Phytochemistry, 1973, Vol. 12, pp. 2727 to 2732. Pergamon Press. Printed in England.

STEROLS OF THE BROWN ALGA *SARGASSUM FLUITANS**

LELAND L. SMITH, AMIYA K. DHAR†, JAMES L. GILCHRIST and YONG YENG LIN

Division of Biochemistry, Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550, U.S.A.

(*Received* 7 *March* 1973. *Accepted* 15 *May* 1973)

Key Word Index—*Sargassum fluitans*; Sargassaceae; brown algae; sterols; fucosterol; cholesterol; 24-methylenecholesterol; 24-ketocholesterol.

Abstract—The sterol composition of the warm-water brown alga *Sargassum fluitans* Børgesen of the Gulf of Mexico was determined by TLC, GLC and IR measurements. The presence of over ten sterols was suggested, of which four (fucosterol, cholesterol, 24-methylenecholesterol, and *trans*-22-dehydrocholesterol) were identified and four (a 24-methylcholesterol, a 24-ethylcholesterol, a 24-methyl-*trans*-22-dehydrocholesterol and a 24-ethyl-*trans*-22-dehydrocholesterol) were recognized but not definitively identified. Saringosterol and 24-ketocholesterol were not found. The crude sterol mixture from *S. fluitans* was oxidized by osmium tetroxide to 24-ketocholesterol in poor yield.

INTRODUCTION

IN ORDER to obtain substantial amounts of 24-ketocholesterol (3β-hydroxycholest-5-en-24-one) for the synthesis of the mammalian brain sterol cerebrosterol (cholest-5-ene-3β, 24-diol), we sought a suitable supply of fucosterol (24-ethylcholesta-5, *E*-24(28)-dien-3β-ol) from which 24-ketocholesterol could be prepared. Although a chemical synthesis of 24-ketocholesterol from 3β-acetoxychol-5-enic acid has been reported,[1] the synthesis in our hands and in those of others[2] has been troublesome, and the route via fucosterol had appeal. However, those species of brown algae (Phaeophyta) from which fucosterol has been previously recovered in abundance were all cold-water species of the orders Laminariales and Fucales from the north Atlantic and Pacific Oceans or from Scandinavian waters.[3] In order to avoid the difficulties of collection of these species we sought to determine whether common *Sargassum* species (order Fucales) of seaweed found in abundance in the warm waters of the Gulf of Mexico were potential sources of fucosterol. The chief sterol of *Sargassum* species previously examined[3–5] is fucosterol, found with smaller amounts of its putative precursor 24-methylenecholesterol (24-methylcholesta-5, 24(28)-dien-3β-ol) and of cholesterol (cholest-5-en-3β-ol).[3,5] With the possible exception of reports of the C-20 epimer of fucosterol (sargosterol)[6] and saringosterol (24-ethylcholesta-5,28-diene-3β, 24 ξ-

* Part XXVII of the series "Sterol Metabolism".

† Present address: American Health Foundation, New York City, NY 10021, U.S.A.

[1] RIEGEL, B. and KAYE, I. A. (1944) *J. Am. Chem. Soc.* **66**, 723.

[2] VAN ALLER, R. T., CHIKAMATSU, H., DE SOUZA, N. J., JOHN, J. P. and NES, W. R. (1969) *J. Biol. Chem.* **244**, 6645.

[3] PATTERSON, G. W. (1971) *Lipids* **6**, 120.

[4] REINER, E., TOPLIFF, J. and WOOD, J. D. (1962) *Can. J. Biochem. Physiol.* **40**, 1401.

[5] IKEKAWA, N., MORISAKI, N., TSUDA, K. and YOSHIDA, T. (1968) *Steroids* **12**, 41.

[6] TSUDA, K., HAYATSU, R., KISHIDA, Y. and AKAGI, S. (1958) *J. Am. Chem. Soc.* **80**, 921.

diol)[5,7] in certain species of *Sargassum*, there was no indication from the literature that our goal could not be attained. We present herein results of our examination of the sterols of *Sargassum fluitans* Børgesen[8,9] of the Gulf of Mexico.

RESULTS

The crystalline sterol preparation from *S. fluitans*, m.p. 123–125°, homogeneous on TLC plates, superficially resembled fucosterol in these properties. However, GLC of the preparation and of the acetylated preparation on 3% SE30 established that at least seven sterols were present and that fucosterol constituted only about half of the total. Although analysis on 3% QF1 suggested only six components, additional analyses on 3% OV210 and on 3% SP2401 supported seven components, which number, from consideration of retention data from the literature,[10] was likely to be only a minimum.

Preparative GLC of the sterol mixture on 3% SE30 gave several sterol fractions which were homogeneous by TLC and GLC and for which satisfactory IR spectra were obtained. It was thus possible to identify *trans*-22-dehydrocholesterol (cholesta-5, *trans*-22-dien-3β-ol), cholesterol, 24-methylenecholesterol, and fucosterol as the major sterols, and to establish the presence of 24-methyl-*trans*-22-dehydrocholesterol (24-methylcholesta-5, *trans*-22-dien-3β-ol) and 24-ethyl-*trans*-22-dehydrocholesterol (24-ethylcholesta-5, *trans*-22-dien-3β-ol). Adequate IR spectra could not be acquired on other sterols whose presence was indicated but which were not recovered in a pure state from preparative GLC.

That each of these sterols was indeed a Δ^5-sterol was supported not only by their GLC behavior but by their typical red coloration on TLC plates with 50% aqueous sulfuric acid and by absorption in the 795–790 cm^{-1} region,[11–13] both of which are characteristic of Δ^5-sterols.

Of key importance to recognition of the structural features of the unsaturated side-chains of the sterols recovered were the out-of-plane bending frequencies of the olefinic hydrogen atom (C=C–H). The several *trans*-Δ^{22}-sterols encountered were recognized as such by prominent absorption in the 968–965 cm^{-1} region characteristic of that structural feature,[14,15] whereas prominent absorption at 880 cm^{-1} (terminal methylene) uniquely characterized 24-methylenecholesterol.[16–19] Characteristic triplet absorption in the 840–795 cm^{-1} region ascribed to the out-of-plane bending of the C-6 (840 and 795 cm^{-1}) and C-28 (825 cm^{-1}) olefinic hydrogen atoms characterized fucosterol.[20,21] Spectra of the two minor sterols matched in detail spectra of reference samples of brassicasterol (24β_F-methylcholesta-5, *trans*-22-dien-3β-ol) and stigmasterol (24α_F-ethylcholesta-5, *trans*-22-dien-3β-ol)

[7] IKEKAWA, N., TSUDA, K. and MORISAKI, N (1966) *Chem. Ind. (London)* 1179.
[8] TAYLOR, W. M (1940) *Papers Michigan Acad. Sci., Arts, Letters* **26,** 69.
[9] EDWARDS, P. (1970) *Contrib. Marine Sci.* **15,** Supplement No. 1, pp. 10, 28, 29, 82, 83
[10] PATTERSON, G. W. (1971) *Anal Chem* **43,** 1165
[11] JONES, R N., HUMPHRIES, P, PACKARD, E and DOBRINER, K. (1950) *J. Am. Chem Soc.* **72,** 86.
[12] BLADON, P., FABIAN, J. M, HENBEST, H. B, KOCH, H. P. and WOOD, G W (1951) *J. Chem. Soc.* 2402.
[13] HIRSCHMANN, H. (1952) *J. Am. Chem Soc* **74,** 5357.
[14] TURNBULL, J. H, WHIFFEN, D H. and WILSON, W. (1950) *Chem. Ind. (London)* 626.
[15] JONES, R N. (1950) *J. Am. Chem. Soc.* **72,** 5322
[16] IDLER, D. R. and FAGERLUND, U. H. M. (1955) *J. Am. Chem. Soc* **77,** 4142.
[17] FAGERLUND, U. H M. and IDLER, D R. (1956) *J Org. Chem.* **21,** 372.
[18] IDLER, D R and FAGERLUND, U H. M (1957) *J Am Chem Soc* **79,** 1988.
[19] BARBIER, M., HUGEL, M -F. and LEDERER, E (1960) *Bull Soc. Chim. Biol* **42,** 91.
[20] DUSZA, J. P (1960) *J. Org Chem* **25,** 93
[21] GIBBONS, G. F., GOAD, L. J and GOODWIN, T W. (1968) *Phytochemistry* **7,** 983.

respectively, thus supporting the 24-methyl- and 24-ethyl-*trans*-22-dehydrocholesterol structures.

TABLE 1. CHROMATOGRAPHIC CHARACTERIZATION OF *Sargassum fluitans* ACETYLATED STEROLS

Argentation thin-layer zone	Component No.	R_C*	Sterol acetates found	Relative retention time, t_R 3% SE30	3% QF1	3% OV210	3% SP2401
S fluitans sterol acetates							
A	1	1 12	Unidentified components	—	—	—	—
B	2	1 00	Cholesterol (69%)†	1 00	1 00	1 00	1 00
	3		24-Methylcholesterol (6%)†	1 31	1 32	1 31	1·31
	4		24-Ethyl-*trans*-22-dehydrocholesterol (15%)†	1 43	1 32	1 36	1 35
	5		24-Ethylcholesterol (10%)†	1 65	1 55	1 57	1 57
C	6	0 90	24-Methyl-*trans*-22 dehydrocholesterol	1 12	1 10	1 10	1 09
D	7	0 81	*trans*-22-Dehydrocholesterol	0 90	0 90	0 89	0 89
E	8	0 74	Unidentified C_{26}-sterol (?)	0 64	0 64	0 66	0 66
	9		Fucosterol	1 67	1 55	1 54	1 53
F	10	0 59	Unidentified sterol (25%)†	1 09	‡	1 08	1 08
	11		Unidentified sterol (70%)†	1 73	‡	1 58	1 57
G	12	0 46	Unidentified sterols	—	—	—	—
H	13	0 23	24-Methylenecholesterol	1 28	1 32	1 27	1 30
I	14	0 15	Unidentified components	—	—	—	—
J	15	0 00	Unidentified origin components	—	—	—	—
Reference sterol acetates							
		1 00	Cholesterol	1 00	1 00	1 00	1 00
		0 99	Campesterol	1 32	1 30	1 31	1 30
		0 98	Stigmasterol	1 44	1 35	1 34	1 34
		1 01	Sitosterol	1 66	1 56	1 58	1 56
		0 90	Brassicasterol	1 11	1 09	1·10	1 09
		0 84	*trans*-22-Dehydrocholesterol	0 92	0 89	0 94	0 88
		0 72	*cis*-22-Dehydrocholesterol	0 91	0 88	0 94	0 88
		0 74	24-Norcholesta-5, *trans*-22-dien-3β-ol	0 63	0 64	0 66	0 65
		0 74	Fucosterol	1 67	1 54	1 54	1 53
		0 23	24-Methylenecholesterol	1 28	1 29	1 28	1 28

* Mobility on argentation TLC relative to cholesterol acetate as one.

† The proportion (as measured on the 3% SE30 system) of the named sterol in the purified argentation TLC zone with the indicated R_C is given in parenthesis.

‡ Not analyzed on 3% QF-1.

Despite these results, it was obvious that the *S. fluitans* sterol mixture was even more complex, and a second independent analysis of the acetylated sterols using argentation TLC resolved the mixture into nine components (Table 1). Subsequent repeated argentation TLC afforded the nine zones free from one another. GLC analysis of each zone established that several zones were heterogenous and that over ten sterols were present. From retention time data four of these components were identified as fucosterol acetate, cholesterol acetate, 24-methylenecholesterol acetate, and *trans*-22-dehydrocholesterol acetate. Fucosterol was recognized as the most abundant sterol, constituting approximately half of the sterol mixture, with cholesterol and 24-methylenecholesterol being the next most abundant sterols, as previously indicated.

By the same means four other lesser sterol acetate components were recognized as most probably being 24-methylcholesterol acetate, 24-ethylcholesterol (24-ethylcholest-5-en-3β-ol) acetate, 24-methyl-*trans*-22-dehydrocholesterol acetate, and 24-ethyl-*trans*-22-dehydrocholesterol acetate. Definitive identification of these 24-alkylsterols was not possible from our data. The notorious failure of GLC (with or without associated MS or IR spectrometry)[2,5,10,22,23] to distinguish between epimeric 24-alkylcholesterol derivatives limits our

[22] GERSHENGORN, M. C., SMITH, A. R. H., GOULSTON, G., GOAD, L. J. and GOODWIN, T. W. (1968) *Biochemistry* **7**, 1698.

[23] KNIGHTS, B. A. (1970) *Phytochemistry* **9**, 903.

assignment of identities to the three major sterols fucosterol, cholesterol, 24-methylenecholesterol, and to the minor sterol *trans*-22-dehydrocholesterol unsubstituted in the C-24 position. Thus, the 24-methylcholesterol detected (Table 1) may be campesterol (24α_F (or 24*R*)-methylcholest-5-en-3β-ol) or ergostenol (24β_F (or 24*S*)-methylcholest-5-en-3β-ol); the 24-ethylcholesterol may be sitosterol (24α_F (or 24*R*)-ethylcholest-5-en-3β-ol) or clionasterol (24β_F (or 24*S*)-ethylcholest-5-en-3β-ol). The 24-methyl-22-dehydrocholesterol found may be brassicasterol (24β_F (or 24*R*)-methylcholesta-5, *trans*-22-dien-3β-ol) or the epimeric 24α_F (or 24*S*)-methylcholesta-5, *trans*-22-dien-3β-ol. The homologous 24-ethyl-22-dehydrocholesterol detected may be stigmasterol (24α_F (or 24*S*)-ethylcholesta-5, *trans*-22-dien-3β-ol) or poriferasterol (24β_F (or 24*R*)-ethylcholesta-5, *trans*-22-dien-3β-ol). Neither the stereochemical identity nor the stereochemical purity of the four 24-alkylsterol components can be considered from our data.

A digression at this point into stereochemical nomenclature of these 24-alkylsterols is pertinent. Although the revised 24α_F-ethyl configurations of sitosterol and stigmasterol and the epimeric 24β_F-ethyl configurations of clionasterol and poriferasterol are broadly disseminated[24–26] and the matter has been reemphasized,[27] reports still appear using the old (opposite) nomenclature for these sterols. To compound this problem the recommended use of the Cahn–Ingold–Prelog Sequence Rule for the C-24 configurations of these sterols[28] has found acceptance, but frequent misuse of the rule and confusion in its application obtains, particularly for the related 24α_F-ethylsterols sitosterol and stigmasterol and their C-24 epimers.[2,22,29] Sitosterol is properly 24α_F (or 24*R*)-ethylcholesterol, but stigmasterol is correctly 24α_F (or 24*S*)-ethyl-*trans*-22-dehydrocholesterol, account being taken of the altered preference of the C_{23}-Δ^{22}-sterol residue over the terminal isopropyl group in this case.[30] In order to appreciate the relationship between algal taxonomy and sterol distribution patterns, retention of the 24α_F- and 24β_F-Fischer projection nomenclature[28] for C-24 alkyl substituents is recommended.[31]

We did not recognize the presence of desmosterol (cholesta-5, 24-dien-3β-ol) or of isofucosterol (24-ethylcholesta-5, Z-24(28)-dien-3β-ol) among the *S. fluitans* sterols, although very minor components 10 and 11 (Table 1) exhibit both argentation TLC and GLC behavior of these two sterol dienes respectively. Furthermore, we did not encounter polar sterols of gas chromatographic retention times greater than those of fucosterol. Thus, saringosterol[5,7,23] and 24-ketocholesterol[23,32] detected in some brown algae were not found in our work.

The unidentified sterol diene (component 8 of Table 1) with very short gas chromatographic retention times cannot be identified with any of the natural sterols for which extensive comparative retention time data have been published,[10] but this sterol may be a

[24] SHOPPE, C. W. (1964) *Chemistry of the Steroids*, 2nd Edn, pp. 14, 45, 62, 63, 75, 77, 80, Butterworths, London.
[25] HEFTMANN, E. (1970) *Steroid Biochemistry*, pp. 20–22, Academic Press, New York
[26] ANON (1967) IUPAC-IUB 1967 Revised Tentative Rules for Steroid Nomenclature, *Biochim. Biophys. Acta* **164**, 453; (1969) *J. Org. Chem.* **34**, 1517; (1969) *Biochemistry* **8**, 2227
[27] ROWE, J. W. (1965) *Phytochemistry* **4**, 1.
[28] PETTIT, G. R. (1963) *Experientia* **19**, 124.
[29] BRIGGS, M. H. and BROTHERTON, J. (1970) *Steroid Biochemistry and Pharmacology*, p. 18, Academic Press, London.
[30] ANON (1970) *J. Org. Chem.* **35**, 2866.
[31] THOMPSON, M. J., DUTKY, S R., PATTERSON, G. W. and GOODEN, E. L. (1972) *Phytochemistry* **11**, 1781.
[32] MOTZFELDT, A.-M. (1970) *Acta Chem. Scand* **24**, 1846.

C_{26}-diene of the type (24-norcholesta-5, *trans*-22-dien-3β-ol) previously detected in molluscs,[33–38] marine plankton,[39] and red algae.[40]

Oxidation with ozone or with osmium tetroxide, of the *S. fluitans* sterol mixture estimated to contain 55% fucosterol and 8% 24-methylenecholesterol, both of which should afford 24-ketocholesterol on oxidation yielded 24-ketocholesterol but in poor (10%) yield.

DISCUSSION

Our present analyses of the sterols of *S. fluitans* support the previously established status of fucosterol as the principal sterol of brown algae and extend this generality to include for the first time species confined totally to warm waters. The relative amount of fucosterol in this case is substantially less and the composition of the sterol mixture from *S. fluitans* is much more complex than has been heretofore suggested for other brown algae. The lower proportion of fucosterol and the added complexity of the sterol mixture precludes use of *S. fluitans* as a ready source of pure fucosterol.

The more complex pattern of sterols of *S. fluitans* affords a basis for some speculation on possible biosynthetic relationships among these sterols in brown algae. Fucosterol and 24-methylenecholesterol appear to be related in a product-precursor relationship, both being derived from desmosterol,[2] which has been tentatively identified in the brown algae *Laminaria faeroensis* and *L. digitata*[41] and which may occur at very low levels in *S. fluitans*. The presence of the three *trans*-Δ^{22}-sterols *trans*-22-dehydrocholesterol, 24-methyl-*trans*-22-dehydrocholesterol, and 24-ethyl-*trans*-22-dehydrocholesterol suggests that *S. fluitans* possesses an active sterol 22-dehydrogenase system capable of metabolizing sterols with and without C-24 alkyl substitution. Although 24-alkylcholesterol derivatives have been but infrequently detected among the sterols of brown algae,[23] the presence of cholesterol and of the 24-methylcholesterol and 24-ethylcholesterol derivatives found in this study suggest that sterol 24- and 24(28)-reductase systems exist in *S. fluitans*. The stereospecificity of the putative 24- and 24(28)-reductase systems cannot be assessed from our studies, but for the phytoflagellate *Ochromonas danica*[22] and for the green algae[3] stereospecific formation of 24β_F-alkylsterols appears to be of importance.

EXPERIMENTAL

Sterol isolation. Specimens of *S. fluitans* Børgesen[9,10] collected from beaches of Galveston Island, Galveston County, Texas, in September and October 1971 were cleansed from sand and other foreign material, washed with H_2O, and dried at room temp. in air. The dried seaweed (750 g) was ground in a mortar and extracted with acetone in a Soxhlet for 48 hr. The acetone extracts were evaporated under vacuum, the residue dissolved in 500 ml of 10% NaOH in EtOH and warmed overnight. The solution was reduced to about half vol. under vacuum, diluted with an equal vol. H_2O, and extracted 3 × with 2 l. Et_2O. The Et_2O extracts were washed with H_2O until neutral, dried, and evaporated under vacuum, to afford 11·5 g of a crude unsaponifiable fraction. The fraction was dissolved in boiling MeOH and cooled to give fine needles which were filtered. The mother liquor was concentrated under vacuum and cooled again to give additional crops of crystalline material, and all crystalline fractions were combined and recrystallized from MeOH

[33] WAINAI, T., TAMURA, T., TRUSCOTT, B. and IDLER, D. R. (1964) *J. Fish. Res. Bd. Can.* **21,** 1543.
[34] VOOGT, P. A. (1969) *Comp. Biochim. Physiol.* **31,** 37.
[35] IDLER, D. R., WISEMAN, P. and SAFE, L. M. (1970) *Steroids* **16,** 451.
[36] VOOGT, P. A. (1971) *Comp. Biochem. Physiol.* **39B,** 139.
[37] IDLER, D. R. and WISEMAN, P. (1971) *Intern. J. Biochem.* **2,** 516.
[38] TECHIMA, S.-I., KANAZAWA, A. and ANDO, T. (1972) *Comp. Biochem. Physiol.* **41B,** 121.
[39] BOUTRY, J.-L., ALCAIDE, A. A. and BARBIER, M. (1971) *Compt. Rend.* **272,** 1022.
[40] IDLER, D. R. and WISEMAN, P. (1970) *Comp. Biochem. Physiol.* **35,** 679.
[41] PATTERSON, G. W. (1968) *Comp. Biochem. Physiol.* **24,** 501.

3×, thereby yielding 1·0116 g (0·135%) of purified mixed sterols, m.p (Kofler block) 123–125°, migrating as a single component with the mobility and color reaction with 50% H_2SO_4 of cholesterol in the usual TLC systems.

TLC Analytical TLC was conducted using Silica Gel HF_{254} chromatoplates 0 25 mm thick as previously described.[42] Argentation TLC of sterol acetates was conducted by techniques adapted from the literature [43,44] A solution of 6 g $AgNO_3$ in 80 ml dist. H_2O was slurried with 30 g of silica gel $HF_{254+366}$ (E. Merck GmbH.) and spread onto 20 × 20 cm glass plates (in the dark) so as to give 0·25 mm thick layers. The plates were air dried in the dark for 1 hr, activated at 100° for 1 hr, and stored in a light-tight storage chamber over $CaCl_2$ Sterol acetate samples were applied to the plates in the usual manner, which were irrigated 4× (in the dark) by ascent with hexane–C_6H_6 (5.3). Each solvent rise was complete within 25 min; intermediate drying in air between each irrigation was for 15 min. Components were visualized by viewing under 254 nm UV light and by spraying the dried plate with 50% aq. H_2SO_4 Preparative TLC was conducted in exactly the same manner. Recovery of each resolved sterol fraction was achieved by shaking the silica gel in a separating funnel with 10% v/v HCl and $CHCl_3$. The $CHCl_3$ layer was dried and evaporated under vacuum to give the purified sterol acetate fraction. Sterol samples were acetylated with Ac_2O–pyridine.

Gas chromatography. Analytical and preparative GLC was conducted as previously described[45,46] on 3% SE30 on 80–100 mesh Gas-Chrom Q or 3% QF1 on 100–120 mesh Gas-Chrom Q and on 3% OV210 or 3% SP2401 on 100–120 mesh Supelcoport Relative retention times were measured vs cholesterol as unit time where the free sterols were being examined, vs cholesterol acetate as unit time where sterol acetates were under analysis. Effluxing sterols collected in glass capillaries or in glass pipettes drawn to a fine tip were rinsed from the collecting tube with spectral grade $CHCl_3$ for further analyses IR spectra were measured in KBr disks.

Sterol analysis Direct GLC analysis of the *S fluitans* sterol mixture gave the following data (relative retention time on 3% SE30, relative retention time on 3% QF1): I, 0·62, 0 67; II, 0·91, 0·90; III, 1 00, 1 00; IV, 1·10, 1·05; V, 1 27, 1 29; VI, 1·42, not resolved on 3% QF1; VII, 1·64, 1·48. Relative retention times on 3% SE30 and on 3% QF1 of key reference sterols are· 24-norcholesta-5, *trans*-22-dien-3β-ol, 0·62, 0·65, *trans*-22-dehydrocholesterol, 0 91, 0 89; *cis*-22-dehydrocholesterol, 0 87, 0 88, cholesterol, 1·00, 1·00; campesterol, 1·31, 1 29; sitosterol, 1·64, 1 54; brassicasterol, 1 10, 1·07, stigmasterol, 1·44, 1 32; 24-methylenecholesterol, 1·27, 1·24, fucosterol, 1·64, 1·48; 24-ketocholesterol, 1·67, 3 28 Argentation TLC and GLC data for the sterol acetates are summarized in Table 1

24-Ketocholesterol. A solution of 310 mg of crystalline *S. fluitans* mixed sterols, m p. 123–125°, in 15 ml of 80% aq. dioxane was stirred with 31 mg OsO_4 and 350 mg $NaIO_4$ for 24 hr. The solvent was removed under vacuum, 20 ml H_2O was added, and the sterols were extracted with EtOAc (3 × 20 ml). The dried extract was evaporated under vacuum and the sterols were chromatographed on 80 g of silica gel irrigated with 3 l C_6H_6, the latter portions of which eluted 24-ketocholesterol which was rechromatographed on TLC plates irrigated 2× with C_6H_6–EtOAc (5:1). The purified 24-ketocholesterol was recrystallized from hexane to give 30 mg, m p. 125–128°, identical in TLC, GLC and IR spectral properties with an authentic sample

Acknowledgements—The authors gratefully acknowledge financial support for these studies from the U.S. Public Health Service (Grant NS-08106) Reference sterol samples were kindly provided by Dr W. Bergmann (deceased), Yale University; Dr. D R. Idler, Memorial University of Newfoundland, Dr. A.-M. Motzfeldt, University of Trondheim; Dr G. W. Patterson, University of Maryland; Dr. A Romeo, University of Rome; and Dr. M. J. Thompson, U.S Department of Agriculture, Beltsville, Md.

[42] SMITH, L. L., MATTHEWS, W. S , PRICE, J. C., BACHMANN, R. C and REYNOLDS, B. (1967) *J. Chromatog.* **27,** 187.
[43] VROMAN, H. E. and COHEN, C. F (1967) *J. Lipid Res.* **8,** 150.
[44] IDLER, D. R. and SAFE, L. M. (1972) *Steroids* **19,** 315.
[45] VAN LIER, J. E. and SMITH, L. L (1968) *Anal. Biochem* **24,** 419
[46] VAN LIER, J E. and SMITH, L. L (1968) *J. Chromatog.* **36,** 7