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AMES LABORATORY

RESEARCH AND DEVELOPMENT REPORT

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ABSTRACT

The vapor pressures of several amino acids have been determined by the Knudsen cell effusion method. The standard thermodynamic quantities for the heat of sublimation, the entropy of sublimation and the free energy of sublimation are calculated.

The sensitivity of the mass spectrometer is calculated for an amino acid from its vapor pressure, its ionization cross-section and its intensity of the $(P\text{-}COOH)^+$ ion currents relative to the total ion intensity.

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*This report is based on an M. S. thesis submitted by Dale Dean Clyde November, 1963, to Iowa State University, Ames, Iowa.

Errata

Dale Dean Clyde and Harry Svec, "Vapor Pressures of Some Amino Acids", USAEC Rept. IS-790 (1963).

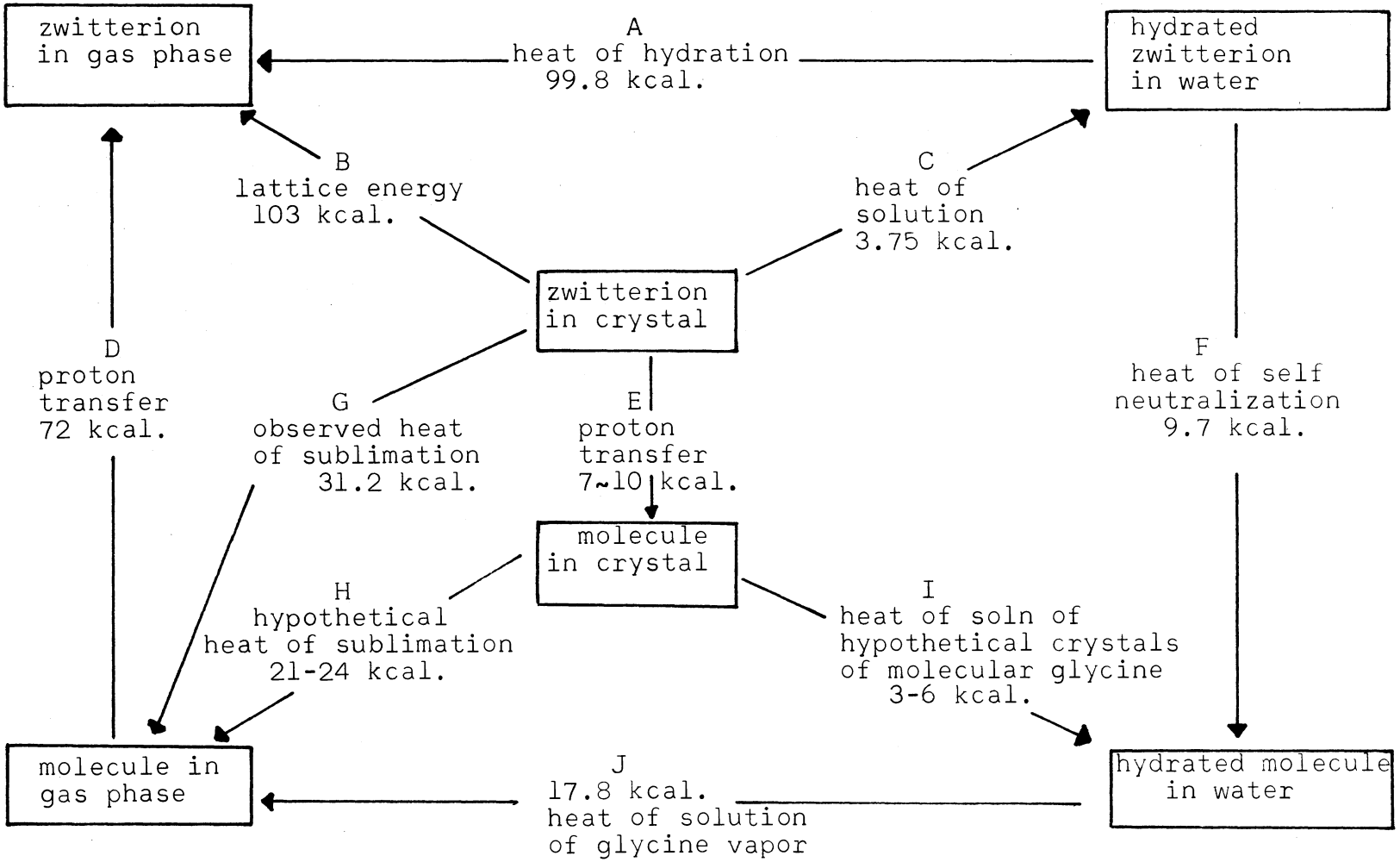
p. 19, Table 1. - The second column heading should read
"Pressure (Torr)
$\times 10^3$"
instead of "Pressure (Torr)"

INTRODUCTION

The relationship of the vapor pressure of crystalline compounds to their structure makes the study of the sublimation process of considerable practical and theoretical interest. Interpretation of the vapor pressure data can suggest the relative energy required for molecules or ions to break crystal bonds during the sublimation process. The vapor pressures of several amino acids have been measured and their heats of sublimation and other thermochemical properties have been calculated. The presentation of these data with a discussion of the results is the purpose of this report.

With the exception of glycine, values for the heats of sublimation or the vapor pressure of the amino acids have not been reported in the literature. At most, only time intervals required to sublime an approximate amount of acid have been recorded over a temperature range of ten degrees (1).

In 1961, Takagi, Chihara and Seki (2) published values for the vapor pressure of glycine at various temperatures and its heat of sublimation. They were interested in the structural form of glycine in the vapor and the solid phases. Realizing the existence of the zwitterion, a dipolar ion characteristic of the amino acids, they considered possible ionic or molecular species present in glycine vapor. As an aid in determining the correct processes, these workers proposed the energy cycle shown in Figure 1.



Energy-cycle of glycine (in kcal./mole) arrows indicate the direction of endothermic changes.

Figure 1. Heat of solution of hypothetical crystals of molecular glycine

The values for A (3) and C (4) are obtained from published experimental results. The lattice energy (B) is based on Kirkwood's theory using heats of solution and partial molal volumes of solutions with varying dielectric constants. A value of 21-24 kcal. for H is based on van der Waals' force energies and hydrogen bond energies. Takagi has determined the value for G.

The process of self-neutralization, F, in solution is evaluated from two reactions which are

$$H_3N^+CH_2COO^- + H^+ = H_3N^+CH_2OOH - \Delta H_a$$

and

$$H_3N^+CH_2COOH = H_2NCH_2COOH + H^+ + \Delta H_b.$$

The value of ΔH_a is determined from the temperature variation of pK_a, giving 1.16 kcal.-mole^{-1}. The heat change associated with the reaction

$$H_3N^+CH_2COO^- = H_2NCH_2COO^- + H^+$$

is 10.8 kcal.-mole^{-1} which is approximately equal to ΔH_b. The heat of self-neutralization is then 9.7 kcal.-mole^{-1}.

The remaining processes D, E, J and I are calculated from the completion of each energy cycle. These values are 72 kcal., 7-10 kcal., 3-6 kcal., and 17.8 kcal., respectively.

From the above information Takagi et al. (2) has been able to make the following conclusions:

1. If the sublimation does not accompany a proton transfer, the lattice energy would be the energy necessary for sublimation. Since the estimated lattice energy is larger

than the calculated heat of sublimation, Takagi concluded that glycine sublimes with the molecular configuration. The observation (5) of the absence of the zwitterion in the vapor by means of mass spectrometry confirms this conclusion.

2. The energy required to transfer a proton within the crystal is D of Figure 1. The energy required to transfer a proton within a molecule in glycine vapor is E. The process requiring the least amount of energy, and therefore the more likely to occur, is the transformation from a zwitterion to a molecule within the crystal before sublimation takes place.

Dr. H. J. Svec and Greg Junk (6) have developed a mass spectrometric method for the quantitative analysis of the amino acids in which the instrumental sensitivity for different amino acids is obtained empirically. In order to analyze a mixture of amino acids, the sample must be vaporized in the mass spectrometer. The relative amounts of the amino acids in the vaporized sample depends on their vapor pressures. An analyst using this procedure could be more selective in his analysis if he knew the vapor pressures of a variety of amino acids over a range of temperature. One of the purposes for this research then was to increase the usefulness and potential of this analytical method by providing some needed vapor pressure data.

THEORY AND METHOD

Due to the strong bonding of the dipolar ions present in solid amino acids, vapor pressures are low and the heats of sublimation are much higher than those for corresponding analogous straight chain aliphatic acids. As a result a special apparatus and technique must be used to measure the vapor pressures. A procedure capable of measuring low vapor pressures is the Knudsen effusion method which has been used for this research.

At the beginning of the twentieth century, Knudsen (7) noted a direct relationship between the vapor pressure of a sample and the weight lost during a measured interval of time. The molecules of the sample passed through a hole of known area in a vessel containing vapor in equilibrium with the solid or liquid sample. By assuming that the Maxwellian velocity distribution of the molecules can be averaged, he expressed the number of molecules striking an area of wall equal to the area of the orifice by

$$\sigma = 1/4 \; Z \; \bar{c} \qquad (1)$$

where Z = the number of molecules per cm^3 and $\bar{c}$ = average mean velocity. From the equation for a perfect gas, $PV = \frac{NRT}{A}$, (2) Z can be calculated according to $Z = \frac{N}{V} = \frac{AP}{RT}$ = the number of molecules per cm^3, (3) where N = number of molecules of the gas and A = Avogadro's number.

The total number of molecules (8) effectively leaving

a surface was considered equal to the difference between the number leaving ($\sigma_{1\rightarrow 2}$) and the number returning ($\sigma_{2\rightarrow 1}$) to the solid phase or

$$\sigma_{1\rightarrow 2} - \sigma_{2\rightarrow 1} = 1/4\ A\ (\frac{P_1\bar{c}}{RT} - \frac{P_2\bar{c}}{RT}). \quad (4)$$

The pressure P_1 on one side of the orifice is lower than the pressure P_2 on the other side of the orifice such that the mean free paths of the molecules are at least ten times the orifice diameter.

If the effusion vessel is placed in a vacuum, then $P_2 = 0$ and

$$\sigma_{1\rightarrow 2} = \frac{A}{4}\ \frac{P_1\bar{c}}{RT} \quad (5)$$

From the Maxwell-Boltzmann distribution of velocities, the arithmetical average velocity is

$$\bar{c} = (\frac{8RT}{\pi M})^{1/2} \quad (6)$$

and

$$\sigma_{1\rightarrow 2} = \frac{AP}{4RT}\ (\frac{8RT}{\pi M})^{1/2} \quad (7)$$

where M = molecular weight of the gas.

It follows that the amount leaving the surface can be expressed as

$$m = \sigma_{1\rightarrow 2}\ \frac{M}{A} \quad (8)$$

Substituting Equation (7) in (8) gives

$$m = P(\frac{M}{2\pi RT})^{1/2} \quad (9)$$

and

$$P = m\ (\frac{2\pi RT}{M})^{1/2} = \frac{w}{at}\ (\frac{2\pi RT}{M})^{1/2} \quad (10)$$

For $R = 8.314 \times 10^7$ erg-deg^{-1}-mole^{-1}, Equation (10) becomes

$$P = (2.286 \times 10^4)\frac{w}{at}\sqrt{\frac{T}{M}}\ \frac{\text{dynes}}{\text{cm}^2} \qquad (11)$$

where

a = area in cm^2,
t = time in seconds,
w = weight lost in grams,
T = temperature in absolute degrees and
M = molecular weight.

Converting to observable units gives

$$P = 17.14\ \frac{w}{at}\sqrt{\frac{T}{M}}\ \text{mm.} \qquad (12)$$

Clausing (9) realized subsequently that if the hole were longer than the mean free path of a molecule, some of the molecules would collide with the inside wall of the effusing hole. This meant that some of the molecules could be reflected back into the cell. Thus a factor to correct this effect was needed. The equation for the calculation of vapor pressures became,

$$P = \frac{17.14}{W_o}\ \frac{w}{at}\ \sqrt{\frac{T}{M}} \qquad (13)$$

where W_o is the Clausing factor.

The factor which he introduced was experimentally shown to be a function of the radius of the cylinder through which the molecules traveled and thus the Clausing factor in Equation (13) is the correction factor for the effusion orifice. Following the work of Clausing, Demarcus (10) theoretically calculated more accurate values for the Clausing factors.

The ideal equation developed above represents a larger

number of molecules than actually escape through real holes. Only when the cell has no opening, does a true equilibrium pressure exist. In reality the equation given by Knudsen represents a steady state pressure. Speiser (11) considered a material balance for the sublimation of a sample from a Knudsen cell. The rate of vaporization equals the summation on the rate of condensation and the rate of effusion. From the kinetic theory of gases, the following relationships can be derived:

$$R_c = \alpha P_{ss} (2\pi MRT)^{-1/2} H_s A, \tag{14}$$

$$R_v = \alpha P_{eq} (2\pi MRT)^{-1/2} H_s A \text{ and} \tag{15}$$

$$R_e = P_o (2\pi MRT)^{-1/2} H_o A \tag{16}$$

where

H_s = area of the sample
H_o = area of the orifice
α = vaporization coefficient
P_o = pressure at the orifice
P_{ss} = steady state pressure
R_c = rate of condensation
R_v = rate of vaporization
R_e = rate of effusion
A = Avogadro's number
P_{eq} = equilibrium pressure.

From the equation accounting for a material balance,

$$R_v = R_c + R_e, \tag{17}$$

P_{eq} can be obtained and becomes

$$P_{eq} = P_{ss} + \frac{P_o H_o}{\alpha H_s} . \tag{18}$$

Whitman (12) was the first to apply a Clausing factor to correct for the shape of the cell in the vaporization process. Later Motzfeldt(13) developed a more rigorous

equation

$$P_{ss} = \frac{P_{eq}}{1 + \frac{W_o' H_o}{H_s} \left(\frac{1}{\alpha} + \frac{1}{W_a'} - 2\right)} \qquad (19)$$

for the steady state pressure P_{ss} where W_o' and W_a' are Clausing factors for the orifice and the cell, respectively. If the depth of the cell is as long as the diameter of the cell, the Clausing factor for the cell is approximately 0.5. If $\alpha = 1$, then the equation for the measured steady state pressure becomes equivalent to Speiser's expression.

APPARATUS

The apparatus used for the study of the vapor pressure of the amino acids consisted of a fused quartz microbalance by which weight-loss measurements were made. Figure 2 shows a schematic diagram of the apparatus. The microbalance was patterned after the one described by Defayette (14) except that the one used in this study has greater capacity with a resulting lower sensitivity. Free use was made of the work of Edwards and Baldwin (15) who discuss the construction of microbalances and their operation. The balance (shown at A in Figure 2) was made from a 4 mm quartz tube approximately 20 cm long, trussed with a quartz rod. Quartz fibers were pulled from stubs on each side of the main beam at its center and fused to a supporting quartz frame. A piece of Cunife (General Electric Co., Schenectady, N. Y.) rod was machined to fit in the center of the main beam. After the metal rod was sealed in place by pieces of quartz rod fused to the main beam, it was magnetized by a force of 200,000 ampere turns. Quartz hooks were placed on each end of the beam and a quartz fiber was drawn from one end of the beam to serve as an index.

The balance which behaves like a tangent galvanometer is operated by a passing current through the two solenoids shown at B in the figure. A variation of the current changes the electromagnetic field perpendicular to the Cunife magnet and thus the orientation of the balance beam is controlled.

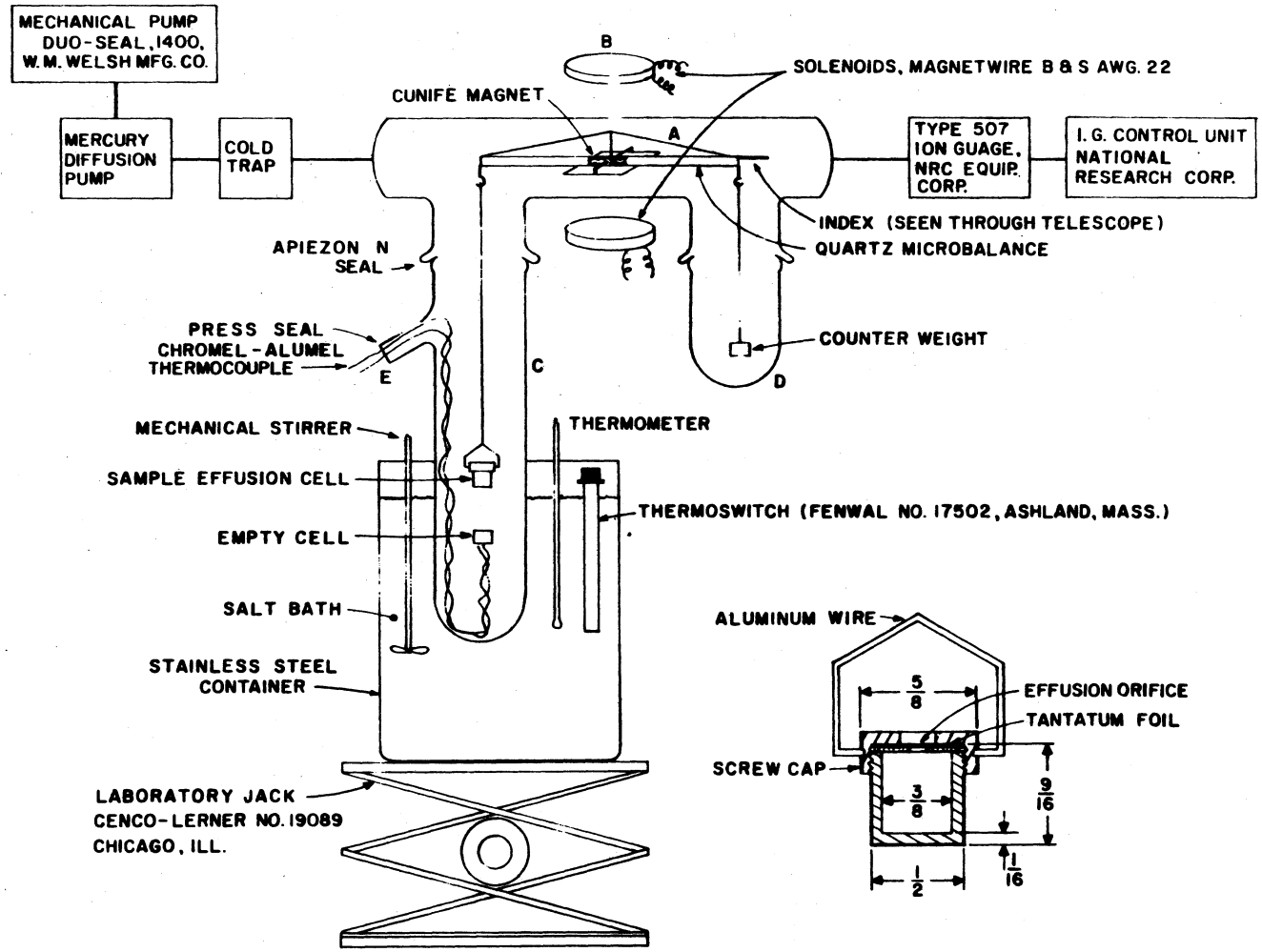
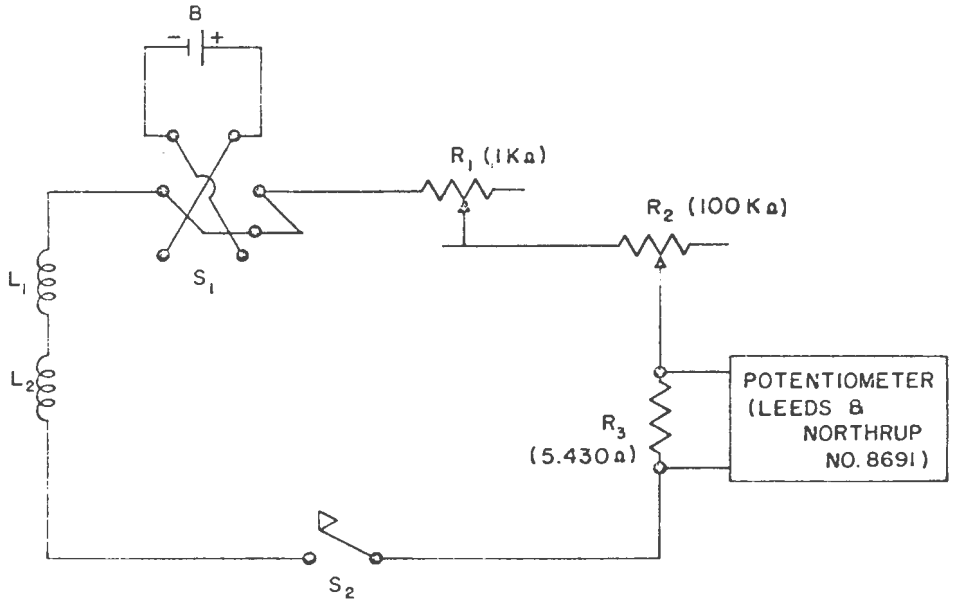


Figure 2. Gravimetric apparatus for studying the vapor pressures of several amino acids

The solenoid current is related directly to weight differences. Each coil was wound on a brass spool 15 cm in diameter and 3 cm wide and consisted of approximately 1100 turns of Formvar insulated AWG 22 copper wire. The circuit was connected as shown in Figure 3. The coils were connected in series with helical variable resistors, four six-volt batteries, a constantan wire resistor and the circuit polarity was changeable by means of a polarity reversing switch. An accurate measurement of the current flowing through the coils was made by measuring the IR drop across the manganin resistor with a potentiometer.

The balance was used as a null instrument. A telescope with ocular cross hairs (Gaertner Scientific Co.) allowed the observation of the quartz index extending from one end of the microbalance. All weighings were made by adjusting the microbalance index to coincide with the point of intersection of the ocular cross hairs. The sample and the counterweights were suspended from the ends of the balance beam at A and B. The balance was calibrated by observing the change in the coil current required to restore the balance to the null position after a precision weight was added to one side of the balance under its working load. The weights used for the calibration of the quartz microbalance were made of platinum and aluminum wire and were accurately weighed on a conventional balance (Mettler Type H16). A plot of millivolts (potentiometer readings of the IR drop across the precision resistor) versus



L_1 AND L_2 ARE SOLENOIDS (B&S AWG 22 MAGNETWIRE).

S_1 IS A DPDT SWITCH.

S_2 IS A SPST SWITCH.

R_1 AND R_2 ARE 10 TURN VARIBLE RESISTORS (HELIPOT, BECKMAN INSTRUMENT INC.).

R_3 IS A PRECISION RESISTOR CONSTANTAN WIRE.

B IS A UNIT OF FOUR SIX-VOLT STORAGE BATTERIES CONNECTED IN SERIES.

Figure 3. Circuit diagram used to control micro balance

milligrams gave a straight line for weights of 0 to 50 mg. The sensitivity of the balance was calculated from the slope by the method of least squares. Since the beam of the microbalance is suspended from the basic frame by very small quartz fibers, the load was limited and changes in the position of the balance had to be made slowly and carefully. On one occasion, the balance was accidentally given a sudden jolt during its operation and was broken. It was repaired and recalibrated. The sensitivity of the original balance and of the repaired balance were 0.075 $\pm$ 0.009 mg-mv^{-1} and 0.072 $\pm$ 0.008 mg-mv$^{-1}\pm$, respectively. These two values appear in the record of this work which appears in the author's research notebook.

A thermostated salt bath was used to heat tube C of Figure 2. The salt bath container was made from a stainless steel cylinder six inches in diameter and twenty-two inches long which was wrapped with a layer of asbestos followed by approximately forty turns of resistance wire (Chromel A, AWG 20, Hoskins Manufacturing Co., Detroit) which acts as a heater. Another layer of asbestos was placed over the wires and the container was further insulated with a layer of fiber glass pipe insulation. A 0-130 volt variable autotransformer (Superior Electric Co., Bristol, Conn.) and a thermoswitch (Fenwal no. 17502, Ashland, Mass.) were connected in series with the heater. The bath mixture (16) consisted of 18 weight per cent $NaNO_3$, 30 weight per cent $LiNO_3$, and 52 weight per

cent KNO_3 which has an eutectic freezing point at 120°C. A mechanical stirrer and a mercury thermometer were also placed in the bath. A laboratory jack (Cenco-Lerner no. 19089, Chicago, Ill.) was used to adjust the height of the bath around the tube in which the effusion cell was suspended.

A chromel-alumel thermocouple was used to measure the temperature in tube C. The thermocouple wires were introduced through the side of the tube at E and extended along the inside wall of the tube to the bottom as are shown in Figure 2. The fused junction of the thermocouple was located directly below the suspended effusion vessel. During the operation of the apparatus, a blackened effusion cell was placed upside down over the thermocouple junction. The wires leading from the hot junction were connected in series with a cold junction kept at 0°C and a potentiometer.

A mercury diffusion pump and a liquid-N_2 cooled trap, backed by a rotary mechanical pump maintained a sufficiently low pressure within the system. An ionization guage with an electronic guage control unit indicated the pressure in the apparatus. All joints were sealed with Apiezon W vacuum wax except the joint of tube C. Apiezon N was used for this joint since this made it more convenient to introduce samples into the system.

The conditions necessary for molecular flow were satisfied in the design of the effusion cell used in this work

(see detail in Figure 2). The effusion aperture measured 0.32 mm in diameter. At a pressure of 10^{-3} Torr, this is less than ten times the shortest mean free path calculated for the largest amino acid molecule studied. A microdrill was used to bore the hole in a tantalum disc, 0.05 mm thick. After the hole was polished with Crocus cloth, its size was measured with a metallographic microscope fitted with a filar eyepiece.

The effusion vessels (see detail in Figure 2) were made from one half-inch diameter aluminum rod and were one inch long. A concentric hole 3/8 inch in diameter and 9/16 inch deep was drilled in the rod. With a sample packed in the vessel, the free space of the cell was approximately as long as the inside diameter of the vessel. To insure good heating by radiation from the furnace, each container was painted with Aquadag (Acheson Colloid Corp., Port Huron, Mich.) and baked. The tantalum disc with the aperture was firmly held in place by means of a screw cap designed to fit the top of the cell. The screw cap had a large enough hole in its center so that the effusion aperture was not covered.

After the effusion vessel was filled with an amino acid and placed inside the vacuum apparatus which was then pumped down to a pressure of 5×10^{-4} Torr or less, the salt bath was raised and the stirrer started. In thirty to forty-five minutes the temperature of the effusion vessel reached equilib-

rium as indicated by the thermocouple and the bath thermometer. The variable resistors in the balance circuit were adjusted until the balance was at the null position. Balance readings were recorded at fifteen minute to one hour intervals, depending upon the volatility of the sample. Timing was by means of an electrical timer (Lab-Chron 1501). The bath stirrer had to be stopped during the time of each reading in order to avoid vibration of the balance. Several readings at several different temperatures were made during a single run.

EXPERIMENTAL RESULTS

Data for the amino acids studied are tabulated in Table 1. The values for log P were plotted against reciprocal absolute temperatures. This gave a straight line according to the equation

$$\log P = -\frac{A}{T} + B \qquad (20)$$

where A is the slope and B is the intercept. Equations for the best line fitting the data were obtained by the least squares method and are listed in Table 2. The uncertainties in the slope and the intercept were also calculated by the least squares method.

The equation used in the calculation of the standard thermodynamic quantities of sublimation is

$$\Delta F^{o} = \Delta H^{o} - T\Delta S^{o} = -RT \ln P(atm) \qquad (21)$$

or

$$\Delta F^{o} = -2.303\ RT\ [\log P(mm) - \log 760] . \qquad (22)$$

Substitution of Equation (20) for log P gives

$$\Delta F^{o} = -2.303\ RT\ [-\frac{A}{T} + B - \log 760]. \qquad (23)$$

The rearranged equation is

$$\Delta F^{o} = 4.576\ A - 4.576\ [B - 2.881]T . \qquad (24)$$

Comparison of Equation (24) with Equation (21) shows that

$$\Delta F^{o} = 4.576\ A \text{ and}$$

$$\Delta S^{o} = 4.576\ [B - 2.881].$$

The standard thermodynamic quantities calculated for

Table 1. Vapor pressure of some amino acids

Temp. ^{o}k	Pressure (Torr)	$1/T \times 10^{-3}$	+ log P
	Glycine		
453	0.0587	2.2075	1.23136
457	0.0859	2.1882	1.06601
466	0.159	2.1459	0.79860
471	0.243	2.1231	0.61439
	l-alanine		
453	0.0759	2.2077	1.11976
460	0.122	2.1741	0.91364
465	0.203	2.1496	0.69250
469	0.258	2.1324	0.58840
	l-α-amino-n-butyric acid		
449	0.0972	2.2274	1.01233
452	0.1290	2.2102	0.88941
455	0.163	2.1980	0.77781
462	0.360	2.1624	0.48149
	dl-norvaline		
439	0.0404	2.2769	1.39362
446	0.0664	2.2422	1.17783
452	0.1010	2.2124	0.99568
461	0.1930	2.1692	0.71443
	l-valine		
438	0.0395	2.2857	1.40340
444	0.0682	2.2548	1.16622
448	0.103	2.2346	0.98716
452	0.150	2.2124	0.82391
456	0.233	2.1930	0.63264
	l-leucine		
464	0.216	2.1552	0.66555
454	0.0936	2.2026	1.02872
452	0.0844	2.2124	1.07366
446	0.0440	2.2422	1.35655
	l-methionine		
463	0.0384	2.1598	1.41567
472	0.0622	2.1186	1.20621
478	0.105	2.0920	0.97881
485	0.163	2.0619	0.78781

Table 1. (Continued)

Temp. ^{o}k	Pressure (Torr)	$1/T \times 10^{-3}$	+ log P
	l-phenylalanine		
451	0.0252	2.2173	1.59860
457	0.0463	2.1888	1.33630
463	0.0758	2.1598	1.12133
469	0.119	2.1322	0.92445
	l-proline		
442	0.0675	2.2655	1.17070
448	0.107	2.2321	0.97062
451	0.171	2.2173	0.76700
457	0.210	2.1882	0.67778
465	0.307	2.1487	0.51286
467	0.299	2.1487	0.52433
	dl-norleucine		
435	0.0190	2.2999	1.72125
449	0.0576	2.2272	1.23958
461	0.129	2.1692	0.88941
469	0.184	2.1313	0.73518
	isoleucine		
442	0.0763	2.2599	1.11748
448	0.106	2.2321	0.97469
453	0.172	2.2075	0.76447
456	0.209	2.1930	0.67985
461	0.262	2.1692	0.58170
	cycloleucine		
443	0.0666	2.2573	1.17653
450	0.112	2.2222	0.95078
456	0.166	2.1930	0.77989
462	0.269	2.1645	0.57025
468	0.351	2.1368	0.45469
	α-amino isobutyric acid		
462	0.451	2.1691	0.34582
452	0.218	2.2126	0.66154
441	0.108	2.2691	0.96658
439	0.078	2.2795	1.10791

Table 2. Vapor pressure-temperature relationships for some amino acids

glycine	$+\log P = -\frac{(7.12 \pm .215)}{T} + (14.47 \pm 0.46)$
L-alanine	$\log P = -\frac{(7.22 \pm 0.47)}{T} + (14.81 \pm 1.02)$
L-α-amino-n-butyric acid	$\log P = -\frac{(8.49 \pm 0.39)}{T} + (17.86 \pm 0.86)$
DL-norvaline	$\log P = -\frac{(6.27 \pm 0.08)}{T} + (12.89 \pm 0.55)$
DL-leucine	$\log P = -\frac{(5.98 \pm 0.34)}{T} + (12.05 \pm 0.66)$
isoleucine	$\log P = -\frac{(6.27 \pm 0.45)}{T} + (13.05 \pm 2.25)$
cycloleucine	$\log P = -\frac{(6.44 \pm 0.19)}{T} + (13.36 \pm 0.61)$
α-amino isobutyric acid	$\log P = -(\frac{6.57 \pm 0.28}{T}) + (13.90 \pm 0.28)$
L-valine	$\log P = -\frac{(8.49 \pm 0.36)}{T} + (17.99 \pm 0.81)$
L-leucine	$\log P = -\frac{(7.86 \pm 0.42)}{T} + (16.28 \pm 0.93)$
L-methionine	$\log P = -\frac{(6.53 \pm 0.46)}{T} + (12.67 \pm 0.98)$
L-phenylalanine	$\log P = -\frac{(8.04 \pm 0.37)}{T} + (16.22 \pm 0.81)$
L-proline	$\log P = -\frac{(5.04 \pm 0.40)}{T} + (10.32 \pm 0.88)$

Table 3. Standard thermodynamic quantities

	Takagi (2)	this research
heat of sublimation	31.18 ± 0.49 kcal/mole	32.6 ± 1.0
entropy of sublimation	49.33 ± 1.17 cal/deg-mole	53.0 ± 2.2
free energy of sublimation	16.47 ± 0.60 kcal/mole	16.8 ± 1.2

glycine agree with the values reported by Takagi *et al.* within experimental error. These values are given in Table 3 along with the values obtained in this work. The same thernodynamic quantities have been calculated for all of the amino acids studied and are tabulated in Table 4.

L-glutamic acid, L-cystine, L-tyrosine, L-tryptophan and L-serine were not stable enough to determine their heats of sublimation in the temperature range of 150° to 200°C. No attempts were made at lower temperatures.

Table 4. Standard thermodynamic quantities of some amino acids

	ΔS^o cal/deg-mole	ΔF^o kcal/mole	ΔH^o kcal/mole	Density gm/cm^3	Solubility(17) at 25°C gm/100 gm of H_2O
Glycine	53.0 ± 2.2	16.8 ± 1.2	32.6 ± 1.0	1.601	24.99
L-alanine	54.6 ± 4.7	18.1 ± 2.6	33.0 ± 2.2	1.401	16.65
L-α-amino-n-butyric acid	68.6 ± 3.9	18.4 ± 2.1	38.9 ± 1.8	1.231	18.56 20°C
DL-norvaline	45.8 ± 2.5	15.0 ± 0.9	28.7 ± 0.4	1.316	7.09
DL-leucine	42.0 ± 3.0	14.8 ± 1.8	27.4 ± 1.6	1.191	0.991
isoleucine	46.5 ±10.3	14.8 ± 3.7	28.7 ± 2.1	--	--
cycloleucine	48.0 ± 2.8	15.2 ± 1.2	29.5 ± 0.9	--	--
α-amino iso-butyric acid	50.4 ± 1.3	15.0 ± 1.4	30.1 ± 1.3	--	--
L-valine	69.1 ± 3.7	18.2 ± 1.9	38.9 ± 1.6	--	--
L-leucine	61.3 ± 4.3	17.7 ± 2.3	36.0 ± 1.9	--	--
L-methionine	44.8 ± 4.5	16.5 ± 2.5	29.9 ± 2.1	1.278	13.71 20°C
L-phenylalanine	61.0 ± 3.7	18.6 ± 2.0	36.8 ± 1.7	1.230	8.85
L-proline	34.0 ± 4.0	12.9 ± 2.2	23.1 ± 1.8	1.165	2.426

DISCUSSION OF ERRORS

According to Phipps, Seifert, Simpson and coworkers (17), the errors occurring in the calculated vapor pressure can be divided into four types--inherent, systematic, statistic and basic errors.

Inherent errors are due to an incomplete knowledge of some quantity not measured in the experiment. Such errors are of concern in Avogadro's number, the molecular weight of the compound and the gas constant. Since the values for these constants are considered correct, the error introduced by them is insignificant.

Systematic errors represent the uncertainty in a measured quantity in a nonrandom manner. Errors of this type involved in this research are (1) in the measurement of the orifice area, (2) in the weights used to calibrate the balance and to determine the conversion factor, (3) the Clausing factor, and (4) in the thermocouple calibration. In addition a systematic error occurs when the measured vapor pressure is assumed to equal the true equilibrium pressure. Use of the equation given by Motzfeldt (13)

$$P_{ss} = \frac{P_{eq}}{1 + \frac{W_o' H_o}{H_s} \left(\frac{1}{\alpha} + \frac{1}{W_\alpha'} - 2\right)} \qquad (22)$$

reveals the magnitude of this error. The diameter of the cell is approximately 0.5. For an orifice of the size used in this

research, the evaporation coefficient can be estimated to be nearly equal to one (19). Thus the equation becomes

$$P_{ss} = \frac{P_{eq}}{1 + \frac{W_o H_o}{H_s}} \tag{23}$$

Since the ratio of the areas is 0.0015, and the product of this ratio and H_o is insignificant, the equilibrium pressure can be assumed to be the same as the measured vapor pressure.

Statistical errors represent the uncertainty in the measured quantity in a random manner. These include errors in the time, the temperature, the Clausing factor, the area and the loss in weight of the sample. The estimated uncertainty in reading the time is 0.03 of a minute or about 2 seconds. Since all of the time intervals were greater than 10 minutes, the maximum error in the time is $\pm$ 0.3%. The error in the temperature is determined by the error in reading the potentiometer and by the error in the spread of the temperature during the experiment.

$$\sigma_{temp} = [\sigma^2_{pot} + \sigma^2_{range}]^{1/2} \tag{24}$$

The maximum spread in the temperature during an experiment corresponded to a potentiometer reading of $\pm$ 0.04 mv or $\pm$ 1°C for the Chromel-Alumel thermocouple. This corresponds to an error of $\pm$ 0.5%. The error in reading the potentiometer was $\pm$ 0.005 mv which was negligible compared to the temperature spread; thus, the total error in the temperature becomes ~0.5%.

The uncertainty in setting the balance during each

reading was estimated to be ± 0.04 mg. Since two readings were taken for each weight-loss interval, the total uncertainty becomes ± 0.08 mg. The error introduced in the vapor pressure is not uniform because the weight varies; however, most of the samples lost over 2 milligrams. The estimated error in the vapor pressure is estimated to be ± 4%.

The Clausing factor was determined by extrapolation of the data given by Demarcus (10). The error in this value depends upon the error in the ratio L/R and the error in reading the graph.

$$\sigma_{c.f.} = [\sigma_{gr}^2 + \sigma_{L/R}^2] \qquad (25)$$

The micrometer used to measure the thickness of the tantalum foil was calibrated against a set of standard guages. Its standard deviation was calculated to be ± 0.0006 mm. From this value the calculated per cent error in the ratio (L/R) is ± 1.0%.

The error in reading the value from the graph used to obtain the Clausing factor is estimated to be ± 0.0003, or a ± 0.04% error. Therefore, there is a total error of ± 1% in the value of this factor.

The uncertainty in the measurement of the diameter of the orifice is ± 0.002 mm. which corresponds to an error of ± 1.2%. The error due to the linear expansion of the tantalum metal is negligible over the temperature range for the sublimation of the amino acids.

The total error in the vapor pressure becomes

$$\sigma_p = [\sigma^2_{temp} + \sigma^2_{time} + \sigma^2_{c.f.} + \sigma^2_{area} + \sigma^2_{wt.}]^{1/2} \qquad (26)$$

$\sigma_p = \pm$ 4.3% error.

Basic errors arise from the failure of the experiment to fulfill ideal conditions. Knudsen's requirements for true molecular flow and the non-ideality of the vapor phase may have introduced a basic error. The magnitude of these are not known but are assumed to be negligible.

DISCUSSION OF RESULTS

Values were calculated for the standard entropy, standard free energy and the heat of sublimation for glycine. The author's values for these quantities agree within experimental error with those published by Takagi et al. (2) (see Table 3).

Other observations noted from the results are as follows:

A. Branching at the end of a hydrocarbon chain results in a high heat of sublimation. Examples are valine, leucine and phenylalanine which have a heat of sublimation of approximately 36-38 kcal./ mole.

B. Branching on any inner carbon atom except the psi and omega atoms of a hydrocarbon chain lowers the heat of sublimation. For example, isoleucine and cycloleucine have a heat of sublimation approximately 7-8 kcal./mole less than leucine.

C. Proline, a heterocyclic amino acid, had the lowest heat of sublimation observed. No other such acids were tested so it is not possible to state that this is generally true.

D. In a homologous straight chain series such as glycine, l-alanine, l-α-amino-n-butyric acid, dl-norvaline and dl-norleucine, there is a gradual decrease in the heat of sublimation with lengthening of the chain. Exception to this is the nonnaturally occurring amino acid, l-α-amino-n-butyric acid which is higher than it should be.

E. In comparing isomers, the heat of sublimation is always greater for the acid in which branching occurs on the omega carbon. For example, valine and leucine have a greater heat of sublimation than norvaline and norleucine, respectively, by approximately 9-10 kcal./mole. The butyric acid isomers are again an exception. The l-α-amino-n-butyric acid is greater than the α-aminoisobutyric acid by approximately 9 kcal./mole.

F. Anomalies for the butyric acids are also observed in the solubilities and the crystal densities (see Table 5).

No attempt has been made to explain the above trends. This might be done if a knowledge of the lattice energy of the crystalline acids and heats of hydration were known. Unfortunately these are unavailable for all of the amino acids except glycine.

A mixture of amino acids can be quantitatively analyzed by means of a mass spectrometer. The sensitivity of the analysis of an amino acid depends upon its vapor pressure, its ionization cross section and the intensity of the $(P\text{-}COOH)^+$ ion current relative to total ion intensity. Svec and Junk (6) have empirically determined the relative sensitivities by using an internal standard in the mass spectrometer. The sensitivities can be calculated from the vapor pressure by the equation

$$S = [\%\ (P\text{-}COOH)^+](I.C.)(V.P.)^{1/2}$$

where $[\%\ P\text{-}COOH^+]$ is the fraction of the total ion currents

Table 5. Sensitivities

	Calc. from This work	Empirically Determined (6)
Glycine	0.88	0.88
Alanine	0.88	0.87
L-α-amino-n-butyric acid	0.84	0.89
Valine	0.96	0.96
Isoleucine	1.00	1.00

from a pure amino acid. Values for these fractions are obtained from Svec and Junk (6). The ionization cross section (I.C.) is calculated by the method of Otvos and Stevenson (20). The vapor pressure is obtained from this work.

The source temperature of the mass spectrometer during the analysis is estimated to be approximately 160°C. The vapor pressures and sensitivities are calculated at this temperature, see Table 5. The agreement between the calculated and empirical sensitivities indicates that the sensitivities can be calculated from the vapor pressures.

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