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MEASUREMENT OF SUBLIMATION HEAT OF INDOLE-3-ACETIC ACID AND EVAPORATION HEAT OF INDOLYLACETONITRILE

F. GALÁN-ESTELLA, J. GONZÁLEZ-JULIÁN and P. AGUADO-RODRÍGUEZ

Laboratory of Biology Faculty of Biology, University of Salamanca, Salamanca 37008, Spain

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Key Word Index—Sublimation heat; indole-3-acetic acid; gas liquid chromatography (ECD)-Knudsen effusion method.

Abstract—The sublimation heat of indole-3-acetic acid and evaporation heat of 3-indolylacetonitrile were measured between 40 and 150° by a combined gas liquid chromatography–Knudsen effusion method. The vapour pressure equation for IAA(s) = IAA(g) proved to be log $P = 6.73-3393.98/T$ (T refers to absolute temp.) and for IAN(l) = IAN(g) was log $P = 6.68-3189.74/T$. These results yield $\Delta H°$ sublimation values of $15.30 \pm 0{,}35$ kcal/mol (IAA) and $\Delta H°$ evaporation 14.59 ± 0.28 kcal/mol (IAN) using the slope and second law determination, respectively. By interpolation, the boiling point of IAN proved to be 160°/0.2 torr, in accordance with results reported in the literature.

INTRODUCTION

According to Mann and Jaworski [1] most losses occurring in the purification of indole-3-acetic acid (IAA) in crude extracts of plant tissues are due to sublimation of IAA upon evaporating the ether fractions at 40°/0.2 mm Hg. However, it has been proposed that such losses are not caused by sublimation but rather by decomposition of IAA during purification [2]. In this case, the determination of the vapour pressure of IAA is desirable. In some cases, better knowledge of sublimation for a particular temperature and pressure may facilitate the application of molecular sublimation as a technique for purifying crude plant extracts, together with the application of the sublimatography technique for this phytohormone as well as others, such as indole-3-acetopyruvic acid, indole-3-lactic acid, 5-methoxyindole-3-acetic acid, 4-chloroindole-3-acetic acid, indole-3-butyric acid and abscissic acid. This technique was used in the purification of IAA in fungal extracts of *Rhizopus suinus* (at 95–105°/10^{-4} mm Hg) [3] and in extracts of *Fusarium oxysporum* f. *cubensé* [4]. Both the study of the sublimation of IAA and of the evaporation of IAN lead to the experimental result that vapours of IAA and IAN have essentially the same composition as the solid liquid, IAA(s) = IAA(g) or IAN(l) = IAN(g); i.e. the sublimation of IAA and the evaporation of IAN are congruent and the kinetic process of surface evaporation is not complicated by the presence of a condensed reaction product phase.

The aim of the present work was to calculate the $\Delta H°$ sublimation and evaporation heats of IAA and IAN by the Knudsen method over a temperature of 40 to 150° and to measure the sublimation rate (dm/dt) of solid IAA.

To calculate the probability of transmission 'W' [5] more commonly known as the evaporation coefficient [6, 7] of the effusion cell for our still, the following equation was used 'Kb = veloc. sublimat. exper./veloc. sublimat. theor. using benzoic acid to test the apparatus, since we knew the log $Ps = A - B/T$ (T refers to absolute temp.) for the 70–114° temperature range [8].

RESULTS AND DISCUSSION

The results of the sublimation and vaporization calculations obtained in the Knudsen effusion studies on indole-3-acetic acid and on 3-indolylacetonitrile are summarized in Tables 1 and 2. The effusion rates of IAA and IAN were measured from the weights of sublimate deposited on the condenser. The sublimate plus sublimand of the IAA and IAN samples were taken as 100% and the mass spectra obtained for both were in agreement with the results of Jamieson and Hutzinger [9] with molecular ions at m/z 175 and 156, as well as the ion at m/z 130, usually very intense and often the base peak of all indole compounds. Furthermore, the quantitative analisis by gas chromatography with an electron capture detector of the heptafluoroderivatives of the sublimate plus sublimand of IAA and IAN samples were 99% IAA or 99% IAN. The transmission probability for this effusion cell was considered to be approximately constant (determined by calibration with benzoic acid). Table 3 shows the values of this factor for benzoic acid in the 70–114° temperature range; this was 0.052 ± 0.009. The transmission probability factor was applied to the calculation of the vapour pressure of IAA and IAN. The data and least squares lane for IAA and IAN are plotted in Fig. 1. Second-law treatment of the data gave a $\Delta H°_{298}$ value of 15.3 ± 0.34 kcal/mol for the reaction heat (molar heat of sublimation) of IAA(s) = IAA(g) at 298K. Calculation of the molar sublimation heats, by the second law or August equation, is fairly exact in its final result and hence it is unnecessary to distinguish between the mean molar heat, ΔH, and the true molar heats, ΔH, corresponding to each of the temperatures. However, the third law method is considered to be an even more exact procedure since in it sublimation heat is considered to vary with temperature. However, this in turn depends on a good knowledge of the thermodynamic functions and free energy functions. In the case of IAA and IAN, until now no data have appeared in the literature relating to the calculation of the fef functions. In our work the temperatures taken (first

Table 1. Sublimation pressure data of solid indole-3-acetic acid

Temp. (°) average	Time (min)	Mass loss (g)	Pressure (torr) Knudsen	Vaporization rate (g/cm/sec)	ΔH° (kcal/mol) average
37	110	$6.01 \cdot 10^{-5}$	$6.24 \cdot 10^{-5}$	$4.56 \cdot 10^{-8}$	15.53
47	150	$5.56 \cdot 10^{-4}$	$4.30 \cdot 10^{-4}$	$3.09 \cdot 10^{-7}$	14.77
56	120	$3.77 \cdot 10^{-4}$	$3.69 \cdot 10^{-4}$	$2.62 \cdot 10^{-7}$	15.30
58	74	$1.88 \cdot 10^{-4}$	$3.00 \cdot 10^{-4}$	$2.12 \cdot 10^{-7}$	15.52
65	170	$7.79 \cdot 10^{-4}$	$5.46 \cdot 10^{-4}$	$3.82 \cdot 10^{-7}$	15.47
70	120	$8.45 \cdot 10^{-4}$	$8.45 \cdot 10^{-4}$	$5.87 \cdot 10^{-7}$	15.38
70	94	$7.40 \cdot 10^{-4}$	$9.45 \cdot 10^{-4}$	$6.56 \cdot 10^{-7}$	15.30
74	90	$7.37 \cdot 10^{-4}$	$9.89 \cdot 10^{-4}$	$6.82 \cdot 10^{-7}$	15.48
74	60	$4.57 \cdot 10^{-4}$	$9.20 \cdot 10^{-4}$	$6.35 \cdot 10^{-7}$	15.53
79	97	$3.47 \cdot 10^{-3}$	$4.35 \cdot 10^{-3}$	$2.98 \cdot 10^{-6}$	14.65
83	170	$4.76 \cdot 10^{-3}$	$3.42 \cdot 10^{-3}$	$2.33 \cdot 10^{-6}$	14.98
84	88	$1.19 \cdot 10^{-3}$	$1.66 \cdot 10^{-3}$	$1.13 \cdot 10^{-6}$	15.53
88	120	$4.12 \cdot 10^{-3}$	$4.23 \cdot 10^{-3}$	$2.86 \cdot 10^{-6}$	15.06
88	200	$3.41 \cdot 10^{-3}$	$2.10 \cdot 10^{-3}$	$1.42 \cdot 10^{-6}$	15.56
91	130	$6.25 \cdot 10^{-3}$	$5.95 \cdot 10^{-3}$	$4.01 \cdot 10^{-6}$	14.93
93	140	$3.26 \cdot 10^{-3}$	$2.89 \cdot 10^{-3}$	$1.94 \cdot 10^{-6}$	15.53
93	130	$7.22 \cdot 10^{-3}$	$6.89 \cdot 10^{-3}$	$4.63 \cdot 10^{-6}$	14.90
102	160	$1.16 \cdot 10^{-2}$	$9.10 \cdot 10^{-3}$	$6.04 \cdot 10^{-6}$	15.07
102	77	$3.01 \cdot 10^{-3}$	$4.91 \cdot 10^{-3}$	$3.26 \cdot 10^{-6}$	15.53
107	130	$5.49 \cdot 10^{-3}$	$5.34 \cdot 10^{-3}$	$3.52 \cdot 10^{-6}$	15.66
112	120	$5.78 \cdot 10^{-3}$	$6.12 \cdot 10^{-3}$	$4.01 \cdot 10^{-6}$	15.74
112	190	$1.21 \cdot 10^{-2}$	$8.10 \cdot 10^{-3}$	$5.31 \cdot 10^{-6}$	15.53
115	160	$1.24 \cdot 10^{-2}$	$9.86 \cdot 10^{-3}$	$6.43 \cdot 10^{-6}$	15.53
119	150	$2.45 \cdot 10^{-2}$	$2.09 \cdot 10^{-2}$	$1.36 \cdot 10^{-5}$	15.09
121	100	$1.02 \cdot 10^{-2}$	$1.31 \cdot 10^{-2}$	$8.49 \cdot 10^{-6}$	15.53
121	140	$3.60 \cdot 10^{-2}$	$3.31 \cdot 10^{-2}$	$2.14 \cdot 10^{-5}$	14.80
121	148	$4.96 \cdot 10^{-2}$	$4.31 \cdot 10^{-2}$	$2.79 \cdot 10^{-5}$	14.60
125	100	$1.91 \cdot 10^{-2}$	$2.47 \cdot 10^{-2}$	$1.59 \cdot 10^{-5}$	15.17
126	60	$7.65 \cdot 10^{-3}$	$1.65 \cdot 10^{-2}$	$1.06 \cdot 10^{-5}$	15.53
126	35	$8.36 \cdot 10^{-3}$	$3.09 \cdot 10^{-2}$	$1.99 \cdot 10^{-5}$	15.03
130	100	$1.59 \cdot 10^{-2}$	$2.07 \cdot 10^{-2}$	$1.33 \cdot 10^{-5}$	15.53
133	111	$3.71 \cdot 10^{-2}$	$4.36 \cdot 10^{-2}$	$2.78 \cdot 10^{-5}$	15.03
135	120	$5.12 \cdot 10^{-2}$	$5.58 \cdot 10^{-2}$	$3.55 \cdot 10^{-5}$	14.90
135	180	$2.18 \cdot 10^{-2}$	$1.58 \cdot 10^{-2}$	$1.01 \cdot 10^{-5}$	15.92
144	15	$5.17 \cdot 10^{-3}$	$4.56 \cdot 10^{-2}$	$2.87 \cdot 10^{-5}$	15.41
144	20	$4.47 \cdot 10^{-3}$	$2.96 \cdot 10^{-2}$	$1.86 \cdot 10^{-5}$	15.77
					Av. 15.30
					St. 0.34

The temperatures taken are the averages of three temperatures. Each experiment (each line of Table) was taken as a single measurement.

Table 3. Benzoic acid kinetic

Temp (°)	Time (sec)	Mass loss (%)	Mass (g)	Mass loss (g) theoric	Mass loss (g) experiment	Transmission probability (W)
70	1500	40.76	0.1239	0.93	$5.05\,10^{-2}$	0.0540
72	1500	41.31	0.1239	1.12	$5.12\,10^{-2}$	0.0456
72	1500	50.91	0.1239	1.12	$6.31\,10^{-2}$	0.0562
73	1500	47.60	0.1239	1.23	$5.90\,10^{-2}$	0.0480
73	1500	57.42	0.1239	1.23	$7.11\,10^{-2}$	0.0579
70	2100	44.19	0.1383	1.31	$6.11\,10^{-2}$	0.0467
70	2100	62.35	0.1383	1.31	$8.62\,10^{-2}$	0.659
75	2100	59.25	0.1383	2.06	$8.19\,10^{-2}$	0.398
75	2100	70.56	0.1383	2.06	$9.76\,10^{-2}$	0.0474
75	420	65.56	0.0282	0.41	$1.85\,10^{-2}$	0.0449
75	420	96.22	0.0282	0.41	$2.71\,10^{-2}$	0.0659
						Av. 0.0520
						Std. 0.009

Table 2. Vapour pressure data of liquid indolylacetonitrile

Temp. (°) average	Time (min)	Mass loss (g)	Pressure (torr) Knudsen	Vaporization rate (g/cm/sec)	Δ H° (kcal/mol) average
124	50	0.7085	$3.23 \cdot 10^{-2}$	0.00118	14.84
130	19	0.4769	$5.76 \cdot 10^{-2}$	0.00209	14.60
130	10	0.1783	$4.09 \cdot 10^{-2}$	0.00149	14.88
128	14	0.4826	$7.90 \cdot 10^{-2}$	0.00287	14.28
157	20	1.7255	$2.05 \cdot 10^{-1}$	0.00719	14.50
157	25	1.4192	$1.35 \cdot 10^{-1}$	0.00473	14.86
157	17	2.1404	$2.99 \cdot 10^{-1}$	0.01049	14.17
50	35	0.0177	$1.04 \cdot 10^{-3}$	0.00004	14.28
35	45	0.0063	$2.80 \cdot 10^{-4}$	0.00001	14.42
85	56	0.1025	$3.96 \cdot 10^{-3}$	0.00015	14.88
62	60	0.0229	$8.00 \cdot 10^{-4}$	0.00003	14.99
160	15	1.6990	$2.70 \cdot 10^{-1}$	0.00944	14.36
160	10	0.8092	$1.93 \cdot 10^{-1}$	0.00674	14.65
					Av. 14.59
					St. 0.28

The temperatures taken are the averages of three temperatures. Each experiment (each line of Table) was taken as a single measurement.

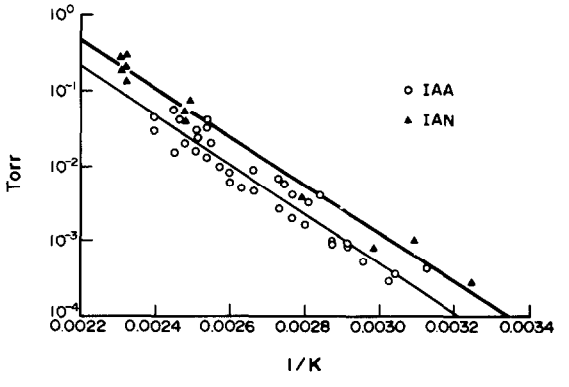

Fig. 1. Plot of log P (torr) as function of reciprocal absolute T (K) for IAA and IAN.

column of Tables 1 and 2) are the means of three temperatures. Accordingly, the values in the last column of Tables 1 and 2 is the mean molar sublimation heat for those mean temperatures. Each experiment (each row of Tables 1 and 2) was taken as a simple measurement.

The molar heat of vaporization for IAN was $\Delta H^\circ_{298} = 14.50 \pm 0.28$ kcal/mol, with a boiling point of 157–160°/1.6 mm Hg. This is in accordance with the data found in the literature and very similar to the latent evaporation heat of 3-methylindole, $\Delta H^\circ_{298} = 15.23$ kcal/mol, whose vapour pressure equation according to its bp (266–265°C/755 torr) is $\log P = 9.05 - 3329.40/T$, similar to that of IAA and IAN as would be expected. Figure 2 shows the dm/dt function for IAA in the 40–150° temperature range. From this function it is possible to predict that the losses due to sublimation of less than a few micrograms of IAA in crude extracts of plant tissues for surfaces not greater than 200 cm^2 at 40° and vacuum values not greater than 0.002 mm Hg are infinitesimal and thus undetectable by currently existing techniques, against what is stated by Mann and Jaworski [1]. The vapour pressure for these two phytohormones are fairly low for the sublimation and evaporation heats of IAA and IAN, similar to the case of the evaporation pressures of most alpha-aminoacids.

evaporation parameters

Sublimation pres. (torr) theorical	Sublimation rate (theor) (g/cm/sec)	Sublimation pres. (torr) experimental	Sublim exp/theor.
$9.00\,10^{-2}$	$3.37\,10^{-5}$	$4.84\,10^{-3}$	18.51
$1.08\,10^{-1}$	$3.41\,10^{-5}$	$4.92\,10^{-3}$	21.93
$1.08\,10^{-1}$	$4.20\,10^{-5}$	$6.06\,10^{-3}$	17.79
$1.18\,10^{-1}$	$3.93\,10^{-5}$	$5.68\,10^{-3}$	20.83
$1.18\,10^{-1}$	$4.74\,10^{-5}$	$6.85\,10^{-3}$	17.27
$9.00\,10^{-2}$	$2.91\,10^{-5}$	$4.10\,10^{-3}$	21.41
$9.00\,10^{-2}$	$4.11\,10^{-5}$	$5.90\,10^{-3}$	15.18
$1.42\,10^{-1}$	$3.90\,10^{-5}$	$5.65\,10^{-3}$	25.13
$1.42\,10^{-1}$	$4.65\,10^{-5}$	$6.73\,10^{-3}$	21.10
$1.42\,10^{-1}$	$4.40\,10^{-5}$	$6.37\,10^{-3}$	22.27
$1.42\,10^{-1}$	$6.46\,10^{-5}$	$9.36\,10^{-3}$	15.17
		Av.	19.69
		Std.	3.15

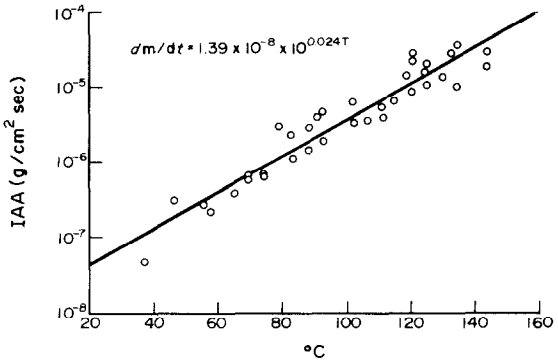

Fig. 2. Rate of molecular sublimation of the IAA.

EXPERIMENTAL

Procedure for measurement. The process of sublimation at high vacuum may be referred to as a molecular sublimation and is very similar to molecular distillation. The material which is collected on the condenser is referred to as the sublimate and the initial crystal is the sublimand. The amount of a crystalline material that is transferred from the vaporizer to the condenser within a given period of time is referred to as the sublimation rate.

The evapn rate of a pure solid at very low pressures is given by the Hertz–Knudsen equation:

$$dm/dt = WP_s (M/2\pi RT_s)^{1/2} = 0.0583\ WP_{torr}(M/T)^{1/2}$$

The sublimation pressure of IAA and the evapn pres. of IAN can be found from the least-squares fits of the data relating to temp. and the mass losses from the effusion cell or sublimate per unit of time, analysed quantitatively by GLC (ECD or FID):

$$P_s = dm/dt\ 1/AW(2\pi RT_s/E)^{1/2}$$

in which P is the vapour pressure calculated with an assumed molecular weight M, dm/dt is the rate of mass loss, W a transmission probabilaty of the effusion cell [5] or coefficient of evapn [16]; that is, the effect of the collisions taking place between the evapd molecules of IAA when effusion occurs in a space in which there are already molecules [7] and which is calcd using benzoic acid. A is the area of the effusion orifice, R is the gas constant and T is temperature. Strictly speaking, this equation is applicable only when the walls of the evacuated chamber are cooled so that vapour molecules striking them condense and do not evaporate again [12].

Vacuum sublimation apparatus. A vacuum sublimation and molecular distillation apparatus for laboratory use was designed to attain pressures below 10^{-5} torr in the still itself. It was of a similar kind to that employed previously [3, 13–15]. It It consisted of a Knudsen effusion cell connected to a condenser tube and then to a vacuum system. The Knudsen effusion cell was a removable vial containing small known quantities of the sample. It was a gold tube with dimensions of either *ca* 5 mm long and 4.4 mm and 4.03 external and internal diameter, or 24 mm long and 4.4 and 4.05 mm external and internal diameter. It was heated electrically by an external 'Thermocoax' furnance. The temperature of the solid IAA was controlled by a calibrated chromel–alumel thermocouple in contact with the effusion cell wall. The temperature of this thermocouple was calibrated in experiments in which a second chromel–alumel thermocouple was inserted into the effusion cell. The thermocouple used for effusion studies recorded a temperature of 1.5–2.0° higher than the temperature of the inner surface of the gold effusion cell, containing μg amounts of IAA powder, over the entire experimental range. The condenser tube was removable pyrex tube *ca* 170 mm long and 6 mm external diameter. One end of the tube was connected to the vacuum system and the other end to the Knudsen effusion cell by a Gaco-R. O ring. The pyrex tube was enclosed in a copper cylindrical refrigerator cooled with liquid N_2 that maintained the temperature of the condenser at *ca* 150°. The distance between the Knudsen effusion cell and condenser was less than 5 mm. Pressure in the system was kept below 10^{-5} torr by means of a diffusion pump. Using this 'molecular still', the procedure was to raise the furnace temperature from 35 to T° below T_c (critical point of substance) and then to hold the oven-enclosure at the same temp. for t sec. The vaccum was then released and the main tube removed (condenser) from the vaccum system. The sublimate, or vaporisate, was dissolved from the condenser with MeOH and Et_2O. For increased accuracy, several measurements of the rate of weight loss of the sample were made at each temp. The time-weighted average of all weight-loss readings was calculated and recorded as one pressure point. These weight losses were measured by GLC (ECD or FID) of the heptafluorobutyric derivative of IAA and IAN, and the methyl ester derivative of benzoic acid, The sensitivity of the GLC system was ≤ 1 ng (ECD) and < 1 μg (FID).

Instruments and conditions of quantitative GLC analysis. GLC assay of IAA was performed essentially according to ref. [16]. The IAA methylester was prepared with diazomethane [17]. The sterifying reagents were removed by a stream of N_2 and the HFBI was added to the ester under rigorously dry conditions.

In the case of IAN, methylation was unnecessary. Reagent was removed with a 0.5 M H_2SO_4 soln. [18]. GLC-ECD was performed in a glass column 1.83 × 0.32 cm) packed with 3% OV-17 on 100-120 mesh Gas Chrom Q using N_2 as the carrier gas (flow rate 28 ml/min) with the column temp. isothermal to 170°; injector temp. was 190° and detector temp. was 200°. 1 μl amounts were injected into the column. GLC-FID was performed on the same glass column (OV-17) with column temp. at 110°; injector temp. 160° and detector temp. 170°.

Chemicals and solvents. The main chemicals used were IAA and IAN (Xpectrix). The main organic solvents were MeOH (GR grade, Merck) and hexane (AR grade, Merck).

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