(2) M. Flocks and H. C. Zweng, *Am. J. Ophthalmol.*, **44**, 380 (1957).
(3) C. S. O'Brein and K. C. Swan, *Trans. Am. Ophthalmol. Soc.*, **39**, 175 (1941).
(4) J. Trzeciakowski and C. Y. Chiou, *J. Pharm. Sci.*, **67**, 531 (1978).
(5) R. Hazard, J. Cheymol, P. Chabrier, A. Sekera, and R. Eche-Fialaire, *Bull Soc. Chim. Fr.*, **1961**, 2087.
(6) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Wiley, New York, N.Y., 1962.
(7) W. H. Morrison, *Am. J. Ophthalmol.*, **37**, 744 (1954).
(8) "The Merck Index," 9th ed., M. Windholz, Ed., Merck & Co., Rahway, N.J., 1976, p. 966.
(9) S. S. Chrai and J. R. Robinson, *Am. J. Ophthalmol.*, **77**, 735 (1974).
(10) P. H. Chung, T. F. Chin, and J. L. Lach, *J. Pharm. Sci.*, **59**, 1300 (1970).
(11) M. A. Nunes and E. Brochmann-Hanssen, *ibid.*, **63**, 716 (1974).
(12) A. Longwell, S. Birss, N. Keller, and D. Moore, *ibid.*, **65**, 1654 (1976).
(13) F. N. Martin and J. L. Mims, *Arch. Ophthalmol.*, **44**, 561 (1950).

ACKNOWLEDGMENTS

J. Trzeciakowski was supported in part by a predoctoral traineeship from the National Institutes of Health (GM-00760). The authors thank the National Eye Institute of the National Institutes of Health (Ey-01624) for research support.

The authors also thank Ms. Marilyn K. Martin for technical assistance.

Effect of Low and High Relative Humidity on Metered-Dose Bronchodilator Solution and Powder Aerosols

F. CHARLES HILLER ˣ, MALAY K. MAZUMDER, J. DOUGLAS WILSON, and ROGER C. BONE

Received August 9, 1979, from the *University of Arkansas for Medical Sciences, Little Rock, AR 72201.* Accepted for publication October 19, 1979.

Abstract □ Physical properties of two metered-dose bronchodilator aerosols packaged as solutions and two aerosols packaged as finely ground powders were measured at low and high relative humidity. The aerodynamic size distribution and particle concentration were measured in real time using the single-particle aerodynamic relaxation time analyzer, which can measure the aerodynamic diameter of single suspended particles in the respirable size range. The count median aerodynamic diameter, the mass median aerodynamic diameter, the total particles per dose, and the total aerodynamic mass per dose were calculated. Significant increases were noted in the count median aerodynamic diameter for three aerosols and in the mass median aerodynamic diameter for two aerosols. The number of particles in the measured size range increased 3.6- and 4.1-fold for the droplet aerosols and 1.4-fold for the powder preparations. The aerodynamic mass per dose in the measured size range increased 5.7- and 11.4-fold for the droplet aerosols and 3.1- and 1.6-fold for the powder aerosols. These data indicate that all aerosols tested increased in size at high humidity and that aerosols dispensed as droplets may be more unstable than those dispensed as powders.

Keyphrases □ Bronchodilators—metered-dose solution and powder aerosols, effect of low and high humidity □ Aerosols—bronchodilators, metered-dose solution and powder preparations, effect of low and high humidity □ Asthma products—metered-dose solution and powder aerosols, effect of low and high humidity □ Metered-dose aerosols—bronchodilators, solution and powder preparations, effect of low and high humidity

Metered-dose bronchodilator aerosols are used widely to treat obstructive lung disease. The efficacy of these medications is determined by their quantity and site of pulmonary deposition. Factors that affect pulmonary retention and distribution are the inhalation technique, airway patency, and particle size.

BACKGROUND

The particle-size distributions of aerosols produced by some of these devices have been reported (1–3), but the effect of hygroscopicity on particle size was not included. Any particle may grow by water condensation or shrink by water evaporation, depending on the humidity and physical characteristics of the particle. Such changes may affect the mass and site of aerosol deposition in the respiratory tract. Although particle growth during exposure to high humidity such as that occurring in the respiratory tract has been predicted for therapeutic aerosols (4) and the effect of humidity on the size of propylene glycol aerosols has been described (5), there is no information documenting or quantitating the effect of high humidity on bronchodilator aerosols.

Measurement of aerosol particle size is most relevant for prediction of deposition when the size is expressed as the aerodynamic diameter, defined as the diameter of a unit density spherical particle having the same terminal settling velocity as the particle in question. Methods currently used to measure the aerodynamic size distribution of aerosols require particle impaction and subsequent analysis of the quantity deposited (4, 6). The instability of water-containing particles has been described (7), and size changes may occur in <1 sec (8). This instability precludes extrapolation of the particle-size measurement following precipitation of unstable particles to the particle size in the suspended state. Measurement in the suspended state is necessary to assess accurately the effect of humidity on the size of unstable particles.

The purpose of this study was to determine the effect of high humidity, similar to that encountered in the respiratory tract, on the particle-size distribution of four commonly used metered-dose aerosol devices. Humidity in the trachea and more distal airways generally is regarded to be in the 99.0–99.8% range (9). The mass median aerodynamic diameter and count median aerodynamic diameter (the sizes below which are 50% of the particles by mass and number, respectively), the geometric standard deviation (84.1% size divided by 50% size), the mass per dose, and the number of particles per dose were measured. A new device, the single-particle aerodynamic relaxation time analyzer (hereafter called the analyzer) that measures the aerodynamic diameter of single particles in real time, was used for these studies.

EXPERIMENTAL

The following medications were studied: isoproterenol hydrochloride[1] and isoetharine mesylate[2], both packaged as solutions, and metaproterenol sulfate[3] and isoproterenol sulfate[4], both packaged as finely ground powders. In each case, the commercially available preparation was used. Each medication was injected into an environmental chamber for subsequent sampling by the analyzer.

The analyzer (Fig. 1) and the theoretical basis for its operation were described previously (3, 10, 11). The sensing volume, in which the signal

[1] Isuprel Mistometer, Winthrop Laboratories, New York, N.Y.
[2] Bronkometer, Breon Laboratories, New York, N.Y.
[3] Metaprel, Dorsey Laboratories, Lincoln, Neb.
[4] Medihaler-Iso, Riker Laboratories, Northridge, Calif.

0022-3549/80/0300-0334$01.00/0

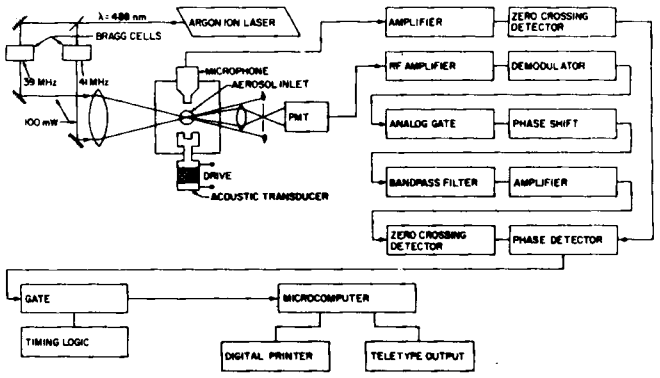

Figure 1—*Schematic outline of the SPART analyzer. The analyzer and ancillary signal processing equipment are shown.*

for particle-size measurement is generated, is formed by the intersection of two laser beams of slightly different frequencies (Fig. 2). Each particle entering the sensing volume is subjected to acoustic oscillation at 24.25 kHz. Particle oscillation lags behind acoustic field oscillation; this delay, the phase lag, is directly related to the aerodynamic diameter.

Particle oscillation is measured by a laser Doppler velocimeter using the Doppler principle (7). The Doppler frequency shift in the light scattered by a particle oscillating in this sensing volume is detected by a photomultiplier, which generates a frequency-modulated (FM) signal. The FM signal, which transmits particle velocity information, is demodulated and compared using data processing circuitry to the acoustic signal generated by a microphone to determine the phase lag. A microprocessor determines the aerodynamic diameter from the phase lag and stores the data for analysis (Fig. 1). The stored data are processed by a minicomputer to determine the count median aerodynamic diameter, mass median aerodynamic diameter, and geometric standard deviation (σg) and to plot the following distributions: (*a*) derivative of the number with respect to the aerodynamic diameter, (*b*) derivative of the aerodynamic mass with respect to the aerodynamic diameter, (*c*) the cumulative number, and (*d*) the cumulative aerodynamic mass. The aerodynamic mass is calculated from the aerodynamic diameter by:

$$\text{aerodynamic mass} = \tfrac{2}{3}\,\pi \text{ aerodynamic diameter}^3 \times \text{density} \quad \text{(Eq. 1)}$$

The density is assumed to be unity. The actual density of these multicomponent particles is difficult to determine. The actual mass differs from the aerodynamic mass as the difference between the actual and assumed density increases. There also is some error introduced for particles with an aerodynamic diameter of <1 μm since those particles behave somewhat differently than larger particles. For these studies, the error should be small since most of the mass is in particles with an aerodynamic diameter of <1 μm. The geometric standard deviation (Table I) was calculated by a computer from the cumulative aerodynamic mass distributions and was only slightly different from the geometric standard deviation calculated from cumulative number distributions.

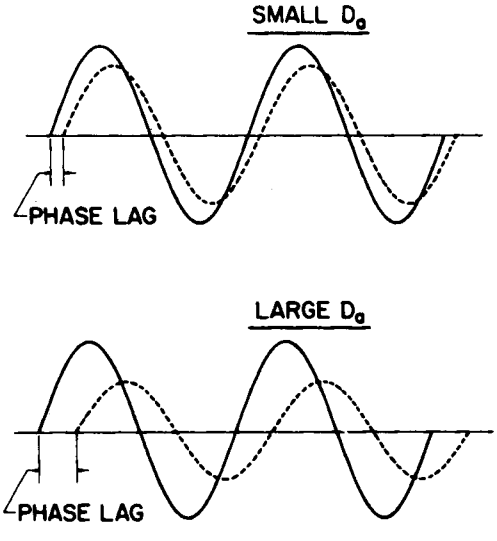

Figure 2—*The solid lines represent the wave form of the oscillatory motion of air caused by acoustic excitation. The broken lines represent particle motion, which lags behind air motion. The relative phase lag depends on the aerodynamic diameter of the particulate.* D_a = *aerodynamic diameter. (Reprinted from Ref. 3 with permission of the publisher.)*

Table I—Physical Properties of Aerosol Produced by Four Metered-Dose Bronchodilator Devices Measured at Low and High Humidity

Drug	Parameter	CMAD[a], μm		MMAD[b], μm		σg[c]	
Isoetharine mesylate	RH[d]	21	95	21	95	21	95
	x[e]	0.74	0.93	3.59	3.79	2.0	1.9
	SD[f]	0.02	0.08	0.73	0.63	0.07	0.06
	p[g]	<0.001		NS[h]		—	
Isoproterenol hydrochloride	RH	20	96	20	96	20	96
	x	0.70	0.92	3.21	4.03	2.1	1.9
	SD	0.03	0.12	0.50	0.38	0.1	0.1
	p	<0.001		<0.01		—	
Metaproterenol sulfate	RH	16	98	16	98	16	98
	x	0.68	0.80	4.05	5.22	2.0	2.0
	SD	0.01	0.04	0.32	0.54	0.1	0.2
	p	<0.001		<0.001		—	
Isoproterenol sulfate	RH	17	96	17	96	17	96
	x	0.70	0.71	2.88	3.26	2.0	2.0
	SD	0.02	0.08	0.24	0.43	0.1	0.1
	p	NS		NS		—	

[a] Average count median aerodynamic diameter (n = 7). [b] Average mass median aerodynamic diameter (n = 7). [c] Average geometric standard deviation (n = 7). [d] Average relative humidity for each group of studies. [e] Average of seven studies for measured characteristics. [f] Standard deviation. [g] Statistical significance of difference between measured characteristic at high and low humidity on basis of t test. [h] Not significant.

The aerosol is drawn from the environmental chamber to the analyzer at a rate of 55 cm^3/min. Only a portion of this sample passes through the sensing volume. The sensing volume is 1.3×10^{-6} cm^3, and the quantity of aerosol passing through it is 1.3×10^{-2} cm^3/sec. The analyzer coincidence loss is <2% at a counting rate of 40 particles/sec or less. In the present study, the count rate was maintained below this level by maintaining aerosol concentrations in the environmental chamber at <3000 particles/cm^3.

The environmental chamber was a 200-liter insulated plastic box. The temperature was maintained at 37 ± 0.5° by applying thermostatically controlled heat strips to the chamber walls and was monitored using a digital thermometer. The chamber was humidified for high humidity studies by bubbling chamber air through a heated water bath and was monitored with a hygrometer[5]. Ambient humidity studies were at 16–21% relative humidity (RH), and high humidity studies were at 95–98% RH. The analyzer was operated at 37°, and the relative humidity of the efflux from the analyzer was monitored[6] to ensure that no significant loss of humidity occurred during transport of the aerosol to the sensing volume.

Prior to each study, the environmental chamber was checked to ensure that it was free of residual aerosol. The aerosol and thermal mixing was provided using a fan inside the chamber. The decay rate for this chamber has been measured as a function of the aerodynamic diameter and is sufficiently low for the particle sizes involved in this study so as to be insignificant for the sampling time used. During testing, the environmental chamber was placed above the analyzer. The aerosol was sampled vertically with laminar flow in the sampling tubes to minimize particle loss from impaction in the sampling tubing.

For each of the four metered-dose devices, seven trials were performed. When appropriate environmental conditions were attained, the drug was injected into the chamber. The count rate was assessed, and the injection was repeated until the chamber concentration reached 3000 particles/cm^3. Injection was completed in <1 min. Following loading of the chamber with the aerosol, 30 sec was allowed for mixing; the analyzer sampling then was begun and continued for 5 min.

RESULTS

Seven trials were performed for each study, with 5000–9000 individual particles sized in each trial. The data reported are the average of these

[5] Model HTAB-176, Abbeon Corp., Santa Barbara, Calif.
[6] Model HM-111, Weathermeasure Corp., Sacramento, Calif.

Table II—Number of Particles and Total Mass Aerosolized in 0.10–10.0-μm Range per Dose by Metered-Dose Devices

Drug	Parameter	Particles per Dose ($\times 10^6$)		Dose, μg		Active Ingredient per Dose, μg[a]
Isoetharine mesylate	RH[b]	21	95	21	95	410
	x[c]	123	447	334	1907	
	SD[d]	38	771	164	566	
	p[e]	<0.001		<0.001		
Isoproterenol hydrochloride	RH	20	96	20	96	125
	x	49	202	89	1016	
	SD	2.5	68	18	297	
	p	<0.001		<0.001		
Metaproterenol sulfate	RH	16	98	16	98	650
	x	288	364	660	2215	
	SD	24	52	54	839	
	p	<0.01		<0.001		
Isoproterenol sulfate	RH	17	96	17	96	75
	x	140	200	225	509	
	SD	6	39	23	135	
	p	<0.01		<0.001		

[a] Active ingredient dispensed per dose according to the manufacturer's package insert. [b] Average relative humidity (n = 7). [c] Average of seven studies. [d] Standard deviation from seven studies. [e] Statistical significance of difference between measured characteristic at high and low humidity on basis of unpaired t test.

trials. The average results for the count median aerodynamic diameter, mass median aerodynamic diameter, and geometric standard deviation for each preparation at low and high humidity are shown in Table I. Also shown is the statistical significance of the comparison between the values at low and high humidity. The information indicates that there was a significant increase at high humidity in the count median aerodynamic diameter for isoetharine mesylate, isoproterenol hydrochloride, and metaproterenol sulfate but not for isoproterenol sulfate. Significant increases in the mass median aerodynamic diameter were noted for isoproterenol hydrochloride and metaproterenol sulfate. The geometric standard deviation for all of the aerosols was similar, did not change much with high humidity, and indicated a heterodisperse aerosol.

Table II describes the total number of particles and the total mass per dose of the aerosol measured in the 0.30–6.0-μm range. At high humidity, the total particles and total mass per dose of all aerosols increased significantly. The increase in total particles was 3.6- and 4.1-fold for isoetharine mesylate and isoproterenol hydrochloride, respectively, an increase considerably greater than the 1.4-fold change noted for both powdered preparations. The increases in total mass of 5.7- and 11.4-fold for isoetharine mesylate and isoproterenol hydrochloride were greater than the 3.4- and 1.6-fold increases in mass noted with the metaproterenol sulfate and isoproterenol sulfate powders.

Representative aerodynamic size distribution curves are shown in Figs. 3 and 4. In Fig. 3, the number distribution of the droplet isoproterenol hydrochloride aerosol measured at low humidity is compared to the number distribution measured at high humidity. The area under each curve represents the total number of particles measured at each humidity.

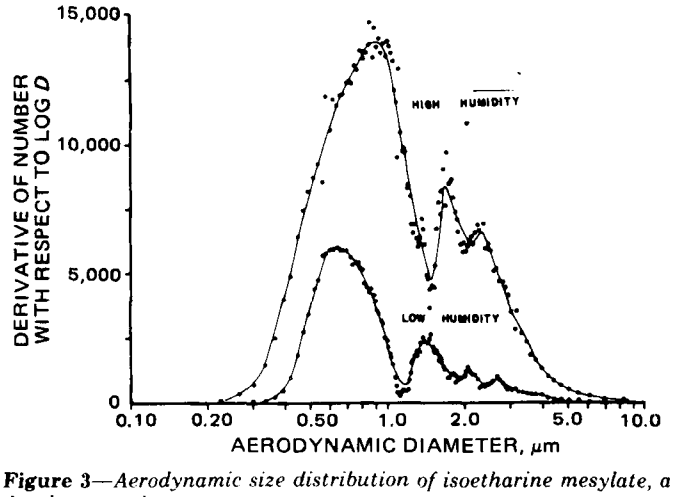

Figure 3—*Aerodynamic size distribution of isoetharine mesylate, a droplet aerosol.*

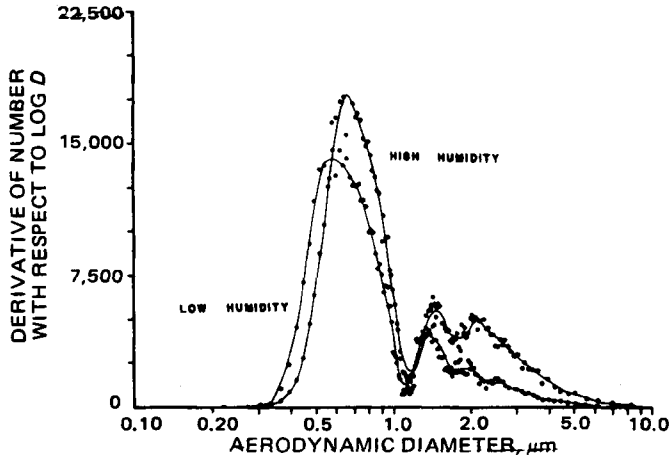

Figure 4—*Aerodynamic size distribution of isoproterenol sulfate, a powdered aerosol.*

The aerodynamic size distribution curves for the powdered isoproterenol sulfate aerosol are shown in Fig. 4, which is arranged similarly to Fig. 3. These figures illustrate the considerably smaller difference in the area under the curve between studies done at low and high relative humidity for the powdered aerosol compared to the droplet aerosol, a difference also shown in the particles per dose column of Table II.

DISCUSSION

The physical properties of all aerosols tested were different at low humidity compared to high humidity. In general, physical properties reflecting particle size increased at high humidity, and these increases were greater for droplet aerosols than for powder aerosols.

The number of particles per dose increased significantly for all four aerosols tested. The increase in particles per dose was especially marked for droplet aerosols. The number of particles per dose increased from 1.23×10^8 to 4.47×10^8 for isoetharine mesylate and from 4.0×10^7 to 2.02×10^8 for isoproterenol hydrochloride. One possible explanation for the increase in the particle number per dose is that many small particles exist at low humidity below the resolution limit (0.3 μm) of the analyzer and that these particles grow into the measurable size range at high humidity.

The mass per dose also increased significantly at high humidity for all four aerosols. The increase was greatest for droplet aerosols, changing from 334 to 1016 μg for isoproterenol hydrochloride at low and high humidity, respectively. The count median aerodynamic diameter increased significantly for three aerosols, while the mass median aerodynamic diameter increased for two aerosols.

The increase in the total mass and the number of particles per dose observed in these studies was considerable, especially for droplet aerosols, and may seem inconsistent with the modest increases in the count median aerodynamic diameter and mass median aerodynamic diameter. There are several possible explanations. At low humidity, the aerosol may contain many particles smaller than the lowest size the analyzer can measure. When the humidity increases, these particles grow and become measurable. Thus, in the measurable size range, there is an apparent increase in the number of particles in the smaller size range. This increase in the number of small particles would offset the increase in the count median aerodynamic diameter and the mass median aerodynamic diameter that would be expected had the aerosol not contained at low humidity many particles below the measurable size range. The size attained by a particle subjected to a given set of conditions is called the equilibrium diameter. The equilibrium diameter is determined by the relative humidity, hygroscopicity of the particle, and size of the dry particle. The severalfold increase in the total aerosol mass per dose without a significant change in the mass median aerodynamic diameter may be due in part to the fact that small hygroscopic particles have larger surface-to-mass ratios than do larger particles and undergo a higher relative growth when exposed to high humidity.

Another phenomenon pertains to the fact that the measured aerodynamic diameter depends in part on the particle density, ρ. If ρ of the dry particle is greater than 1 g/cm^3, then water absorbed by the particle increases the volume but reduces ρ, and, consequently, the aerodynamic diameter does not increase in proportion to the geometric diameter.

Therefore, the total particulate volume may increase out of proportion to the observed change in the mass median aerodynamic diameter. In considering these data and the hypothesized explanations, it is important to recognize that the relative contribution to particle growth at high humidity in a complex system of multicomponent particles, air, water vapor, and other vapors such as fluorocarbons and ethanol is not clear.

The increase in the number density and the mass of aerosol particles at high humidity must not be interpreted as an increase in the content of active ingredients. Although the growth is caused by the water uptake, any accompanying change in the mass distribution of the active ingredient may significantly change the amount and site of deposition of the active ingredient inside the lung. Additional information is needed to determine the content of the active ingredient at various size intervals, especially at high humidity.

The total mass of dry aerosol in the 0.30–6.0-μm range shown in Table II is similar for three of the four aerosols to the mass of the active ingredient claimed by the manufacturer to be dispensed from each device per valve activation. All aerosols contain stabilizing and dispensing agents that may contribute to the total mass. The portion of the active ingredient in the aerosol mass is not known. The active ingredient of droplet aerosols is dissolved in alcohol, which may contribute to the aerosol mass, although it probably evaporates fairly rapidly. The fluorocarbon propellants also contribute to the particle size as these aerosols leave the metered-dose device, but most are highly volatile and evaporate almost immediately upon aerosolization. In addition, all of these devices contain a dispersing agent present in variable quantities. The mass of isoproterenol sulfate measured in this study was greater than the mass of active ingredient reported to be dispensed per dose by the manufacturer. This difference probably was due to the relatively high ratio of dispersing agent to active ingredient for isoproterenol sulfate (~2.5:1) compared to metaproterenol sulfate (~1:1)[7].

The increase in particle size found in this study probably was not greatly influenced by particle aggregation since the aerosol concentration after injection into the environmental chamber was relatively dilute. Aggregation, which is related to the square of the concentration, may contribute along with water condensation to particle growth in the respiratory tract where the concentration should be much higher than in the chamber used for these studies.

Although the content of active ingredient per particle at high humidity is not known, the increase in total aerosol mass, which is considerable for the droplet aerosols, implies that the content of active ingredient shifts to particles of a larger size. A size shift similar to that observed in these high humidity studies may occur when these aerosols are inhaled into the human respiratory tract. Failure to consider particle-size changes may result in inaccurate prediction of respiratory tract deposition, especially for apparently highly unstable droplet aerosols.

[7] I. Porush, Director, Quality Assurance, Riker Laboratories, personal communication.

REFERENCES

(1) I. Porush, C. G. Thiel, and J. G. Young, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 70 (1960).

(2) T. T. Mercer, R. F. Goddard, and R. L. Flores, *Ann. Allergy*, **23**, 314 (1965).

(3) C. Hiller, M. Mazumder, D. Wilson, and R. Bone, *Am. Rev. Resp. Dis.*, **118**, 311 (1978).

(4) P. E. Morrow, *ibid.*, **110**, 88 (1974).

(5) S. S. Davis and M. D. Budd, *Int. J. Pharm.*, **1**, 303 (1978).

(6) T. T. Mercer, *Arch. Intern. Med.*, **131**, 39 (1973).

(7) W. R. Lane, in "Proceedings of the 5th International Cystic Fibrosis Conference," D. Lawson, Ed., Cambridge University, Cambridge, England, 1969, pp 103–111.

(8) S. El Golli, G. Arnaud, J. Bricardi, and C. Treiner, *J. Aerosol Sci.*, **8**, 39 (1977).

(9) J. Porstendorfer, *ibid.*, **2**, 73 (1971).

(10) M. K. Mazumder and K. J. Kirsh, *Rev. Sci. Instrum.*, **48**, 622 (1977).

(11) M. K. Mazumder, *Appl. Phys. Lett.*, **16**, 462 (1970).

ACKNOWLEDGMENTS

Supported by National Institutes of Health Grant HL20024.

The authors thank Melinda Smith and Cindy Burris for assistance in manuscript preparation, Dr. D. L. Lattin for advice during manuscript preparation, and Dr. M. K. Testerman for support and encouragement.

Substituent Constants of Azulene

M. R. LICHTENWALNER * and T. J. SPEAKER ˣ

Received September 4, 1979, from the *School of Pharmacy, Temple University, Philadelphia, PA 19140.* Accepted for publication October 24, 1979. *Present address: Center for Laboratory Medicine, Trevose, PA 19047.

Abstract □ The ionization constants in water for six 3-substituted azuloic acids were determined spectrophotometrically. Conversion of these physical constants to their pKa values allowed a set of Hammett-type σ values for the substituents on these acids to be calculated. Determination of partition coefficients for nine 1-substituted azulenes allowed Hansch-type π values to be determined, using azulene as the model compound.

Keyphrases □ Azulenes, various—substituent constants, ionization and partition coefficients, spectral characteristics □ Substituent constants—various azulenes □ Ionization constants—various azuloic acids □ Partition constants—various azulenes

The σ constant of Hammett (1) and the π constant of Hansch *et al.* (2) are useful in estimating the relative effects of substituents on the reactivity (σ) and partitioning (π) characteristics (3) of aromatic compounds. There are extensive collections of correlations of biological activity with these substituent values within one or another series of benzenoid compounds (4–6). Thus, they can guide the selection of substituents with which to modify the bioactivity and other properties of benzenoid compounds (7).

BACKGROUND

Replacement of the benzenoid nucleus with nonbenzenoid aromatic systems has been used to prepare local anesthetic esters, amides, and carboxamides (8–10) and oxidative enzyme inhibitors (11). Few experimentally determined σ or π values are available for nonbenzenoid aromatic systems. Except for the work of McDonald *et al.* (12), only scattered reports of pKa and σ values and even fewer reports of partition coefficients and π values for nonbenzenoid aromatic compounds are available. These studies include the ionization constants of azuloic acids as determined in 50% aqueous ethanol (12, 13), the ionization constants of variously substituted ferrocenecarboxylic acids (14), and the octanol–water partition coefficient of azulene (15). This small set of values is restricted further in utility because, as Bright and Briscoe (16) reported, apparent

0022-3549/80/0300-0337$01.00/0