# Sublimation Thermodynamic Parameters for Cholesterol, Ergosterol, $\beta$ -Sitosterol, and Stigmasterol

# Vahur Oja

Department of Chemical Engineering, Tallinn University of Technology, Tallinn, Estonia

# Xu Chen<sup>†</sup>

Philip Morris USA Postgraduate Research Program, 4201 Commerce Road, Richmond, Virginia 23234

## Mohammad R. Hajaligol\* and W. Geoffrey Chan

Philip Morris USA Research Center, 4201 Commerce Road, Richmond, Virginia 23234

Vapor pressure and enthalpies of sublimation data for sterols are desirable engineering parameters in the separation of sterols from plant materials by vacuum pyrolysis or supercritical fluid extraction. In this study, vapor pressures of cholesterol, ergosterol,  $\beta$ -sitosterol, and stigmasterol were measured by an isothermal Knudsen effusion method. The vapor pressure correlations were fitted to the following equations: cholesterol  $\ln(p/Pa) = -17136/(T/K) + 39.88$  (from (386 to 414) K); ergosterol  $\ln(p/Pa) = -17686/(T/K) + 40.61$  (from (381 to 412) K);  $\beta$ -sitosterol  $\ln(p/Pa) = -17295/(T/K) + 39.7$  (from (389 to 410) K); and stigmasterol  $\ln(p/Pa) = -20254/(T/K) + 46.31$  (from (390 to 417) K).

## Introduction

Literature review shows that over the last few decades there have been two research areas where vapor pressure of sterols has been of interest. In the first area, a large number of papers were published on supercritical extraction of sterols and other plant materials. These studies were related to food production with lower cholesterol content<sup>1,2</sup> or isolating steroids ( $\beta$ sitosterol and stigmasterol, for example) for the pharmaceutical industry.<sup>3,4</sup> One important physical property in modeling and correlating the solubility of solute in supercritical fluids is the vapor pressure of solute. However, only a few sets of estimated and measured vapor pressure data on solid sterols were used in calculations or in modeling of sterol solubility in supercritical fluids.<sup>1,3,5–8</sup>

The second area which contains only a few papers deals with the evolution of sterols from plant materials during pyrolysis via vacuum pyrolysis.<sup>9,10</sup> It is worthy to note that under pyrolytic conditions some sterols, like cholesterol and stigmasterol, have been identified as the precursors of polycyclic aromatic hydrocarbons (PAHs),<sup>11–14</sup> which are important environmental contaminants. The interest of this work is related to the vaporization of biomass pyrolysis tars.<sup>15</sup> Knowledge of the vapor pressures of sterols is important to predict the behavior of these compounds in the pyrolysis process. As part of our work to study the vaporization process of biomass pyrolysis tars, the vapor pressures of cholesterol, ergosterol,  $\beta$ -sitosterol, and stigmasterol were examined.

In general, information on the vapor pressure of sterols and sterol-like compounds is limited. There are only a few vapor pressure data sets available for cholesterol in the literature. A comparison of the reported vapor pressure data shows that the cholesterol vapor pressure values are the most established,<sup>17</sup> whereas for other sterols, considerable disagreements among each other and/or with the predicted values can be seen.

The vapor pressures of liquid cholesterol and ergosterol were measured by Hickman et al.<sup>16</sup> using a direct determination method. However, a review of the data indicates that the compounds were measured in the temperature range bracketing the generally reported melting point regions of the compounds. More recently, limited amounts of data for solid cholesterol, stigmasterol, and ergosterol were reported using a gas saturation technique.<sup>5</sup> However, due to difficulties in measuring very low vapor pressures, the emphasis was not on obtaining data with a high degree of accuracy. In addition, due to the lack of experimental data, Kosal et al.<sup>3</sup> tried to estimate vapor pressures of cholesterol based on supercritical fluid solubility data and a thermodynamic model. Recently, vaporization and sublimation enthalpies of cholesterol were evaluated by correlation gas chromatography.<sup>17</sup>

The aim of the present work is to provide new vapor pressure data for solid sterols where practically few reliable data were available in the literature.

## Experimental

*Materials.* Cholesterol [57-88-5] with a formula of  $C_{27}H_{46}O$ , a melting temperature of (420 to 422) K, and a molecular weight of 386.65 was purchased from Aldrich (Sigma-Aldrich Co) with a purity of 99 + %.

Ergosterol [57-87-4] with a formula of  $C_{28}H_{44}O$ , a melting temperature of (429 to 431) K, and a molecular weight of 396.65 was purchased from Sigma (Sigma-Aldrich Co) with a purity of 98 %. Another batch with a purity of 98 % from Alfa Aesar was used for thermal stability studies.

<sup>\*</sup> Corresponding author. E-mail: mohammad.r.hajaligol@altria.com. Tel.: 804-335-2333. Fax: 804-335-2096.

<sup>&</sup>lt;sup>†</sup> Current Address: Cummins Inc., Corporate Research and Technology, Columbus, IN.

 $\beta$ -Sitosterol [83-46-5] with a formula of C<sub>29</sub>H<sub>50</sub>O, a melting temperature of (409 to 413) K, and a molecular weight of 414.71, with a purity of 98 % was supplied by Fluka (Sigma-Aldrich Co). In thermal stability measurements,  $\beta$ -sitosterol with a purity of 98 % from Sigma (Sigma-Aldrich Co) was used.

Stigmasterol [83-48-7] with a formula of  $C_{29}H_{48}O$ , a melting temperature of (438 to 440) K, and a molecular weight of 412.69, was purchased from Sigma (Sigma-Aldrich Co.) with a purity better than 93 %. Stigmasterol used in a field ionization mass-spectrometric (FIMS) study had a purity of 95 %, purchased from Sigma a few years earlier.

#### **Experimental Techniques**

*Knudsen Effusion Technique.* Vapor pressures in the range of  $(10^{-3} \text{ to } 10^{-1})$  Pa were measured by a Knudsen effusion method in isothermal step mode. The Knudsen effusion method is a recommended method for vapor pressure measurements for pressures lower than 1 Pa. Different experimental setups and procedures have been described in the literature since 1909.<sup>18–24</sup>

The vapor pressure measurement technique and procedure applied here were in principle the same as that used by Oja and Suuberg.<sup>23,25</sup> The experimental setup of this work was described in detail in previous publications.<sup>26,27</sup> Briefly, about 10 mg of test material is placed in a hermetic cell with a pinhole located on the center of the cell cover. The cell cover was fabricated from 0.0254 mm thick stainless steel foil. The pinhole was made by either drilling or electrochemical corrosion methods which produced similar results. The diameter of holes varied from 0.65 mm to 1.1 mm depending on the compound studied. Effects of pinhole were studied in earlier work,<sup>23,25,27</sup> and it was shown that for the specific cell configuration pinholes in the range of (0.6 to 1.1) mm do not have a significant effect on the vapor pressure of the compounds in the vapor pressure region below 1 Pa.

The mass loss rate from the cell under high vacuum (absolute pressure of as low as  $10^{-5}$  Pa) was measured by a Cahn 121 thermogravimetric analyzer (Thermo Cahn, Madison, WI). The temperature was measured by a type K thermocouple with an uncertainty of 0.1 K. The performance of the device was checked by measuring vapor pressures of anthracene (99+ % purity) and at higher temperatures verified by naphthacene (98 % purity), both purchased from Sigma-Aldrich Co. Anthracene has been used as a vapor pressure calibration compound in earlier studies.<sup>23,25,27</sup>

A commonly used effusion equation, expressed as eq 1, was applied to calculate the vapor pressure data as follows

$$P = \frac{m}{tA_0 W_0} \left(\frac{2\pi RT}{MW}\right)^{0.5} \tag{1}$$

where *P* is the vapor pressure; *m* is the mass loss of the sample through the orifice during time *t*;  $A_0$  is the area of the orifice; *R* is the ideal gas constant; *T* is the temperature of the sample; MW is the molecular weight of the sample; and  $W_0$  is the Clausing factor, which can be calculated by eq 2 shown as

$$W_0 = \frac{1}{1 + \frac{3L}{8r}}$$
(2)

where *L* and *r* are the thickness of the cell cover and the radius of the orifice (pinhole), respectively. Detailed discussions of the validity of the equations applied in this work, possible errors, and additional corrections can be found elsewhere.<sup>29–33</sup>

To remove volatile impurities and traces of absorbed water, about 5 % to 15 % of total mass was evaporated in the Knudsen device in the low to moderate temperature range of measurement before actual data were taken. During a run, at least two repetition cycles in the chosen temperature region were carried out. The highest temperatures of vapor pressure measurement were kept (10 to 15) K below the melting temperature of the compound, as determined by a differential scanning calorimeter (DSC). Temperatures as high as the melting temperature of the compound were approached in only a few cases and toward the end of each individual test. The reliability of vapor pressure data obtained by the Knudsen effusion technique depends not only on the performance of the system and the purity of samples but also on the thermal behavior of compounds studied in the experimental temperature region.

Techniques to Verify Thermal Behavior of Compounds. A thermal analyzer, STA 409 TG/DSC/MS (Netzsch Instrument, Inc., Burlington, MA), was used to study the thermal behavior of the compounds of interest and to verify their melting points. Standard thermal experiments were performed under flowing helium (50 mL·min<sup>-1</sup>) at heating rates ranging from (2 to 10) K·min<sup>-1</sup>.

Field ionization mass spectrometry (FIMS) analysis was performed on a stigmasterol sample with 95 % purity. The experiment was performed at SRI International, Menlo Park, CA. Specific details regarding the experimental procedure can be found elsewhere.<sup>10,34</sup> Briefly, about 50  $\mu$ g of sample was placed in a capillary tube inside a direct heating probe, and the sample was heated at a heating rate of 3 K · min<sup>-1</sup> under vacuum (10<sup>-3</sup> Pa). Mass spectra were collected stepwise in (20 to 30) K intervals.

Thermal stabilities of all four sterols were examined by comparing the purities of the original sample, the vapor pressure measurement residue, and the heat treated sample of each compound. The purity of the original samples and the residues were compared using proton nuclear magnetic resonance spectroscopy (<sup>1</sup>H NMR) with a Varian Unity 400 spectrometer (Varian Inc., Palo Alto, CA). The purity of heat treated cholesterol,  $\beta$ -sitosterol, and stigmasterol samples was examined by gas chromatography/mass spectrometry (GC/MS) using a HP6890 GC equipped with an HP 5973 quadrupole MSD analyzer in the scanning mode. The purity of the heat treated ergosterol sample was examined by high-performance liquid chromatography (Agilent series 1100 model HPLC with a diode array UV detector at 326 nm wavelength). The detailed description can be found elsewhere.<sup>27</sup> For the heat treated sample preparation, about 10 mg of compound was placed into the Knudsen cell without the orifice on the cell cover. The cell was held at the highest testing temperature of vapor pressure measurement for 3 h in the Knudsen effusion device in high vacuum. The total mass loss was less than 2 % during heat treatment.

#### **Results and Discussions**

Verification of thermal stability of biomolecules such as sugars, steroids, or other lipids under experimental conditions is fundamental for obtaining reliable vapor pressure data. In addition to the possibility of thermal decomposition, some biomolecules can undergo phase transitions accompanied by significant enthalpy change.<sup>25</sup> Literature review indicated that polymorphic transition occurs for cholesterol at 304.8 K and for  $\beta$ -sitosterol at 342.7 K accompanied by enthalpy change of (2.5 and 2.9) kJ·mol<sup>-1</sup> respectively.<sup>35</sup> Additional information for these sterols at higher temperatures or for other sterols studied was not found.

In this work, the first insights into the thermal behavior of sterols were obtained from TG/DSC/MS experiments. The samples showed no sign of thermal decomposition or polymorphic phase transition in the vapor pressure measurement regions. Only traces of absorbed water were detected. The melting temperature was determined as 421 K for cholesterol, 431 K for ergosterol, 411 K for  $\beta$ -sitosterol, and 435 K for stigmasterol.

FIMS experiments on stigmasterol of 95 % purity showed that there was no increase in the intensity ratio of masses m/z 412 and m/z 394 in the higher temperature range of (396 to 411) K compared to the lower temperature range of (353 to 390) K. The intensity ratios were 0.06 and 0.07, respectively. It was assumed that the m/z 412 corresponds to stigmasterol, and the mass m/z 394, among others, could indicate the presence of dehydrated stigmasterol (stigmasterol m/z 412 – water m/z 18). The full FIMS spectra for the stigmasterol were not shown here, as they were published elsewhere.<sup>10</sup>

The <sup>1</sup>H NMR spectra of the heated samples did not show any sign of decomposition, except for ergosterol, in which some small new peaks were visible compared with the spectrum of the original sample. HPLC analysis showed the change in purity levels of heated ergosterol was less than 0.5 % relative to the original sample. For cholesterol,  $\beta$ -sitosterol, and stigmasterol samples, based on GC/MS analyses, the changes in purity level were all below 0.5 %. Accordingly, based on the experimental results of DSC/TG/MS, FIMS, <sup>1</sup>H NMR, GC/MS, and HPLC, the possible thermal decomposition could be of little importance under our experimental conditions. The data from vapor pressure measurements presented below confirmed the same. Although in this work decomposition of samples was not detected in the temperature region of interest, it is important to note that the existence of dehydrogenation or hydrogenation reactions<sup>36,37</sup> has been reported in the literature under very different reaction conditions.

Tables 1(a) through (c) present experimental temperatures, effusion rates, and calculated vapor pressures using eq 1 for anthracene and naphthacene. Anthracene and naphthacene were used to calibrate and verify the performance of the Knudsen device. The sublimation enthalpies ( $\Delta_{sub}H$ ) of these compounds were calculated in accordance with an integrated form of the Clausius–Clapeyron given by eq 3 and are tabulated in Table 2 along with their standard deviations.

$$\ln P = -\frac{A}{T} + B = -\frac{\Delta_{\rm sub}H}{RT} + B \tag{3}$$

The results of this study agree well with the available literature values. Information for anthracene vapor pressures and sublimation enthalpy were given by Oja and Suuberg,<sup>23</sup> Chen et al.,<sup>27</sup> Hansen and Eckert,<sup>28</sup> and Ribeiro da Silva et al.,<sup>24</sup> and the vapor pressure and sublimation enthalpy of naphthacene was given by Oja and Suuberg.<sup>23</sup>

Experimental data for sterols (effusion rates, experimental temperatures) along with calculated vapor pressures using eq 1 are tabulated in Tables 3(a) through (d). Vapor pressure of cholesterol was measured in the temperature range of (387 to 414) K using a pinhole size of 0.65 mm in diameter. The sample of ergosterol was examined in the temperature range of (370 to 412) K using a pinhole with diameter of 1.04 mm. Vapor pressure data for  $\beta$ -sitosterol was measured over the temperature range of (381 to 410) K using a pinhole with a diameter of 1.09 mm. Vapor pressure of stigmasterol, the compound with lowest vapor pressure, was determined in the temperature range of (380 to 417) K using a pinhole of 1.05 mm in diameter. To keep temperatures as low as possible, the biggest orifice sizes

Table 1. Vapor Pressure of Anthracene (a) Before and (b) AfterSterol Vapor Pressure Measurements and (c) Vapor Pressure ofNaphthacene<sup>a</sup>

*		
(a) <i>T</i> /K	effusion rate/( $g \cdot s^{-1}$ )	vapor pressure/Pa
334.0	$6.687 \cdot 10^{-8}$	0.0651
354.8	$5.065 \cdot 10^{-7}$	0.5077
342.0	$1.511 \cdot 10^{-7}$	0.1487
337.0	$9.374 \cdot 10^{-8}$	0.0916
332.2	$5.575 \cdot 10^{-8}$	0.0541
326.8	$3.160 \cdot 10^{-8}$	0.0304
319.6	$1.366 \cdot 10^{-8}$	0.0130
349.7	$3.181 \cdot 10^{-7}$	0.3165
(b) <i>T</i> /K	effusion rate/( $g \cdot s^{-1}$ )	vapor pressure/Pa
332.1	$5.455 \cdot 10^{-8}$	0.0532
326.9	$3.118 \cdot 10^{-8}$	0.0300
319.6	$1.353 \cdot 10^{-8}$	0.0129
349.7	$3.136 \cdot 10^{-7}$	0.3121
334.2	$6.779 \cdot 10^{-8}$	0.0659
339.4	$1.153 \cdot 10^{-7}$	0.1131
323.9	$2.230 \cdot 10^{-8}$	0.0214
331.7	$5.453 \cdot 10^{-8}$	0.0529
(c) <i>T</i> /K	efusion rate/( $g \cdot s^{-1}$ )	vapor pressure/Pa
404.4	$2.217 \cdot 10^{-8}$	0.0210
409.5	$3.560 \cdot 10^{-8}$	0.0339
414.6	$5.665 \cdot 10^{-8}$	0.0542
419.6	$8.784 \cdot 10^{-8}$	0.0846
424.7	$1.319 \cdot 10^{-7}$	0.1278
410.6	$4.429 \cdot 10^{-8}$	0.0422
405.6	$2.690 \cdot 10^{-8}$	0.0255
415.8	$6.954 \cdot 10^{-8}$	0.0667
420.8	$1.070 \cdot 10^{-7}$	0.1032
416.6	$6.658 \cdot 10^{-8}$	0.0639
430.1	$2.201 \cdot 10^{-7}$	0.2146
399.0	$1.559 \cdot 10^{-8}$	0.0146
418.6	$8.151 \cdot 10^{-8}$	0.0784

<sup>*a*</sup> Data are given in the order data collected (orifice diameter  $6.5 \cdot 10^{-4}$  m, cell cover thickness  $2.5 \cdot 10^{-8}$  m).

Table 2. Vapor Pressure Correlation Parameters for Anthracene(with Data Collected Before (B) and After (A) Sterol VaporPressure Measurements) and Naphthacene from the IntegratedClausius-Clapeyron Equation Fit, Along with ExperimentalTemperature Ranges and Calculated Enthalpies of Sublimation

	temperature range	parameters for $\ln(p/Pa) = -A/(T/K) + B$		enthalpies
compound	T/K	Α	В	$\frac{\Delta_{\rm sub}H}{(\rm kJ\cdot mol^{-1})}$
anthracene (B) anthracene (A) naphthacene	320 to 355 320 to 350 399 to 430	$\begin{array}{c} 111778 \pm 67 \\ 111838 \pm 89 \\ 15014 \pm 315 \end{array}$	$\begin{array}{c} 32.53 \pm 0.21 \\ 32.71 \pm 0.27 \\ 33.34 \pm 0.76 \end{array}$	$97.9 \pm 0.6$ $98.4 \pm 0.7$ $124.8 \pm 2.6$

were used in the case of the three latter compounds. The data show no change in vapor pressure behavior when the temperature was first increased and then decreased or vice versa.

The agreement between our measured vapor pressures and the only available data for solid sterols and specifically cholesterol, given by Wong and Johnson,<sup>5</sup> is very poor: a difference of up to 2 orders of magnitude in vapor pressure can be observed when our data are extrapolated to the experimental temperature region of (308 to 333) K. It should be noted that the objective of Wong and Johnson<sup>5</sup> was to study the solubility of sterols, and the vapor pressure measurement was not systematic and used to estimate the solubility. Furthermore, Wong and Johnson<sup>5</sup> did not claim to have provided vapor pressure values of high accuracy.

Variation of the natural logarithm of vapor pressure data of cholesterol, ergosterol,  $\beta$ -sitosterol, and stigmasterol as a function of reciprocal of temperature is plotted and compared graphically in Figure 1. It can be seen that the vapor pressure

Table 3. Vapor Pressure of (a) Cholesterol,<sup>*a*</sup> (b) Ergosterol,<sup>*b*</sup> (c)  $\beta$ -Sitosterol<sup>*c*</sup>, and (d) Stigmasterol<sup>*d*</sup>

(a) <i>T</i> /K	effusion rate/ $(g \cdot s^{-1})$	vapor pressure/Pa
408.2	$4.439 \cdot 10^{-7}$	0.1251
397.3	$1.420 \cdot 10^{-7}$	0.0395
386.6	$4.302 \cdot 10^{-8}$	0.0118
392.0	$7.724 \cdot 10^{-8}$	0.0213
403.1	$2.548 \cdot 10^{-7}$	0.0714
413.8	$7.564 \cdot 10^{-7}$	0.2146
(b) <i>T</i> /K	effusion rate/( $g \cdot s^{-1}$ )	vapor pressure/Pa
402.3	$4.725 \cdot 10^{-8}$	0.0354
381.3	$1.612 \cdot 10^{-8}$	0.0031
383.8	$4.294 \cdot 10^{-9}$	0.0044
386.5	$5.970 \cdot 10^{-9}$	0.0056
394.4	$7.630 \cdot 10^{-9}$	0.0142
399.6	$1.914 \cdot 10^{-8}$	0.0260
404.6	$3.490 \cdot 10^{-8}$	0.0447
407.2	$5.954 \cdot 10^{-8}$	0.0591
412.3	$7.851 \cdot 10^{-8}$	0.1027
(c) <i>T</i> /K	effusion rate/( $g \cdot s^{-1}$ )	vapor pressure/Pa
395.2	$7.027 \cdot 10^{-8}$	0.0172
389.9	$3.875 \cdot 10^{-8}$	0.0094
400.3	$1.212 \cdot 10^{-7}$	0.0299
405.6	$2.094 \cdot 10^{-7}$	0.052
397.7	$9.071 \cdot 10^{-8}$	0.0223
392.4	$5.207 \cdot 10^{-8}$	0.0127
410.7	$3.612 \cdot 10^{-7}$	0.0902
(d) <i>T</i> /K	effusion rate/( $g \cdot s^{-1}$ )	vapor pressure/Pa
401.2	$6.077 \cdot 10^{-8}$	0.0161
396.1	$3.126 \cdot 10^{-8}$	0.0082
390.9	$1.506 \cdot 10^{-8}$	0.0039
393.5	$2.122 \cdot 10^{-8}$	0.0056
398.7	$4.338 \cdot 10^{-8}$	0.0115
406.3	$1.107 \cdot 10^{-7}$	0.0296
411.5	$1.999 \cdot 10^{-7}$	0.0538
416.6	$3.526 \cdot 10^{-7}$	0.0954
408.9	$1.532 \cdot 10^{-7}$	0.0411
414.0	$2.680 \cdot 10^{-7}$	0.0723
403.9	$8.252 \cdot 10^{-8}$	0.022
397.2	$3.546 \cdot 10^{-8}$	0.0094
392.0	$1.767 \cdot 10^{-8}$	0.0046

<sup>*a*</sup> Data are given in the order data were collected (orifice diameter  $6.5 \cdot 10^{-4}$  m, cell cover thickness  $2.5 \cdot 10^{-8}$  m). <sup>*b*</sup> Data are given in the order data were collected (orifice diameter  $1.04 \cdot 10^{-3}$  m, cell cover thickness  $2.5 \cdot 10^{-8}$  m). <sup>*c*</sup> Data are given in the order data were collected (orifice diameter  $1.09 \cdot 10^{-3}$  m, cell cover thickness  $2.5 \cdot 10^{-8}$  m). <sup>*d*</sup> Data are given in the order data were collected (orifice diameter  $1.05 \cdot 10^{-3}$  m, cell cover thickness  $2.5 \cdot 10^{-8}$  m).

curves of the first three sterols are well represented by straight lines. Only in the case of stigmasterol the data points at extreme temperatures tend to be lower and in the middle slightly above the fitted straight line. The reason for this slightly curved line is unclear, as the experimental data were reproducible when temperature was increased or decreased. The DSC studies of stigmasterol did not show any evidence of phase change in the experimental temperature range, and no data were found in the literature for changes in the heat capacity with temperature to include the effects of heat capacity.

Table 4 provides the two constants of vapor pressure temperature dependency as shown in Figure 1, and the calculated sublimation enthalpies ( $\Delta_{sub}H$ ) with their standard deviations in accordance with an integrated form of the Clausius–Clapeyron given by eq 3. The sublimation enthalpies obtained from this equation correspond to the average values over the experimental temperature ranges studied.

Since the vapor pressures of all sterols were measured in narrow temperature ranges and reliable heat capacity values for the sterols do not exist, except for cholesterol, the sublimation enthalpies of the sterols at 298 K were not calculated. Taking into account



**Figure 1.** Comparison of vapor pressures of  $\blacklozenge$ , cholesterol;  $\blacklozenge$ , ergosterol;  $\blacksquare$ ,  $\beta$ -sitosterol; and  $\Box$ , stigmasterol.

 Table 4. Vapor Pressure Correlation Parameters for Different

 Sterols Studied Using the Integrated Clausius-Clapeyron Equation

 Fit, Along with Temperature Range and Calculated Enthalpies of

 Sublimation

	temperature range	parameters for $\ln(p/Pa) = -A/(T/K) + B$		enthalpies
compound	T/K	Α	В	$\frac{\Delta_{\rm sub}H}{(\rm kJ\cdot mol^{-1})}$
cholesterol ergosterol 3-sitosterol stigmasterol	386 to 414 381 to 412 389 to 410 390 to 417	$\begin{array}{c} 17136 \pm 107 \\ 17686 \pm 109 \\ 17295 \pm 60 \\ 20254 \pm 163 \end{array}$	$\begin{array}{c} 39.88 \pm 0.27 \\ 40.61 \pm 0.27 \\ 39.70 \pm 0.15 \\ 46.31 \pm 0.41 \end{array}$	$\begin{array}{c} 142.5\pm0.89\\ 147\pm0.91\\ 143.8\pm0.5\\ 168.4\pm1.36 \end{array}$

polymorphic transition of cholesterol at 304.8 K, we derived from our results an enthalpy of about 156 kJ·mol<sup>-1</sup> at 298 K—extrapolated by using the corresponding equation and heat capacity values from Nichols et al.<sup>17</sup>—while Nichols et al.<sup>17</sup> report a value of (163.6  $\pm$ 4.4) kJ·mol<sup>-1</sup>. The sublimation enthalpy from this study (142.5 kJ·mol<sup>-1</sup>) for cholesterol also agrees well with the results from Hickman et al.,<sup>16</sup> where their vaporization enthalpy of 114.9 plus fusion enthalpy 29.9 kJ·mol<sup>-1</sup> (taken from Nichols et al.<sup>17</sup>) gives a sublimation enthalpy of 144.8 kJ·mol<sup>-1</sup>. It is necessary to point out that though vapor pressure measured by Hickman et al.<sup>16</sup> is in the temperature range bracketing the generally reported melting temperature region of cholesterol, it is reasonable to say that the estimated enthalpy corresponds to vaporization<sup>17</sup> rather than sublimation.

The structure of sterols reported in this study are presented in Figure 2, and they are derivatives of the perhydrocyclopentanophenanthrene ring system with one hydroxyl group at the C-3 position and an alkyl group of different chain lengths and degree of unsaturation at the C-17 position. The chain length and unsaturation of the alkyl groups, and also the endocyclic unsaturation, must be responsible for the variation of the vapor pressures and sublimation enthalpies. For example, it is obvious that the difference between cholesterol (MW = 386.65) and  $\beta$ -sitosterol (MW = 414.71) is the result of an increase in chain length of the alkyl substitution, whereas the difference between  $\beta$ -sitosterol (MW = 414.71) and stigmasterol (MW = 412.69) is due to the unsaturation in the alkyl side chain. While the exocylic double bond (stigmasterol vs  $\beta$ -sitosterol) lowers the vapor pressure, the effect of the endocyclic double bond is not clear. It is possible that the slight change in conformation of the B ring due to the additional endocyclic double bond may not have any significant impact on the vapor pressure so that ergosterol (MW = 396.65) has a higher vapor pressure than stigmasterol (MW = 412.69).





Ergosterol: C28H44O, MW 396.65

Cholesterol: C277H46O, MW 386.65





Stigmasterol: C29H48O, MW 412.69

β-Sitosterol: C<sub>29</sub>H<sub>50</sub>O, MW 414.71

Figure 2. Structures of sterols studied.

### Conclusion

This paper reports a new set of vapor pressure data for solid cholesterol, ergosterol,  $\beta$ -sitosterol, and stigmasterol. Previously available vapor pressure data were limited, and their use as physical-chemical properties in engineering calculations was uncertain.

No evidence of decomposition of the samples was observed under the vapor pressure measurement conditions reported here, confirming that these results should be representative of compounds studied.

## Acknowledgment

We thank Drs. Ping Li, Steve Haut, and Jan Wooten (Philip Morris USA Research Center) for their helpful discussion and assistance. We thank Anthony Brown, Yvonne Bezjak, Jennifer Ricketts, and Richard Seitz (Lancaster laboratory, c/o Philip Morris USA Research Center) for technical support. The authors are very grateful to Mr. B. Waymack and Dr. D. Kellogg for their assistance. Technical assistance by Dr. R. Malhotra from SRI International, who produced the FIMS data, is greatly appreciated.

#### **Literature Cited**

- Singh, H.; Yun, S. L. J.; Macnaughton, S. J.; Tomasko, D. L.; Foster, N. R. Solubility of cholesterol in supercritical ethane and binary gas mixture containing ethane. *Ind. Eng. Chem. Res.* **1993**, *32*, 2841–2848.
   Lin, T. Y.; Wang, Y. J.; Lai, P. Y.; Lee, F. J.; Cheng, J. T.-S.
- (2) Lin, T. Y.; Wang, Y. J.; Lai, P. Y.; Lee, F. J.; Cheng, J. T.-S. Cholesterol content of fried-shredded pork extracted by supercritical carbon dioxide. *Food Chem.* **1999**, *67*, 89–92.
- (3) Kosal, E.; Lee, C. H.; Holder, G. D. Solubility of progesterone, testosterone, and cholesterol in supercritical fluids. *J. Supercrit. Fluids* **1992**, *5*, 169–179.
- (4) Subra, P.; Berroy, P.; Vega, A.; Domingo, C. Process performance and characteristics of powders produced using supercritical CO<sub>2</sub> as solvent and antisolvent. *Powder Technol.* 2004, *142*, 13–22.
- (5) Wong, J. M.; Johnston, K. P. Solubilization of biomolecules in carbon dioxide based supercritical fluids. *Biotechnol. Prog.* **1986**, *2*, 29–39.
- (6) Valderrama, J. O.; Gonzalez, N. A.; Alvarez, V. H. Gas solid equilibrium in mixtures containing supercritical CO2 using a modified regular solution model. *Ind. Eng. Chem. Res.* 2003, 42, 3857–3864.
- (7) Yun, S. L. J.; Liong, K. K.; Gurdial, G. S.; Foster, N. R. Solubility of cholesterol in supercritical carbon dioxide. *Ind. Eng. Chem. Res.* 1991, 30, 2476–2482.
- (8) Chen, P.-C.; Chen, Y.-P.; Wong, D. S. H. Correlation of steroid solubilities in supercritical carbon dioxide. *Fluid Phase Equilib.* 1993, 83, 175–182.
- (9) Pakdel, H.; Roy, C. Separation and characterization of steroids in biomass vacuum pyrolysis oils. *Bioresour. Technol.* **1996**, *58*, 83–88.
- (10) Oja, V.; Hajaligol, R. H.; Waymack, B. E. The vaporization of semivolatile compounds during tobacco pyrolysis. J. Anal. Appl. Pyrolysis 2006, 76, 117–123.
- (11) Badger, G. M.; Donnelly, J. K.; Spotswood, T. M. The formation of aromatic hydrocarbons at high temperatures. XXIV. The pyrolysis of some tobacco constituents. *Aust. J. Chem.* **1965**, *18*, 1249.
- (12) Stevenson, R. F.; Schlozhauer, W. S.; Chortyk, O. T.; Arrendale, R. F.; Snook, M. E. Precursors of polynuclear aromatic hydrocarbons in

tobacco smoke. Polynuclear Aromatic Hydrocarbons. Ann Arbor, MI: Ann Arbor Science, 1979, 277.

- (13) Shin, E.; Nimlos, M. R.; Evans, R. J. The formation of aromatics from the gas-phase pyrolysis of stigmasterol: kinetics. *Fuel* 2001, 80, 1681–1687.
- (14) Britt, P. F.; Buchanan, A. C.; Kidder, M. M.; Owens, C.; Ammann, J. R.; Skeen, J. T.; Luo, L. Mechanistic investigation into the formation of polycyclic aromatic hydrocarbons from the pyrolysis of plant steroids. *Fuel* **2001**, *80*, 1727–1746.
- (15) Oja, V.; Hajaligol, M. R. The volatility of tars from pyrolysis of biomass materials. In *Progress in Thermochemical Biomass Conversion*; Bridgewater, A. B., Ed.; 2001; Vol. 2, pp 1226–1233.
- (16) Hickman, K. C. D.; Hecker, J. C.; Embree, N. D. Direct determination of low vapor pressures. *Ind. Eng. Chem., Anal. Edition* **1937**, *9*, 264–7.
- (17) Nichols, G.; Kweskin, S.; Frericks, M.; Reiter, S.; Wang, G.; Orf, J.; Carvallo, B.; Hillesheim, D.; Chickos, J. Evaluation of the vaporization, fusion, and sublimation enthalpies of the 1-alkanols: the vaporization enthalpy of 1-, 6-, 7-, and 9-heptadecanol, 1-octadecanol, 1-eicosanol, 1-docosanol, 1-hexa-cosanol, and cholesterol at *T*=298.15 K by correlation gas chromatography. *J. Chem. Eng. Data* **2006**, *51*, 475–482.
- (18) Knudsen, M. Effusion and the molecular flow of gases through openings. *Ann. Phys.* **1909**, *28*, 999–1016.
- (19) Knudsen, M. Experimental determination of vapor pressure of mercury at 0° and higher temperatures. Ann. Phys. 1909, 29, 179–193.
- (20) Murray, J. J.; Pottie, R. F.; Pupp, C. The vapor pressures and enthalpies of sublimation of five polycyclic aromatic hydrocarbons. *Can. J. Chem.* **1974**, *52*, 557–563.
- (21) Kaisersberger, E.; Hadrich, W.; Emmerich, W.-D. Measurement of low vapor pressures according to the Knudsen effusion method. *Thermochim. Acta* **1985**, *95*, 331–336.
- (22) Mathews, C. K.; Baba, M. S.; Narasimhan, L. Vapor pressure and enthalpy of sublimation of C<sub>70</sub>. Fullerene Sci. Technol. **1993**, 1, 101–109.
- (23) Oja, V.; Suuberg, E. M. Development of a non-isothermal Knudsen effusion method and application to PAH and cellulose tar vapor pressure measurement. *Anal. Chem.* **1997**, *69*, 4619–4626.
- (24) Ribeiro da Silva, M. A. V.; Monte, M. J. S.; Santos, L. M. N. B. F. The design, construction, and testing of a new Knudsen effusion apparatus. J. Chem. Thermodyn. 2006, 38, 778–787.
- (25) Oja, V.; Suuberg, E. M. Vapor pressures and enthalpies of sublimation of D-glucose, D-xylose, cellobiose, and levoglucosan. J. Chem. Eng. Data 1999, 44, 26–29.
- (26) Shim, H.-S.; Oja, V.; Hajaligol, M. R. Vapor pressure measurements of tobacco pyrolysis tar by a non-isothermal Knudsen effusion method. *J. Anal. Appl. Pyrolysis* 2003, *66*, 183–190.
- (27) Chen, X.; Oja, V.; Chan, W. G.; Hajaligol, M. R. Vapor pressure characterization of several phenolics and polyhydric compounds by Knudsen effusion method. J. Chem. Eng. Data 2006, 51, 386–391.
- (28) Hansen, P. C.; Eckert, C. A. An improved transpiration method for the measurement of very low vapor pressures. *J. Chem. Eng. Data* **1986**, *31*, 1–3.
- (29) Motzfeldt, K. The thermal decomposition of sodium carbonate by the effusion method. J. Phys. Chem. 1955, 59, 139.
- (30) Zaitsau, D. H.; Verevkin, S. P.; Paulechka, Y. U.; Kabo, G. J.; Servuk, V. M. Comprehensive study of vapor pressures and enthalpies of vaporization of cyclohexyl esters. *J. Chem. Eng. Data* **2003**, *48*, 1393–1400.
- (31) Carlson, K. D.; Gilles, P. W.; Thorn, R. J. Molecular and hydrodynamical effusion of mercury vapor from Knudsen cells. *J. Chem. Phys.* 1963, 38, 2064.
- (32) Ward, J. W.; Fraser, M. V. Some of the parameters affecting Knudsen effusion. IV. Monte Carlo calculations of effusion probabilities and flux gradients for Knudsen cells. J. Chem. Phys. **1968**, 49, 3743.
- (33) Ribeiro da Silva, M. A. V.; Monte, M. J. S. The construction, testing and use of a new Knudsen effusion apparatus. *Thermochim. Acta* 1990, *171*, 169–183.
- (34) Malhotra, R.; McMillen, D. F.; Watson, E. L.; Huestis, D. L. Characterization of coal liquefaction resins by field ionization mass spectrometry: correlating spectral features with processing parameters. *Energy Fuels* **1993**, *7*, 1079–1087.
- (35) Petropavlov, N. N.; Tsygankova, I. G.; Teslenko, L. A. Microcalorimetric investigation of polymorphic transitions in organic crystals. *Sov. Phys. Crystallogr.* **1988**, *33*, 853–855.
- (36) Smith, L. L.; Lulig, M. J.; Teng, J. I. Sterol metabolism. XXVI. Pyrolysis of some sterol allylic alcohols and hydroperoxides. *Steroids* 1973, 22, 627–635.
- (37) Gassiot-Matas, M.; Juliá-Danés, E. Pyrolysis gas chromatography of some sterols. *Chromatographia* 1972, 5, 493–501.

Received for review June 2, 2008. Accepted October 30, 2008. V. Oja appreciates the support of Estonian Scientific Foundation Grant G7222.

JE800395M