Correlations between Substituent Parameters of 4-Substituted Benzoic Acids and Their *In Vitro* Dissolution and Partitioning

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Abstract □ The *in vitro* dissolution and partitioning of some 4-substituted benzoic acids from nondisintegrating disks was investigated using a water–octanol system. The rates of appearance of benzoic acids in the aqueous phase differed from the rates of appearance in octanol, probably due to back-transfer from the octanol phase. The suitability of the procedure for investigating the influence of molecular modification on *in vitro* dissolution and partitioning is considered. Correlations among the rates of appearance in octanol, the rates of appearance in an aqueous phase, and substituent parameters of benzoic acids were investigated. The best statistical fit was obtained using molecular orbital substituent indexes.

Keyphrases □ Benzoic acids, 4-substituted—*in vitro* dissolution and partitioning from nondisintegrating disks □ Dissolution—4-substituted benzoic acids, nondisintegrating disks, *in vitro* □ Partitioning—4-substituted benzoic acids, nondisintegrating disks, *in vitro* □ Structure–activity relationships—4-substituted benzoic acids, *in vitro* dissolution and partitioning from nondisintegrating disks

There has been considerable interest in the development of techniques capable of simulating drug absorption processes from solid dosage forms. Absorption processes involve dissolution of the drug followed by partitioning of the dissolved drug into a lipid membrane. However, these processes generally have been considered independently as dissolution (1, 2) and as partitioning systems (3). A single procedure combining the two systems was reported for simulation of the overall absorption process (4). The report was confined to the dissolution and partitioning of one drug.

In this work, the dissolution and partitioning of some substituted benzoic acids were measured. Relationships between the rates of appearance in aqueous and octanol phases and substituent parameters (5) of the compounds were investigated. Such relationships would be useful for the assessment of the effects of molecular modification on *in vitro* availability of drugs as measured by partitioning rates.

Previous correlations between structure and biological activity were limited to studies in which drugs were administered in solution. Results from these *in vitro* correlations may indicate the feasibility of assessing effects of molecular modification on biological activity following administration of solid dosage forms.

EXPERIMENTAL

Materials—Nondisintegrating disks of benzoic acid[1] and its 4-hydroxy, chloro, bromo, iodo, nitro, and methoxy[2] derivatives were prepared in a hydraulic press[3]. The compression pressure was 300 kg/m^2. Laboratory grade 1-octanol[2], which did not interfere with spectrophotometric assays, was used as the organic solvent; 200-ml volumes of 0.1 *N* HCl were used as the dissolution medium.

Apparatus—A modified rotating disk-type apparatus was used. The dissolution cell was described previously (6). The stirrer shaft was modified to take account of the two-phase system. A machined titanium disk holder was fitted into the base of the stirrer shaft so that a constant surface of disk was exposed to the dissolution medium.

Three propeller blades were attached to the shaft 1.5 cm above the upper edge of the disk holder. When octanol was in the cell, the lower edge of each blade was 2.3 cm above the octanol–aqueous phase boundary. The stirrer shaft was driven by an 80-rpm asynchronous motor[4].

Procedure—Octanol was presaturated with 0.1 *N* HCl; 0.1 *N* HCl was presaturated with octanol for 24 hr at 25 ± 0.1° prior to use.

A disk was fitted into the disk holder. Then 200 ml of 0.1 *N* HCl solution was placed in the dissolution cell, and the unit was assembled but not stirred. An equal volume of octanol was superimposed on the aqueous phase. After the stirrer motor was started, samples of octanol were removed at appropriate time intervals and assayed spectrophotometrically[5]. An equal volume of octanol was added to the cell after sampling.

A flow-through procedure was used to sample the aqueous medium. The solution was pumped by way of 1-mm diameter polyethylene tubing into a 0.1- or 1.0-cm path length flowcell in a spectrophotometer[6]. The flow rate of the solution was 7 ml/min. At any one time, less than 2 ml of the aqueous solution was out of the bulk solution.

RESULTS AND DISCUSSION

The rates of appearance of the benzoic acids in the aqueous phase were obtained from slopes of the concentration of drug dissolved in the aqueous phase as a function of time. Rates of appearance followed zero-order kinetics for at least the first 30 min prior to the attainment of a steady-state concentration in the aqueous phase. Rates of appearance in the octanol phase were obtained from slopes of the drug concentration in octanol against time. After an initial lag period, the rate of appearance in the octanol phase also could be described by zero-order kinetics.

A typical plot of the drug concentration in the aqueous and organic phases as a function of time is shown in Fig. 1. Rate constants for appearance of each compound in the aqueous and octanol layers are given in Table I. Included in the same table are rate constants for dissolution into an aqueous phase alone. The dissolution rate of drug into 0.1 *N* HCl and its appearance in the organic solvent phase were not equivalent as reported previously (2). However, according to Niebergall *et al.* (7), the results for benzoic acid into cyclohexane–octanol were a specific example of a general case.

The rate constant for the appearance in octanol of compounds other than benzoic acid and 4-hydroxybenzoic acid are similar to those for dissolution into an aqueous phase alone. This discrepancy can be accounted for. Khalil and Martin (3) reported that a lipid phase need not act as a perfect sink, and back-transfer of the drug from the lipid to aqueous phase can occur. Consequently, the appearance in octanol would be less than expected if back-transfer did not occur. Such a situation does not detract from using this system for assessment of the effects of structural changes in a molecule on its dissolution and partitioning. It does emphasize the

[1] Fisons A. R.
[2] Laboratory reagent, British Drug Houses Ltd.
[3] Apex type A14.
[4] Crouzet Ltd., France.
[5] Unicam SP 500.
[6] Cecil type CE202.

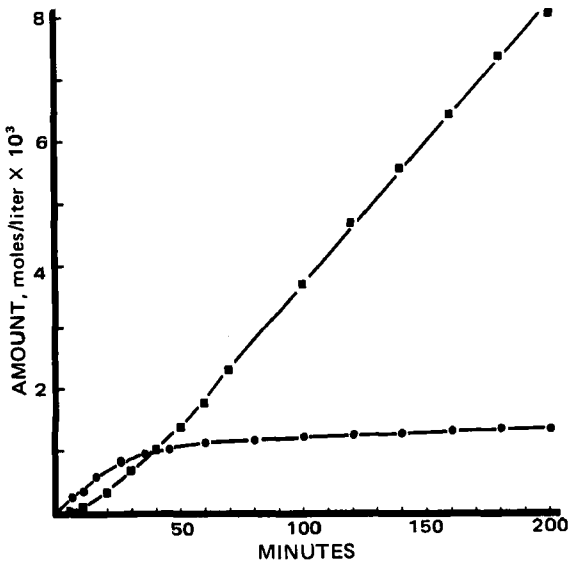

Figure 1—*Amounts of 4-hydroxybenzoic acid in 0.1* N *HCl* (●) *and octanol* (■) *as a function of time.*

need for care in the choice of an organic solvent as a model lipid layer.

Octanol has been used successfully as a reference solvent in studies correlating structure with biological activity (5). Substituent parameters used in quantitative structure–activity studies include π (where $\pi = \log P_x - \log P_H$; P_H is the partition coefficient of a parent compound, and P_x is that of a derivative); σ, the Hammett constant; and molecular orbital substituent indexes (8). Values of substituent indexes are given in Table II.

The relationship between the rate constant for appearance (k_p) in octanol and π is:

	n	r	s	F	
$\log k_p = -1.70(\pm 0.36)\pi - 5.17$	7	0.905	0.505	22.6	(Eq. 1)

when using a combination of σ and π, one obtains:

	n	r	s	F	
$\log k_p = -1.56(\pm 0.20)\pi - 0.63(\pm 0.05)\sigma - 5.15$	7	0.929	0.491	12.6	(Eq. 2)

Recently, Cammarata and Rogers (8) correlated π values for benzoic acids with appropriate electronic indexes. Excluding the values for nitro and iodobenzoic acids, the following equation relates the appearance in octanol to molecular orbital substituent indexes:

	n	r	s	F	
$\log k_p = 1.53(\pm 0.06)\ \Sigma_r' \lvert Q_r^T \rvert - 0.90(\pm 0.09)\ \Sigma_r' S_r^E - 3.99$	5	0.992	0.199	66.9	(Eq. 3)

There is only a slight improvement in correlation by inclusion of π and σ in the same relationship. The best fit is seen when molecular orbital substituent indexes are used. These indexes are specific for the substituent groups of benzoic acid, and this specificity may account for the improved fit over that seen with the more general π. The correlation of the partition rate with electronic indexes indicates that the change in the solvation shell around the substituent controls partitioning from a polar to nonpolar environment.

Correlations between rates of appearance (k_0) into the aqueous phase beneath octanol with the same substituent parameters lead to the following equations:

	n	r	s	F	
$\log k_0 = -1.74(\pm 0.36)\pi - 5.48$	7	0.910	0.503	24.1	(Eq. 4)
$\log k_0 = -1.60(\pm 0.20)\pi - 0.67(\pm 0.06)\sigma - 5.47$	7	0.935	0.479	14.0	(Eq. 5)
$\log k_0 = 1.69(\pm 0.07)\Sigma_r' \lvert Q_r^T \rvert - 0.92(\pm 0.09)\Sigma_r' S_r^E - 4.34$	5	0.992	0.199	66.9	(Eq. 6)

Table I—Rate Constants[a] for the Appearance of 4-Substituted Benzoic Acids in 0.1 *N* HCl and Octanol

Substituent	$k_0 \times 10^4$, moles/min/liter	$k_w \times 10^4$, moles/min/liter	$k_p \times 10^4$, moles/min/liter
H	13.67	38.10	30.66
OH	25.97	61.90	45.58
Cl	0.14	0.40	0.38
Br	0.06	0.15	0.16
I	0.03	0.06	0.06
NO_2	0.60	1.30	1.27
CH_3O	0.80	1.75	1.74

[a] k_0 is a zero-order rate constant for appearance in 0.1 *N* HCl layered with octanol; k_w is a zero-order dissolution rate constant into 0.1 *N* HCl; and k_p is a zero-order rate constant for appearance in octanol.

Table II—Substituent Parameters for 4-Substituted Benzoic Acids

Substituent	π[a]	σ[a]	$\Sigma' S^E$[b]	$\Sigma' \lvert Q^T \rvert$[b]
H	0	0	0.857	0.121
OH	−0.30	−0.37	1.870	0.945
Cl	0.87	0.23	2.863	0.081
Br	0.98	0.23	3.083	0.100
I	1.14	0.18	—	—
NO_2	0.02	0.78	—	—
CH_3O	0.08	−0.26	3.148	0.566

[a] From Ref. 5. [b] From Ref. 8.

The regression coefficients in each series are similar to those for (k_p), an indication of a rate-limiting process. Again, the best fit is obtained with electronic indexes. However, this is hardly surprising since these indexes are essentially a representation of solvation energy, which is a controlling factor in dissolution. The interrelationship between dissolution and π can be accounted for in the following manner. Hansch *et al.* (9) related the solubility of an organic liquid in water (S) to its partition coefficient between octanol and water (P) as:

$$\log \frac{1}{S} = a \log P + b \qquad \text{(Eq. 7)}$$

The dissolution rate of drug, k_{diss}, from a disk of fixed area under sink conditions may be expressed as:

$$k_{diss} = k' S_{sat} \qquad \text{(Eq. 8)}$$

where S_{sat} is the saturation solubility, and k' is a first-order rate constant. In saturated solutions with low solubility:

$$\log \frac{k'}{k_{diss}} = a \log P + b \qquad \text{(Eq. 9)}$$

i.e., the dissolution rate is related to the partition coefficient and π. While correlations between dissolution and partitioning and substituent parameters have been found, inferences regarding the effects of molecular modification on *in vivo* absorption cannot be made from these relationships. Nonetheless, the dissolution and partitioning system can be used as a screen for the effects of molecular modification on *in vitro* availability. Correlations with *in vivo* data may be possible when biological data are obtained following the administration of congeneric series of drugs as solid dosage forms.

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Sinoacutine from *Glaucium contortuplicatum* Boiss.

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Abstract □ A phytochemical investigation of *Glaucium contortuplicatum* Boiss. (Papaveraceae) resulted in the isolation of sinoacutine from this plant for the first time. Spectral evidence for the identity of the isolated compound as sinoacutine is presented.

Keyphrases □ *Glaucium contortuplicatum*—phytochemical investigation, whole plant extract, sinoacutine isolated □ Sinoacutine—isolated from whole plant extract of *Glaucium contortuplicatum* □ Alkaloids—sinoacutine isolated from whole plant extract of *Glaucium contortuplicatum*

A literature survey revealed that no chemical work had been reported on the title plant. A phytochemical investigation was initiated, and the isolation of dicentrine nitrate was reported (1).

The isolation and identification of sinoacutine from this plant are now reported. Sinoacutine was first isolated from the Chinese drug "Ching-fengteng," *Sinomenium acutum* Redh *et* Wils. (Menispermaceae) (2), and later from *Cassytha pubescens* R. Br. (Lauraceae) (3), *Croton flavens* L. (Euphorbiaceae) (4), *Corydalis pallida* var. *tenuis* (Fumariaceae) (5), and *Cocculus carolinus* D.C. (Menispermaceae) (6).

This is the first reported occurrence of sinoacutine in *Glaucium contortuplicatum*. Recently, it was reported to be present in *G. flavum* (7). Sinoacutine had been synthesized (8) and found to elicit mild antitussive properties (9).

EXPERIMENTAL[1]

A quantity (20 kg) of the air-dried and powdered whole plant was extracted with hot petroleum ether (bp 30–60°) for 36 hr and then with hot methanol for 72 hr. The alcoholic extract, after charcoal treatment and concentration, gave a large amount of inorganic nitrates and an alkaloid, dicentrine nitrate (1), which were removed by filtration. After trituration of the filtrate with acetone to remove sugars, the acetone-soluble portion was evaporated to dryness *in vacuo*, dissolved in water, made basic with ammonium hydroxide to pH 9, and extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and chromatographed over a column containing neutral aluminum oxide S.

Elution with benzene and treatment of the dried residue with acetone yielded colorless prisms of sinoacutine (150 mg), mp 194–197° dec. [lit. (4) mp 197–199°]; $[\alpha]_D^{26.5}$ −117.64° (c 0.81, ethanol) [lit. (4, 6) $[\alpha]_D^{23}$ −115.0° (c 1.03, ethanol), $[\alpha]_D^{28}$ −115.8° (c 1.0, ethanol)]; UV: λ_{max} (methanol) 214 (log ϵ 4.22), 240 (4.21), and 280 (3.72) nm [lit. (6) UV: λ_{max} (ethanol) 214 (log ϵ 4.40), 245 (4.30), and 280 (3.80) nm]. Major absorptions in the IR spectrum were at ν_{max} (KBr) 3450 (broad OH stretch), 1670, 1640, and 1610 cm^{-1} (cyclohexadienone system).

The NMR spectrum in deuterochloroform showed a three-proton singlet at δ 2.45 (NCH_3), two three-proton singlets at δ 3.73 and 3.84 (2-OCH_3), two one-proton singlets at δ 6.36 and 7.56 (two olefinic protons), and two barely resolved proton peaks at δ 6.70 and 6.75 (two aromatic protons), which were in close agreement with the literature values (6, 10). In addition, the four methylene bridge protons were located as two triplets centered at δ 1.16 and 3.66 as determined by irradiation.

Deuterium exchange experiments permitted the assignment of a broad peak centered at δ 6.2 to the phenolic proton. The remaining one methine and one methylene protons appeared between δ 2.0 and 3.4 but were complex and somewhat obscured by the NCH_3 signal; no further attempt was made to assign these protons. The mass spectrum gave a molecular ion at *m/e* 327, which was also the base peak, followed by other significant peaks at *m/e* 312 ($M^+ - CH_3$, 40%), 299 ($M^+ - CO$, 22%), and 284 ($M^+ - CO—CH_3$, 55%). The fragmentation pattern is similar to that of salutaridine (11), the enantiomer of sinoacutine.

The isolate was compared with a reference sample of sinoacutine[2] and was found identical in all respects (IR, UV, mass spectrometric, TLC, melting point, and R_f data), and a mixed melting point was not depressed.

[1] The plant material was collected near Shiraz, Iran, in April 1971 and was identified as *Glaucium contortuplicatum* Boiss. (Papaveraceae) by M. H. Bokhari, Department of Biology, Pahlavi University. A voucher specimen (DPH-G2740) representing the collection was deposited in the Department of Pharmacology Herbarium, Pahlavi University, Shiraz, Iran.

UV spectra were taken using a Beckman model D-G grating spectrophotometer. IR spectra were taken using a Beckman model IR 18 A spectrophotometer. Mass spectra were determined using a single-focusing Hitachi Perkin-Elmer model RMU-6D mass spectrometer, operating at 70 ev. NMR spectra were taken using a Varian T-60 instrument operating at 60 MHz. Optical rotations were recorded using a Carl Zeiss optical polarimeter. The melting-point and mixed melting-point determinations were made using a Kofler hot-stage melting-point apparatus.

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[2] A reference sample of sinoacutine was provided by D. J. Slatkin, Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh.