Quantitative Structure–Activity Relationships and Carminative Activity

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Abstract □ Carminative activities of 34 alcohols, esters, ethers, phenols, and carbonyl compounds were determined using the guinea pig isolated ileum preparation and are expressed as the ability to produce a 50% inhibition (ID_{50}) of a standard response to carbachol. Aqueous solubilities were measured at 37° using either UV absorption or GLC. The ratios of solubility to ID_{50} were reasonably constant, suggesting nonspecific biological activity, similar to that previously observed with general anesthetics. Hansch analysis indicated that carminative activities were largely controlled by solubility, as indicated by octanol–water distribution coefficients. The principal remaining factor appeared to be the steric availability of the oxygen atom in the functional group of the compound.

Keyphrases □ Carminative activity—various classes of compounds, related to solubility □ Solubility—various classes of compounds, related to carminative activity □ Structure–activity relationships—various classes of compounds, carminative activity related to solubility

Ferguson (1) investigated various groups of compounds, each having a common pharmacological action; while the biologically effective concentrations of the compounds varied over 1000-fold, the ratios C/C_s for nonvolatile compounds and p/p_s for vapors varied only 10-fold. The symbols C and p represent the biologically active concentration and vapor pressure, respectively; C_s is the aqueous solubility, and p_s is the saturated vapor pressure. Ferguson equated these ratios with thermodynamic activity. This behavior was observed almost exclusively in homologous series, in which biological activity changes with molecular weight, and groups of chemically related molecules such as phenols (2) or quaternary ammonium compounds (3).

Until recently, anesthetics were the only pharmacological group in which molecules of widely different structure had been observed to follow Ferguson's principle. However, Evans *et al.* (4) suggested that the carminatives could fit a similar situation. These substances have a common pharmacological action but no characteristic chemical feature. Since their physical properties are similar (they are all volatile liquids, mainly lipophilic in character but having significant aqueous solubilities), it was considered that their biological activities may be primarily dependent on their physical properties.

In a preliminary investigation (4), the antispasmodic activities of seven compounds, camphor, carvone, cineole, cinnamaldehyde, eugenol, linalol, and thymol, were evaluated and related to their aqueous solubilities. This paper is an extension of that work, in which a larger range of compounds was examined.

EXPERIMENTAL

Materials—All materials were the purest products commercially available and were used without further purification.

Solubility Determinations—Solubilities were taken from Seidell (5) when available. For the remaining compounds, a moderate excess of solute was stirred continuously with water for up to 10 hr in a sealed conical flask immersed in a thermostatically controlled water bath at 37°. The method of sampling varied with the solute.

Saturated solutions of solid solutes were sampled by pipet and immediately passed through a 0.45-μm membrane filter. Mixtures containing liquid solutes were immersed overnight in a water bath at 37° to allow complete separation. When the solute was lighter than water, the solution and excess solute were first transferred to a separator and subsequently sampled through the tap.

Compounds containing conjugated chromophores were assayed by UV spectrophotometry. Samples of saturated solutions were diluted to suitable volumes with spectroscopic ethanol, and absorptions were measured at the appropriate λ_{max}. Wavelength maxima and adherence to Beer's law were established in preliminary experiments.

The remaining compounds were determined by GLC using a hydrogen flame-ionization detection system. Two columns were used, one consisting of 10% Apiezon L on 80–100-mesh Chromosorb W and the other consisting of Poropak Q polymer beads. The columns were conditioned before use for 48 hr at 210–220° with a nitrogen flow rate of 60 ml/min.

Octanol–water distribution coefficients were obtained or calculated (6).

Carminative Activity—The biological activity of each carminative was determined using the guinea pig isolated ileum preparation suspended in aerated Tyrode physiological solution at 37°. A standard submaximal response to the agonist carbachol was first obtained, and then the activity of the carminative was determined by its ability to inhibit the standard response to carbachol.

Three doses of carminative were selected that inhibited the response to the agonist by 20–80%, and six determinations were performed at each dose level. The percentage inhibition of the standard agonist response was plotted against the log of the corresponding dose of carminative. From this plot, the carminative concentration required to inhibit the agonist response by 50% (ID_{50}) was estimated.

The carminative solution was maintained at 37° throughout each investigation.

RESULTS AND DISCUSSION

The traditional carminatives are all essential oils. These complex mixtures, because of their natural origin, are of variable composition and are difficult to standardize. Therefore, known constituents of essential oils and pure chemical compounds of established carminative activity were selected.

Thirty-four compounds, including alcohols, esters, ethers, phenols, and carbonyl compounds, were examined. Their biological activities and solubilities are presented in Table I together with the ratios C/C_s. These ratios are more scattered than those given by Ferguson (1) for general anesthetics, although most of them lie between 0.1 and 1.0; the corresponding ID_{50} values vary over 100-fold. Ferguson's assumptions were criticized recently (7), and the present distribution of "thermodynamic activities" are of the same order as the recalculated values for general anesthetics.

The classical examples of nonspecific biological activity (1–3) represent systems in which equilibrium would be expected to be established rapidly between the site of action and the surrounding environment, a necessary criterion for this concept. Hansch *et al.* (8) suggested that equilibrium is not established in most cases of nonspecific biological action and that biological activity is dependent on the probability of a given molecule reaching the site of action. It was further shown that such a model could be expressed in the general form:

$$\log BR = a \log P + b(\log P)^2 + c \qquad \text{(Eq. 1)}$$

where BR represents biological response; P is the partition coefficient

Table I—Physical and Biological Data

Compound	ID_{50}[a], $M \times 10^3$	Solubility, C_s, at 37°, $M \times 10^3$	Thermodynamic Activity, C_s/ID_{50}	log P
Benzaldehyde	54.4 (0.3)	21.1	2.57	1.48
Butyl acetate	43.4 (0.6)	50.2	0.86	1.74
Camphor	6.1 (0.6)	16.3	0.37	—
Carvone	10.5 (0.4)	11.0	0.96	—
Catechol	94.8 (0.7)	1966[b]	0.05	0.95
Cineole	13.9 (0.3)	9.1	1.54	—
Cinnamaldehyde	12.1 (0.5)	9.1	1.33	—
Citral	7.6 (0.5)	8.8	0.86	—
o-Cresol	22.8 (0.3)	285[b]	0.08	1.95
m-Cresol	28.9 (0.3)	231[b]	0.12	1.99
p-Cresol	28.8 (0.3)	209[b]	0.14	1.93
Dibutyl ether	58.5 (1.5)	17.2	3.40	3.06
Diethyl ether	260 (2.6)	712	0.37	0.80
Diethyl malonate	62.0 (0.6)	145	0.43	—
Diisopropyl ether	196 (2.1)	79.2	0.93	1.63
3,4-Dimethylphenol	12.4 (0.4)	25.3	0.49	2.42
Di-*n*-propyl ether	99.3 (0.7)	107	2.47	2.03
Ethyl acetate	260 (3.1)	817	0.32	0.70
Ethyl vinyl ether	62.2 (0.7)	139	0.45	1.04
Eugenol	3.7 (0.5)	40.2	0.09	2.99
1-Hexanol	33.8 (0.5)	41.0[b]	0.82	2.03
Isopropyl acetate	110 (0.7)	193	0.58	1.02
Linalol	4.7 (0.7)	38.0	0.12	—
Menthol	7.4 (0.3)	8.6	0.86	3.31
o-Methoxyphenol	54.4 (0.5)	10.6	5.12	1.90
p-Methoxyphenol	47.5 (0.3)	4.0	11.8	1.34
1-Pentanol	77.3 (0.4)	213	0.36	1.16
2-Phenoxyethanol	126 (1.1)	261	0.48	1.16
n-Propyl acetate	114 (0.7)	196	0.57	1.50
Quinol	123 (0.7)	193[b]	0.64	0.55
Resorcinol	89.3 (1.2)	6609[b]	0.01	0.79
Salicylaldehyde	20.0 (0.7)	208[b]	0.10	1.76
Thymol	2.2 (0.4)	59.9	0.04	3.30

[a] Figures in parentheses represent confidence limits ($p' = 0.05$). [b] Taken from Ref. 6.

between octanol and water; and a, b, and c are constants. The Ferguson effect was considered a special case, where $b(\log P)^2$ is very small in comparison with the other terms. In the present work, when most of the 34 compounds were considered, Eq. 2 was found to be the best correlation between biological activity, expressed as log $1/ID_{50}$, and log P:

$$\log 1/ID_{50} = 0.632 + 0.447(0.081) \log P \qquad (5.51)$$

n	r	s
31	0.715	0.353

(Eq. 2)

where $F_{1,29} = 31\ \alpha(0.001) = 13.4$. The heading n represents the number of compounds considered, r is the correlation coefficient, and s is the standard deviation. Three compounds were omitted because octanol–water distribution coefficients were not available. The figure in parentheses following the coefficient in log P represents its standard error ($p' = 0.05$), and the figure in parentheses below the coefficient represents the ratio of the coefficient to the standard error. Comparison of this value with the t value obtained from statistical tables indicates that the probability that the relationship is coincidental is negligible. This finding is confirmed by the figures following the equation, which are results of analysis of variance of the observed results about the regression followed by an F test and which indicate a highly significant correlation. Consideration of a term in $(\log P)^2$ did not improve the relationship significantly.

Carminative activity thus appears to be dependent on the balance between aqueous and nonaqueous solubilities; but since Eq. 2 accounts for only 50% of the variation, other factors must also be important. Equation 3 expresses the relationship between ID_{50} and log P when only alcohols and phenols are considered; it accounts for 90% of the variation:

$$\log 1/ID_{50} = 0.429 + 0.598(0.052) \log P$$

n	r	s
17	0.947	0.175

(Eq. 3)

where $F_{1,15} = 131\ \alpha(0.001) = 16.6$. Therefore, the factors causing the additional variation in Eq. 2 must be linked to the nature of the functional groups in the molecule, *i.e.*, hydroxyl, carbonyl, and ester and ether oxygen, since the correlation is considerably improved when compounds containing one specific functional group are considered as in Eq. 3.

The success of Eq. 3 is somewhat surprising because some of the compounds involved contain more than one functional group (*e.g.*, catechol and methoxyphenol). Therefore, the inference is that only one hydroxyl group is required and that hydroxyl has a considerably greater influence on carminative activity than the ether group. The relationship obtained when compounds containing one hydroxyl only are considered is not significantly better than Eq. 3, supporting this view.

Neither esters nor ethers followed Eq. 3, but each group of compounds yielded its own analogous correlation between ID_{50} and P. Since alcohols, which are weakly charged in comparison with phenols, fitted smoothly into the same correlation as the phenols (Eq. 3), it is unlikely that the effect of hydroxyl on carminative activity is influenced by the electronic charge on the group. Similarly, the failure of the ethers to fit the same relationship as the hydroxyl compounds is more likely to result from hindrance to ether oxygen than from electronic considerations, and the group that replaces the hydrogen of hydroxyl to form an ether would be expected to bring about this hindrance.

The contribution of the carbonyl group to carminative activity must be greater than either hydroxyl or ether, because the ID_{50} values of this group were lower. Therefore, it was assumed that the carbonyl oxygen of esters, rather than the ether oxygen, was the center of biological activity. Octanol–water distribution coefficients were available for only two of the carbonyl compounds. This number was not sufficient to submit this group to a similar treatment to that described.

In summary, the carminative activities of alcohols, esters, ethers, and phenols are largely due to their solubilities, as indicated by their octanol–water distribution coefficients. Other factors are involved, the principal one being the nature of the oxygenated groups in the molecule, probably arising from the steric availability of the oxygen therein. Where two such groups are present in one molecule, carminative activity is almost exclusively influenced by only one of them. The order of increasing influence is O < OH < C=O.

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