the studies carried out by Peereboom and Ross (1) as well as by Seher and Homberg (2), which contain extensive reviews of the more recent literature in this field, and, in particular, of the determination of sterols.

Sterols, which may be present in either free or bound form, are very frequently found as mixtures in the material to be examined. Separation of such sterol mixtures, at the present time, is carried out chiefly with the aid of paper or thin-layer chromatography, frequently using impregnated layers (partition chromatography) or proceeding by way of the reaction products of the sterols (acetates, bromides). According to Seher and Homberg (2), no satisfactory results are available regarding the separation of free sterols on non-impregnated layers (adsorption chromatography). Seher and Homberg themselves were able to separate sterols by means of chromatography of the acetylated products on $MgO-Al_2O_3-CaSO_4$ layers.

Experimental work

Our investigations regarding the usability of magnesium oxide as adsorbent agent in thin-layer chromatography (TLC) of plant constituents (3; 4; 5) had 538
revealed that, using appropriate solvent systems, free sterols and their naturally occurring esters (for instance, palmitates and stearates) could also be separated on magnesium oxide layers. Our work is reported in the present paper.

Separation of the sterols - Separation of the total sterols from the material was carried out in accordance with the methods described by Peereboom and Roos (1) by precipitation with digitonin following preceding saponification. Cleavage of the digitonides was carried out by means of pyridine (6). The sterols finally present in the chloroformic solution were separated by us as follows.

Separation of the sterols by means of TLC - Absorbent - As adsorbent we have used magnesium oxide (commercial product, Merck, "MgO for TLC"), which, immediately prior to elutriation (15 g MgO and 68 ml. water) was passed through a No. 6 sieve with the aid of a brush. Coating was done in the usual manner. The plates were dried at 130° for 30 minutes. Over a drying agent, these plates can be stored for an unlimited period of time.

Solvent systems - Of the approximately 30 solvent systems (SS) tested, the following ones appeared to be best suited for our purposes:

SS (a), Cyclohexane-diethyl ether-glacial acetic acid (20:79.5:0.5);
SS (b), Cyclohexane-diethyl ether-glacial acetic acid (50:49.5:0.5);
SS (c), Petroleum ether (40 - 60°)-glacial acetic acid (99.9:0.1);
SS (d), Petroluem ether (40 - 60°)-acetone (98:2); and
SS (e), Carbon tetrachloride.

The chromatograms were developed at room temperature and normal chamber saturation. The length of run amounted to 15 cm, and the time of run, to about 30 minutes.

Detecting agents - Vanillin-sulfuric acid - Three per cent vanillin in concentrated sulfuric acid: Following spraying, the plate was heated for about 10 minutes to 120°. Reddish violet spots on a light ground. Limits of detection, about 5 μg.

Alkaline permanganate solution - 0.1 M $KMnO_4$ solution and equal parts of 10% aqueous NaOH: Following spraying, the plate was heated for about 10 minutes to 120°. Light spots on a violet ground. Limit of detection, about 10 μg.

Water - Following cautious spraying, there appear light spots on a darkish ground. Limit of detection, about 20 μg.

For quantitative determinations, we have used water as detecting agent, which enabled as to spray also the zones from which the spots had been scraped off.

Quantitative determination of sterols - Zaffaroni (7) and, more recently, Levorato (8) have pointed out that adrenocorticosteroids in concentrated sulfuric acid absorb in the ultraviolet region. Our own investigations (9) had shown that sterols, sterol glycosides and triterpenoid compounds in concentrated sulfuric acid also exhibit specific absorption bands in the ultraviolet region and can be determined quantitatively by means of measuring their absorption. The spec- 539
trophotometric measurement is carried out as follows: The material scraped from a given zone of the chromatogram is dissolved in 0.2 ml. of concentrated HCl, mixed with 3 ml. of concentrated sulfuric acid, and well stirred. The mixture is heated for 15 minutes to 60° (or set aside at room temperature for 2.5 hours), and cooled; the completely clear liquid is measured at the appropriate wavelength (for sterols, $\lambda = 318$ nm) against 0.2 ml. of concentrated HCl plus 3 ml. of concentrated sulfuric acid, in which a corresponding quantity of magnesium oxide has been dissolved. If $MgSO_4$ prcipitates, it is removed by means of centrifugation.

If the sterols are to be measured without prior separation by means of TLC, either the substance or its solution is put into a test tube, the solvent is evaporated, and we proceed as outlined further above.

The limit of detection of this measuring method amounts, in the case of sterols, to about 5 μg; the standard deviation is ± 1.4 per cent.

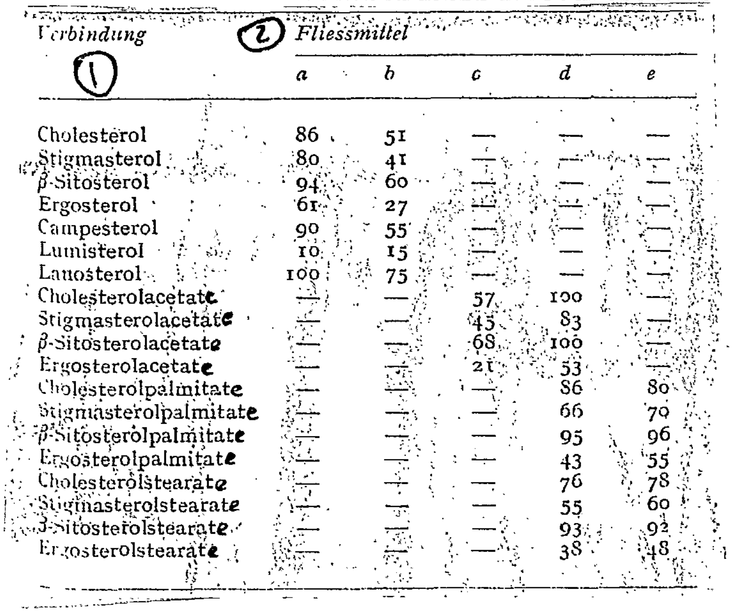
Results and discussion

Qualitative separation of sterols and sterol esters with the aid of solvent systems (a) to (e) - Using SS (a) and (b), the sterols investigated—with the exception of campestrol, which hitherto has been demonstrated only in a few oils—could be separated to a satisfactory degree, and this also in the free state. By means of lengthening the run to 25 cm and using SS (a), it was possible to separate also the critical pairs campestrol (73), cholesterol (78), and β-sitosterol (66). Separation of the sterols in the form of the naturally occurring palmitates and stearates was possible with the aid of SS (c) to (E); this method, however, should be employed only in cases, where separation by way of the digitonides is not necessary (Table 1, Figure 1).

Quantitative determination of sterols - Quantitative determination of the 540
isolated sterols is carried out by means of the afore-described spectrophotometric measurement in concentrated sulfuric acid with great accuracy. The most favorable quantity for TLC separation as well as for spectrophotometric measurement lies between 40 and 100 μg per sterol. The standard deviation of the total determination lies around ± 2.5 per cent. Table 2 and Figure 2 give the extinction values and the calibration curves of several sterols and sterol esters for the purpose of illustration.

The advantage of the determination method described—apparently possessing
a satisfactory limit of detection (about 20 μg) and accuracy (standard deviat- 541
ion of ± 2.5%)—is found, due to the separation of the sterols on magnesium oxide, in the fact that the adsorbent of the scraped zone is completely dissolved in the hydrochloric acid-sulfuric acid mixture, and that, thus, the whole sterol present in the coating (MgO) is subjected to chemical conversion and measurement. Corresponding experiments using weighed quantities of pure substances and spots scraped off the TLC plates yielded identical extinction values.

Table 1 - R_f x 100 values of sterols and sterol esters. Key: 1, Compounds; 2, Solvent system.



Verbindung ①	② Fliessmittel a	b	c	d	e
Cholesterol	86	51	—	—	—
Stigmasterol	80	41	—	—	—
β-Sitosterol	94	60	—	—	—
Ergosterol	61	27	—	—	—
Campesterol	90	55	—	—	—
Lumisterol	10	15	—	—	—
Lanosterol	100	75	—	—	—
Cholesterolacetate	—	—	57	100	—
Stigmasterolacetate	—	—	45	83	—
β-Sitosterolacetate	—	—	68	100	—
Ergosterolacetate	—	—	21	53	—
Cholesterolpalmitate	—	—	—	86	80
Stigmasterolpalmitate	—	—	—	66	70
β-Sitosterolpalmitate	—	—	—	95	96
Ergosterolpalmitate	—	—	—	43	55
Cholesterolstearate	—	—	—	76	78
Stigmasterolstearate	—	—	—	55	60
β-Sitosterolstearate	—	—	—	93	92
Ergosterolstearate	—	—	—	38	48

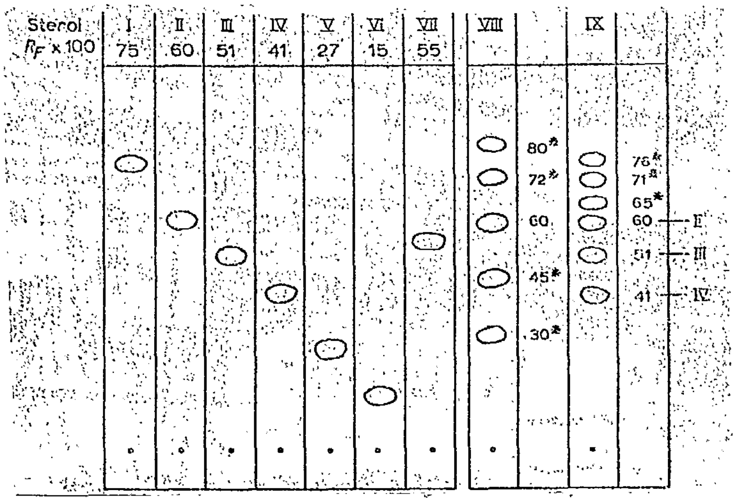


Figure 1 - Separation of sterols and sterol mixtures using solvent system (b). Adsorbent, magnesium oxide; length of run, 15 cm. I, Lanosterol; II, β-Sitosterol; III, Cholesterol; IV, Stigmasterol; V, Ergosterol; VI, Lumisterol; VII, Campesterol; VIII, Olive oil; IX, Peanut oil and ricinus oil (sterols are identical). *Sterol not identifiable.

Table 2 - Extinctions of the calibration curves of several sterols and esters; λ = 318 nm. Key: 1, Compounds; 2, Extinction for.

Verbindung ①	② Extinktion für		
	50 µg	75 µg	100 µg
Cholesterol	0.305	0.450	0.600
Stigmasterol	0.370	0.550	0.740
β-Sitosterol	0.370	0.550	0.740
Cholesterolacetate	0.255	0.375	0.500
Stigmasterolacetate	0.275	0.400	0.540
β-Sitosterolacetate	0.250	0.370	0.490
Cholesterolpalmitate	0.210	0.310	0.420
Stigmasterolpalmitate	0.210	0.320	0.425
β-Sitosterolpalmitate	0.190	0.280	0.375
Cholesterolstearate	0.200	0.285	0.380
Stigmasterolstearate	0.200	0.300	0.410
β-Sitosterolstearate	0.170	0.260	0.350

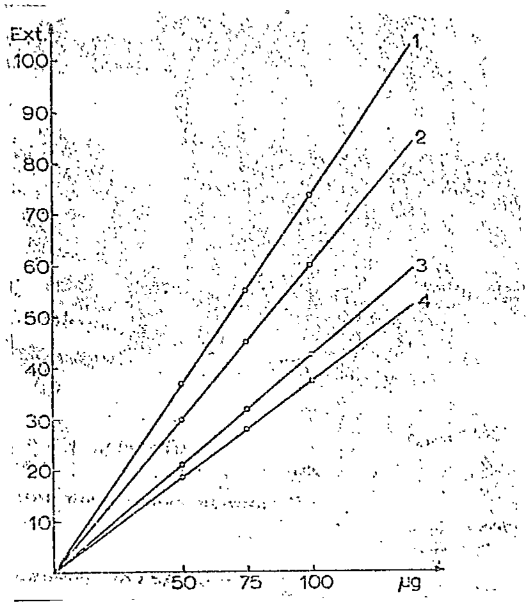


Figure 2 - Calibration curves for several sterols and sterol esters. Ultraviolet spectrophotometric measurements in concentrated sulfuric acid at 318 nm. 1, β-Sito- and Stigmasterol; 2, Cholesterol; 3, β-Sito- and Stigmasterol palmitate; 4, Cholesterol palmitate.

Table 3 - Determination of sterol contents of several oils of the Austrian Dispensatory, 9th Edition. Key: 1, Total sterols; 2, Sterols; 3, free; 4, bound; 5, Qualitative composition of the sterol mixture; 6, Quantitative composition of the sterol mixture; 7, Total sterols; 8, Free sterols; 9, Bound sterols; 10, Olive oil; 11, Peanut oil; 12, Ricinus oil; 13, (not identifiable); weitere, additional; restl., other or remaining.

	① *Gesamt-sterole* (*mg* %)	② *Sterole*		⑤ *Qualitative Zusammensetzung des Sterolgemisches*	⑥ *Quantitative Zusammensetzung des Sterolgemisches*		
		③ *frei* (*mg* %)	④ *gebunden* (*mg* %)		*Gesamtsterole* ⑦	*Sterole frei* ⑧	*Sterole gebunden* ⑨
Oleum ⑩ Olivae	12.94	3.12	9.82	β-Sitosterol 4 weitere Sterols ⑬ (nicht identifizierbar)	78.4 % β-Sitosterol 21.6 % restl. Sterols	78.0 % β-Sitosterol 22.0 % restl. Sterols	78.5 % β-Sitosterol 21.4 % restl. Sterols
Oleum ⑪ Arachidis	28.00	15.56	12.44	β-Sitosterol Stigmasterol Cholesterol 3 weitere Sterols ⑬ (nicht identifizierbar)	50.0 % β-Sitosterol 27.7 % Stigmasterol 13.8 % Cholesterol 8.5 % restl. Sterols	50.0 % β-Sitosterol 27.8 % Stigmasterol 13.8 % Cholesterol 8.4 % restl. Sterols	50.0 % β-Sitosterol 27.7 % Stigmasterol 13.8 % Cholesterol 8.5 % restl. Sterols
Oleum ⑫ Ricini	24.46	21.32	3.14	β-Sitosterol Stigmasterol Cholesterol 3 weitere Sterols ⑬ (nicht identifizierbar)	48.0 % β-Sitosterol 31.3 % Stigmasterol 15.6 % Cholesterol 5.1 % restl. Sterols	48.0 % β-Sitosterol 30.7 % Stigmasterol 16.1 % Cholesterol 5.2 % restl. Sterols	48.0 % β-Sitosterol 31.2 % Stigmasterol 16.5 % Cholesterol 5.3 % restl. Sterols

Application

As application of the method presented, we have determined the sterol contents of several oils listed in the Austrian Dispensatory, 9th edition. The chromatograms of the sterol mixtures of olive oil, peanut oil, and ricinus oil obtained by precipitation with digitonin exhibited, in addition to four known sterols, spots of three to four additional sterols not further identified (cf. Figure 1), with the compositions of the sterol fractions of peanut oil and ricinus oil being practically identical. The total sterol contents listed in Column 1 of Table 3 simultaneously represent the sterol contents after Peereboom and Roos (1).

In all three oils investigated, we found in the free sterols as well as in the bound sterols that the qualitative as well as the quantitative compositions of these two fractions were practically identical.

Acknowledgement - We are indebted to Professor Dr. J.W. Copius Peereboom for a generous gift of highly purified sterols. We are also grateful to Professor Dr. A. Seher for a gift of campestrol.

Bibliography

1 J. W. Copius Peereboom und J. B. Roos, *Fette, Seifen, Anstrichmittel*, 62 (1960) 91.
2 A. Seher und E. Homberg, *Fette, Seifen, Anstrichmittel*, 70 (1968) 481.
3 Th. Kartnig, A. Hiermann und G. Mikula, *Pharm. Zentralhalle*, 108 (1969) 177.
4 Th. Kartnig und G. Mikula, *Pharm. Zentralhalle*, 108 (1969) 457.
5 Th. Kartnig und G. Mikula, *Zentr. Pharm.*, 109 (1970) 251.
6 H. Dam und R. Schoenheimer, *Z. Physiol. Chem.*, 215 (1933) 59.
7 A. Zaffaroni, *J. Am. Chem. Soc.*, 72 (1950) 3828.
8 C. Levorato, *Il Farmaco, Ed. Prat*, 24 (1969) 227.
9 Th. Kartnig und G. Mikula, *Arch. Pharm.*, 303 (1970) 767.