CHROM. 5906

# Separation of β-sitosterol and campesterol on hydrophobic hydroxyalkyl Sephadex LH-20

The separation of individual plant sterols, such as β-sitosterol, from their naturally occurring analogs has been difficult[1] prior to the introduction of lipophilic Sephadex[2]. NYSTRÖM *et al.*[3,4] reported the partial separation of β-sitosterol and campesterol on chromatographic columns of hydroxypropyl ether derivatives of Sephadex G-25 (LH-20) after 20–25 cycles. ELLINGBOE *et al.*[5] demonstrated the separation of microgram amounts of cholesterol ($C_{27}$), campesterol ($C_{28}$), and β-sitosterol ($C_{29}$) on columns of hydroxyalkoxypropyl Sephadex at flow-rates of 1.5 ml solvent per hour. BROOKS AND KEATES[6], using this same type of hydrophobic Sephadex derivatives, demonstrated that quantitative recoveries were possible in chromatographic separations based on different molecular size.

This paper describes the liquid–gel chromatographic separation of 25 mg of a mixture of campesterol and β-sitosterol on columns of hydrophobic long-chain alkyl ethers of Sephadex LH-20. Recycling for six times on a 38- or 88-cm column provides each sterol in the absence of the other as measured by gas–liquid chromatography.

*Experimental procedures*

Hydrophobic long-chain alkyl ethers of Sephadex were prepared from Sephadex LH-20 by the method of ELLINGBOE *et al.*[5] using Needox 1114* ($C_{11}$–$C_{14}$ chain length). This preparation had a weight increase corresponding to a hydroxyalkyl content of 49% (w/w). Solvent-resistant columns (2.5 cm diameter × 45 and 100 cm, Pharmacia) were filled with a homogenous slurry of the Sephadex derivative and a methanol–hexane (95:5) mixture, corresponding to column volumes of 182 and 432 ml, respectively, when packed. After equilibration of the methanol–hexane mixture with the modified Sephadex LH-20 by pumping the solvent through the column at 75 ml/h with a Milton Roy Minipump for 8 h, 25 mg of a commercial sample of β-sitosterol in 25 ml of methanol–hexane was added to the column through the pump. 165 ten-ml fractions were collected immediately for the one-cycle studies and after a calculated volume of solvent mixture had been pumped through the column for three- and six-cycle experiments. Representative fractions were analyzed for their sterol content by gas chromatography of 5 μl on a column of 3% QF-1 on Gas-Chrom Q (6 ft. × 0.25 in. O.D.) under the following conditions: column, 230°; flash heater, 245°; detector, 245°; helium flow, 60 ml/min at 40 p.s.i. (ref. 7). Commercial samples of β-sitosterol were obtained from Aldrich, Schwarz/Mann and Sigma Chemical Companies. Each showed substantially the same chromatographic pattern, two components corresponding to campesterol (43%), RRT = 3.58, and β-sitosterol (56–57%), RRT = 4.26, compared to cholestane as a standard (RRT = 1.0; absolute time = 3.54 min). Aliquots of solutions of weighed samples of each purified sterol were injected into the gas chromatograph and the relative peak areas measured to quantitate the amount of sterol found in each column fraction.

* Needox 1114 is a mixture of olefin oxides of $C_{11}$–$C_{14}$ chain length.

*Results and discussion*

Campesterol and β-sitosterol were partially separated upon one cycle through the 38- or 88-cm columns of modified LH-20 as shown in Fig. 1. Only small amounts of either campesterol or β-sitosterol were obtained in pure form, as reported by SJÖVALL *et al.*[4], using Sephadex LH-20 and recycling the mobile phase for 71.5 h. Campesterol and β-sitosterol were eluted here at 3.4- and 3.6-column volumes, respectively. The separation of the two sterols was complete on the 38-cm column after six cycles and on the 88-cm column after three or six cycles. The elution pattern shows a band of broadening or spreading as cycling continues; β-sitosterol was contained in 240 ml after three cycles and 350 ml after six cycles, presumably due to diffusion in this type of liquid–gel chromatography. Mixtures of 100 mg have been easily separated after three cycles on the 88-cm column. There seems to be no tailing of products and the recoveries were quantitative, in agreement with BROOKS AND KEATES[6].

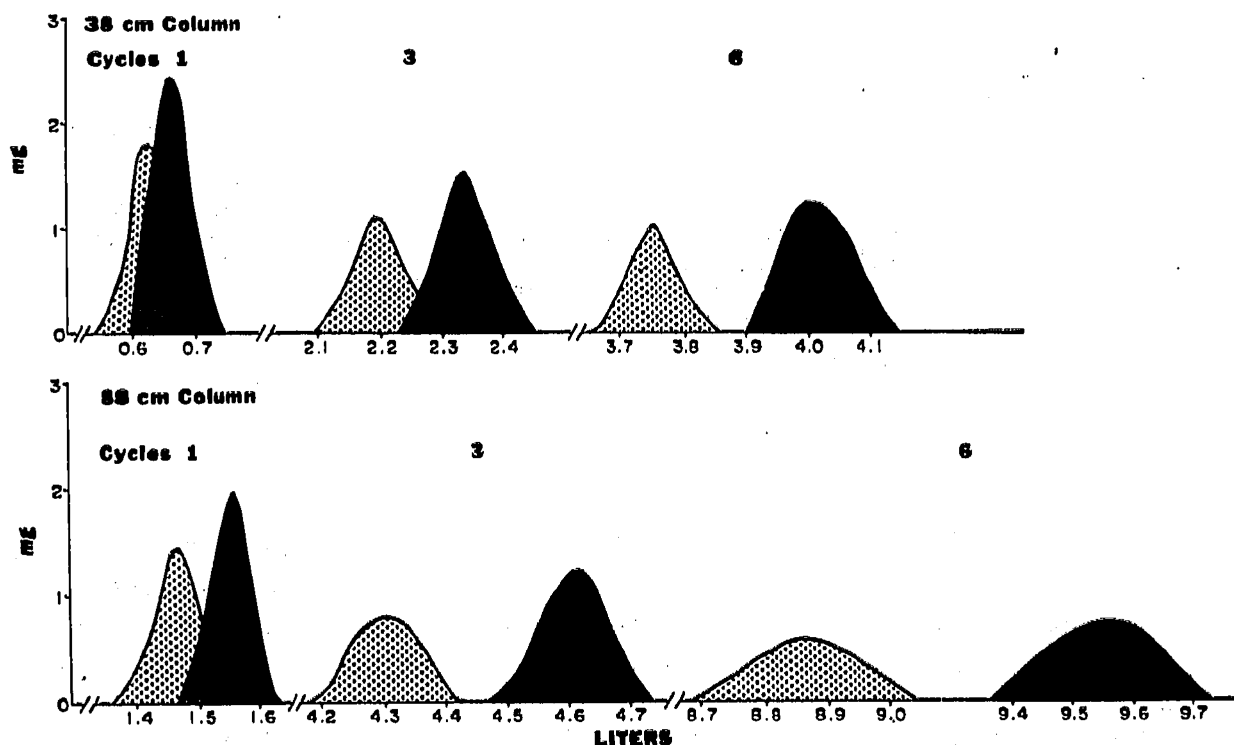


Fig. 1. Chromatographic separation of campesterol and β-sitosterol on N-1114-49% LH-20 columns. Campesterol is represented in dotted areas, β-sitosterol is the solid area.

β-Sitosterol obtained by this procedure exhibited: m.p. 138–139° $(\alpha)_D^{25} = -38°$, RT = 4.26, acetate m.p. 128° (reported for β-sitosterol: m.p. 138–139° (ref. 8), 137–139° (ref. 9), $(\alpha)_D = -37°$ (ref. 10); RRT = 4.25 (ref. 11); acetate, m.p. 127° (ref. 10)). Further evidence of purity was shown by coincident elution of mass and $^{14}C$ upon admixture of 20.3 mg of the sterol and β-[3-$^{14}C$]sitosterol[12]; 98 % of the added material chromatographed with the purified β-[3-$^{14}C$]sitosterol. Campesterol was identified by m.p. 158°, RRT = 3.58 (reported m.p. 158° (ref. 10) 157–158° (ref. 13), RRT = 3.57 (ref. 11)).

Chromatographic separation of milligram amounts of closely related sterols, differing only by one methyl group, is possible and easily reproducible. This form

of liquid–gel chromatography has been used in the separation of radiometabolites from the β-[3-$^{14}$C]sitosterol in metabolism studies[14].

We wish to thank Mrs. PHYLLIS HOUSER for expert assistance in gas chromatography. Samples of Needox 1114 were kindly provided by Mr. H. M. RICHARDSON, Ashland Chemical Co., Columbus, Ohio 43216. This research was supported by U.S.P.H.S. Grant HE-07878.

*Department of Biochemistry,*
*St. Louis University School of Medicine,*
*St. Louis, Mo. 63104 (U.S.A.)*

PAUL M. HYDE*
WILLIAM H. ELLIOTT

1 E. S. WALLIS AND P. N. CHAKRAVORTY, *J. Org. Chem.*, 2 (1938) 335.
2 E. NYSTRÖM AND J. SJÖVALL, *Anal. Biochem.*, 12 (1965) 235.
3 E. NYSTRÖM AND J. SJÖVALL, *Ark. Kemi*, 29 (1968) 107.
4 J. SJÖVALL, E. NYSTRÖM AND E. HAAHTI, *Advan. Chromatogr.*, 6 (1968) 119.
5 J. ELLINGBOE, E. NYSTRÖM AND J. SJÖVALL, *J. Lipid Res.*, 11 (1970) 226.
6 C. J. W. BROOKS AND R. A. B. KEATES, *J. Chromatogr.*, 44 (1969) 509.
7 W. H. ELLIOTT, L. B. WALSH, M. M. MUI, M. A. THORNE AND C. M. SIEGFRIED, *J. Chromatogr.*, 44 (1969) 452.
8 R. S. LUDWICZAK AND K. STOCHOWIAK, *Rocz. Chem.*, 37 (1963) 575; *C.A.* 59 (1963) 10169d.
9 H. WERBIN, I. L. CHAIKOFF AND E. E. JONES, *J. Biol. Chem.*, 235 (1960) 1679.
10 W. BERGMANN, in M. FLORKIN AND H. S. MASON (Editors), *Comparative Biochemistry*, Vol. IIIA, Academic Press, New York, 1962, p. 103.
11 R. F. N. HUTCHINS AND J. N. KAPLANIS, *Steroids*, 13 (1969) 605.
12 P. PH. H. L. OTTO, A. C. BESEMER AND W. H. J. M. WIENTJENS, *J. Label. Compounds*, 6 (1970) 111.
13 E. FERNHOLZ AND H. B. MACPHILLANY, *J. Amer. Chem. Soc.*, 63 (1941) 1155.
14 C. M. SIEGFRIED AND P. M. HYDE, *Fed. Proc.*, 30 (1971) 1105.

Received December 7th, 1971

* Present address: Department of Biochemistry, L.S.U. Medical Center, 1542 Tulane Avenue, New Orleans, La. 70112, U.S.A.