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.

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Substituent Constants of Azulene

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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

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Table I—K_a and pKa Values of 3-Substituted Azuloic Acids and σ Constants of Azuloic and Benzoic Acids

Substituent	$K_a \times 10^6$	pKa	σ Azuloic Acid	σ_m (25)	σ_p (25)	σ_I (26)
H	7.80	5.11 ± 0.03	0	0	—	0
Cl	11.35	4.95 ± 0.05	0.16	0.37	0.23	0.47
Br	13.61	4.87 ± 0.05	0.24	0.39	0.23	0.47
$COCH_3$	17.22	4.76 ± 0.07	0.34	0.31	0.52	0.28
CHO	31.05	4.51 ± 0.04	0.60	0.36	0.22	0.31
NO_2	47.86	4.32 ± 0.06	0.79	0.71	0.78	0.63

Table II—Partition Coefficients of 1-Substituted Azulenes and π Constants for Azulene and Benzene

Substituent	K_p	log K_p	π Azulene	π Benzene
H	1671	3.22	0	0
$COOCH_2CH_3$	6730	3.83	+0.61	+0.51
COOH	754	2.88	−0.34	−0.32
$COCH_3$	743	2.87	−0.35	−0.35
CHO	434	2.64	−0.58	−0.65
NO_2	393	2.59	−0.63	−0.28
$NHCOCH_3$	301	2.48	−0.74	−0.97

pKa values and apparent σ constants calculated from them change substantially as the ethanol concentration in hydroalcoholic solutions changes. They showed that an apparent σ value could change sign as the alcohol concentration of the solution changed.

The present work involved the determination of σ and π values for azulene substituents for use in selecting candidate substances in drug design and the elucidation of drug action.

Although an acidic functional group is not essential (17), Hammett σ values are determined conveniently when the model substance is an acid. The ionization constants of some 3-substituted azuloic acids (Table I) and the partition coefficients of some 1-substituted azulenes (Table II) were measured spectrophotometrically. The corresponding σ and π constants for the azulene series were calculated from the experimental values.

EXPERIMENTAL

Materials—Azulene[1], reagents[1,2], and solvents[3] were purchased commercially. The following compounds were prepared by previously described methods: azuloic acid (18), 3-acetylazuloic acid (19), 3-bromoazuloic acid (19), 3-chloroazuloic acid (19), 3-formylazuloic acid (20), 3-nitroazuloic acid (9), acetamidoazulene (21), acetylazulene (21), azulenecarboxaldehyde (22), nitroazulene (21), and ethyl azuloate (18).

Ionization Constants—The method employed for the determination of ionization constants was adapted from that of Albert and Serjeant (23). The acid of interest was dissolved in 0.01 *N* HCl, 0.01 *N* NaOH, or a graded series of buffers having pH values that bracketed the anticipated pKa. The pH values of the resulting solutions were recorded to a thousandth of a pH unit[4], and the desired spectral characteristics were measured using a recording spectrophotometer[5]. All measurements were made between 20 and 25° to eliminate making temperature corrections (24).

All solutions except buffers were prepared fresh daily. Buffer solutions were prepared as needed from 0.1 *M* stock solutions of acetic acid, hydrochloric acid, sodium acetate, and sodium hydroxide. All water for the preparation of solutions was purified by ion exchange and reverse osmosis and then distilled in glass.

Saturated solutions of the azuloic acids in water containing 1% ethanol (to assist wetting) (23) were prepared as stock solutions from which exact 1:5 dilutions were made with 0.01 *N* HCl, acetate buffer, or NaOH. Volumes of 1% ethanol in water were similarly diluted to provide blanks. Spectra were recorded between 700 and 220 nm using cells with 10-cm light paths.

The absorption spectra of the most basic and most acidic solutions were recorded as superimposed traces on the same section of calibrated chart paper. With the aid of dividers, the optimal wavelength was found; the checklist of priorities regarding selection of this wavelength as described by Albert and Serjeant (23) was observed. All subsequent measurements were made at this wavelength.

In all instances, the pH value of each solution was measured just prior to measuring the absorbance. Sufficient time was allowed for electrode equilibration so that not more than a 0.001-pH unit drift occurred in 1 min.

Since the pKa values were assumed and found to lie between 4 and 5, acetate buffer was used; it has useful buffering properties between pH 4.1 and 5.3. The ionic strength of the buffer in the final measured solution was 8×10^{-3}. The molarity of the acids varied but was never greater than 1.5×10^{-4} mole/liter. A trial determination at pH 4.5 was made to obtain a first measure of the pKa of each acid. Once this value was obtained, a series of buffers with pH values bracketing it was used to provide a set of seven values for each compound. The averages of the K_a and pKa values determined for each compound are given in Table I together with the calculated σ values.

Partition Coefficients—The method for partition coefficients used was derived from that of Hansch and coworkers (25). Monosubstituted azulenes were partitioned between *n*-octanol and water. For the determination of the partition coefficient of azuloic acid only, the water was adjusted with acetic acid to a final acid concentration of 0.01 *N*; the resulting pH was about 3.4. This acid concentration was low enough that it did not interfere with the analysis but was sufficiently high to ensure that azuloic acid was <2% ionized.

Because the specific method employed is critical to the accuracy of the values determined, the procedure is described in detail. The octanol was purified by sequentially washing with nominally 2 *N* NaOH, 4 *N* H_2SO_4, 2 *N* Na_2CO_3, and water followed by distillation under reduced pressure. The water used for partitioning studies was purified by ion exchange, reverse osmosis, and distillation in glass and then was vigorously shaken with purified octanol to ensure saturation. The resulting milky suspension was passed through two 0.22-μm filters[6] saturated with octanol to assist in breaking the emulsion. The phases were separated and stored in ground-glass stoppered bottles.

A few milligrams of each purified 1-substituted azulene was dissolved separately in about 25 ml of water-saturated octanol. These solutions were used as stock solutions. Beer's law plots were established for each compound; correlations between absorbance values and concentration typically had values of r^2 greater than 0.999. A 5.00-ml aliquot of the stock solution and two dilutions of it were pipetted into volumetric flasks containing 1000.0 ml of octanol-saturated water. The mixtures were hand inverted 400 times and allowed to separate, and as much of each octanol layer as possible was withdrawn with the aid of a Pasteur pipet. The octanol layers were clarified by centrifugation at 1000×*g* for 30 min. The absorbances of the octanol solutions were measured immediately at three different absorbance maxima of the azulenoid, and the azulenoid concentration was calculated. Results are reported as the average of these nine values in Table II, expressed as partition coefficients, together with the corresponding values of π.

RESULTS AND DISCUSSION

The ionization constants of six azuloic acids were determined spectrophotometricallly using the method of Albert and Serjeant (23), and the corresponding pKa and Hammett σ values were calculated. These values are presented in Table I together with the corresponding benzenoid σ values. Azuloic acid, with a pKa of 5.11, is a weaker acid than naphthoic acid (pKa 4.17) (27), which is an isomer, or than benzoic acid (pKa 4.18) (27). This finding is consistent with the relatively high charge density of the parent hydrocarbon, azulene, as calculated by the Hückle LCAO–MO (linear combination of atomic orbitals–molecular orbitals) approach (28).

The observation that azuloic acid is about 10-fold weaker as an acid than either naphthoic or benzoic acid was confirmed indirectly by Leermakers and Bowman (13) who determined the apparent pKa of azuloic acid in 50% ethanol to be 6.67. A similar determination of the apparent pKa of benzoic acid gave 5.78.

Comparison of the σ values of the benzene and azulene series shows them to be substantially different. It is possible that inductive and resonance components may correlate with these values; however, the limited number of compounds in this study precludes such a test. The reversal

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Matheson, Coleman and Bell, Norwood, Ohio.
[3] Fisher Scientific Co., Pittsburgh, Pa.
[4] Model 801 pH/mv meter, Orion Research, Cambridge, Mass.
[5] Model 15, Cary Instruments Division, Varian Associates, Monrovia, Calif.
[6] Millipore.

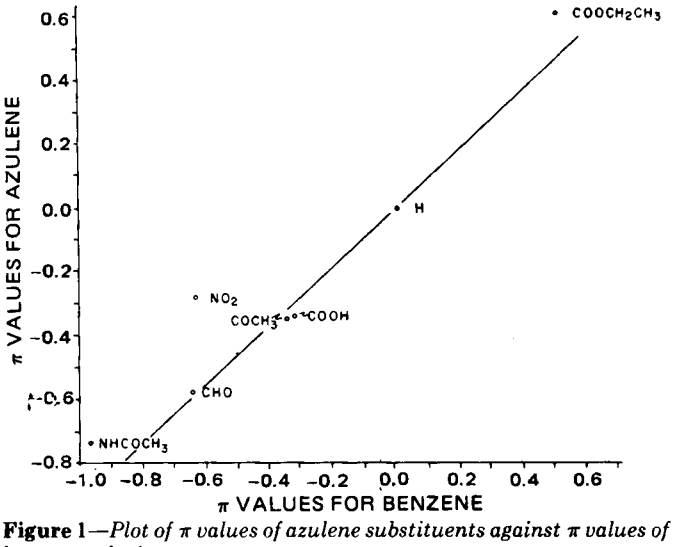

Figure 1—*Plot of π values of azulene substituents against π values of benzene substituents.*

of rank for the acetyl and formyl groups compared to σ_p is deserving of comment since the acetyl and formyl groups usually are considered similar in electronic behavior. They are, however, sterically different, and the difference in σ values may be attributed to a steric interaction between the methyl portion of the acetyl group and the *peri*-proton. Such steric interaction can reduce the coplanarity of the acetyl group with the ring bearing it, thereby decreasing the conjugation between the ring and the carbonyl function. Such a reduction of conjugation would reduce the electron-withdrawing ability of the carbonyl group. This interpretation is consistent with the observation that the UV absorbance maximum of acetylazulene shows a hypsochromic shift of about 7 nm with respect to that of formylazulene.

Another disparity between azulenoid and benzenoid σ values is seen with the bromo and chloro substituents. The benzenoid σ values for these substituents are nearly identical, but the azulenoid values differ distinctly. While this effect may be attributable to differing degrees of steric interaction with the *peri*-proton on the adjacent ring, it is explained more satisfactorily by the greater conjugative effect of the chloro over the bromo substituent.

The octanol–water partition coefficients of seven azulenoid compounds were determined spectrophotometrically, and the corresponding π values (2) were calculated. These values, together with the corresponding benzenoid π values, are presented in Table II.

Comparison of the azulenoid and benzenoid π values in Table II and Fig. 1 shows that a fair correlation exists between the two values (r^2 = 0.841). The correlation improves markedly (r^2 = 0.966) if the values for the nitro substituent are omitted from the regression equation:

$$\pi_{Az} = 0.925\ \pi_{Bz} + 0.041 \qquad \text{(Eq. 1)}$$

where the subscripts Az and Bz indicate azulenoid and benzenoid values, respectively. It is reasoned that the strongly electron-withdrawing character of the nitro group further accentuates the charge separation that exists in azulene itself (μ = 1*D*), thus increasing both its dipole moment and its water solubility.

In summary, while the π constants for azulene may be predicted reasonably and accurately from benzene π values except when the substituents are highly polar, the values of the azulene σ constants may not be inferred reliably from σ values of their benzenoid counterparts.

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