incorporation of appropriate substituents. This compd (**9**) is itself, however, highly effective for antagonizing the effects of nicotine, thiosemicarbazide, and pentylenetetrazole in mice, being only slightly less active than diazepam (**1**) in this regard. On the other hand, **9** was less active than diazepam in the ES and Tr tests and for potentiating the effects of EtOH and pentobarbital. This selective activity was enhanced in the 6-(*o*-chlorophenyl) derivative **17**. This compd was as good or better than diazepam for antagonizing the effects of nicotine, thiosemicarbazide, and pentylenetetrazole in mice, but had little or no activity in any other test.

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Molecular Orbital Calculations on Coumarins and the Induction of Drug-Metabolizing Enzymes

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A structure–activity relationship study has been carried out for the capability of coumarin and 9 of its derivatives to induce drug-metabolizing enzymes in the rat liver. Extended Hückel molecular orbital calculations have been performed to generate various electronic parametric descriptions of the series. Multiple regression analyses, as described by Hansch, simultaneously involving the experimentally determined partition coefficient as well as either the energy of the highest occupied molecular orbital or the first electronic transition energy and the net charge on the carbonyl or α-pyran moiety of the lactone ring of the coumarinic structure, yielded significant multiple correlations ($p < 0.01$) accounting for, resp, over 86 and 91% of the variation in induced enzyme activity. A mechanism of action involving a charge-transfer and/or H-bonded complex formation with at least partial localization of the activity related site to the carbonyl and/or α-pyran moiety of the lactone ring seems to be implicated.

The induction of hepatic drug-metabolizing enzymes by closely related coumarin derivatives has been found to vary widely.[1] Attempts to demonstrate any correlation between the inducing capability of these compounds and their lipid solubility, absorption, or metabolism have been unsuccessful.[1,2] However, electrochemical properties of drugs may be responsible for, or contribute to their pharmacological action.[3-7] Therefore, it has been suggested that this may elucidate the differences in the hepatic action of various coumarins.[1]

A mathematical model relating biological activity to chemical constitution developed by Hansch and coworkers[8] has been applied to numerous biological systems.[8-13] This model can be represented by eq 1

$$\log BA = -a\pi^2 + b\pi + c \log K_{CR} + d \qquad (1)$$

where BA = biological activity, π = log (P_x/P_h), P_x and P_h are partition coefficients of the derivative and parent compound, resp, K_{CR} = rate or equilibrium constant of the critical reaction, and a, b, c, d are constants for a given activity and system.

The model accounts for the arrival of the biologically active compound to its site of action by random walk (π and π^2 terms). The component involving the critical reaction is free energy dependent and may be expressed in terms of a linear combination of the parameters[12-14] that contribute to the total free energy change of the reaction.

These contributions have been tested empirically[12] and they could be of various types: hydrophobic, electronic, steric, H bonding, charge-transfer complexing, or others. Good correlations of the form described by eq 1 have been found using a large variety of quantitative parameters descriptive of one or other of the above factors including some quantum mechanically calcd ones.[13] Accordingly, molecular orbital calculations have been performed on coumarin and 9 of its derivatives, hoping that such an approach might help to elucidate the variation in their activity.

Experimental Section

Molecular Orbital Calculations.—Molecular orbital calcns of the extended Hückel type were performed on the IBM 7094-II computer using a program previously described by Hoffman.[15]

Input to the program consisted of Cartesian coordinates of the atoms, Slater exponents, and Coulomb integrals, H_{ii} for the 2s and 2p orbitals of C and O and the 1s orbital of H. The overlap matrix, **S**, for 1 s and 3 p orbitals for each C and O atom and an s orbital on each H atom was computed explicitly. The

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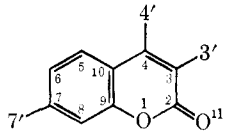
TABLE I
PARAMETERS OF ATOMIC ORBITALS

Atom	Orbital	Slater exponent	Coulomb integral
H	1s	1.000	−13.60
C	2s	1.608	−21.01
	2p	1.568	−11.27
O	2s	2.246	−32.30
	2p	2.227	−14.80

Determination of Enzyme-Inducing Activity.—Drug metabolizing enzymes were induced by coumarin derivatives given to albino rats daily, for 7 days, in oral doses of 1 mmole/kg body weight, dissolved in arachis oil, 5 ml/kg. Control animals were administered arachis oil, 5 ml/kg, orally. Coumarin 3-hydroxylase activity was assayed in liver homogenates using the substrates coumarin and 4-methylcoumarin as described elsewhere.[1,24]

Measurement of Partition Coefficients.—This has been carried out previously[1] using the method of the reverse phase tlc and

TABLE II
DISTRIBUTION OF NET ATOMIC CHARGES OF VARIOUS COUMARINS

Coumarin 

Compd	Position													
	1	2	3	4	5	6	7	8	9	10	11	3′	4′	7′
Coumarin	−0.701	1.294	−0.173	0.068	−0.075	−0.112	−0.058	−0.174	0.556	0.012	−1.186			
Coumarin substituents														
4-Me	−0.712	1.271	−0.265	0.274	−0.085	−0.111	−0.060	−0.173	0.555	−0.012	−1.196		−0.341	
4-OH	−0.713	1.278	−0.282	0.706	−0.073	−0.113	−0.061	−0.174	0.556	−0.027	−1.197		−0.884	
3-Me	−0.700	1.280	−0.036	−0.030	−0.088	−0.112	−0.070	−0.173	0.544	0.017	−1.182	−0.324		
3-OH	−0.695	1.278	−0.476	−0.048	−0.090	−0.111	−0.071	−0.173	0.542	0.027	−1.183	−0.921		
7-OH	−0.702	1.288	−0.186	0.067	−0.060	−0.177	−0.592	−0.253	0.565	−0.016	−1.188			−0.917
4-MeO	−0.713	1.280	−0.277	0.723	−0.076	−0.113	−0.061	−0.174	0.556	−0.023	−1.196		−0.885	
3-MeO	−0.694	1.280	−0.493	−0.049	−0.090	−0.111	−0.071	−0.173	0.542	−0.029	−1.183	−0.908		
4-Me-7-OH	−0.713	1.267	−0.273	0.269	−0.071	−0.178	−0.590	−0.253	0.564	−0.040	−1.198		−0.343	−0.918
4-Me-7-EtO	−0.712	1.268	−0.271	0.270	−0.072	−0.167	−0.592	−0.239	0.564	−0.036	−1.198		−0.343	−0.951

Hamiltonian, **H**, was then constructed on the basis of relation introduced by Mulliken[16] and Wolfsberg and Helmholtz[17]

$$H_{ij} = 0.5K(H_{ii} + H_{jj})\,\mathbf{S}_{ij} \qquad (2)$$

with K being set equal to 1.75, a value found optimal for org molecules.[15]

The set of Hückel equations

$$(H_{ij} - E\mathbf{S}_{ij})C_{ij} = 0 \qquad (3)$$

was solved by 2 matrix diagonalizations by the Jacobi method. A Mulliken population analysis[18] was performed on the resultant wave functions and net atomic charges were calcd on the basis of gross atomic populations.

The Cartesian coordinates of the atoms in the coumarin skeleton were calcd on the basis of the geometry of 4-hydroxycoumarin monohydrate as determined by X-ray analysis.[19] The very slightly puckered, crystalographically determined structure of 4-hydroxycoumarin was found to yield an electron distribution that was only very slightly different from that based on an assumed planar structure for this molecule. Therefore, the skeleton geometry was assumed to be planar and minimal adjustments in bond lengths and angles were applied to ensure closure of the rings. The geometrical characteristics of the various substituents were derived from the compilation of a wide variety of data from experimental and theoretical sources.[20,21] Where applicable, the conformation allowing max bond staggering was used with a view to minimize the total energy.[15]

The characteristics of the atomic orbitals used are shown in Table I. The Slater exponents were LCAO (STO-mimimum basis)-MO-SCF optimized values.[22] The Coulomb integrals were taken to be the valence state ionization potentials used by Houlden and Csizmadia[23] for spectroscopic studies.

The multiple regression analyses were performed on a GE Mark I computer using the technique of least squares.

R_m values calcd as described by Boyce and Milborrow.[25] It has been shown[25] that the change in the value of R_m for a substituent (ΔR_m) is identical with the free energy based constant, π, introduced by Hansch[8] (eq 1).

Results and Discussion

The distribution of net charges at the skeletal and some extraskeletal positions is shown in Table II. Previously published electron density distributions of coumarin report only π electron density distributions[3,26] while the extended Hückel method incorporates both σ and π electrons. Although calculations of this nature have not been performed on substituted coumarins, the effects of substituents on the electron density distribution were similar to those in the case of other heterocyclic compounds.[26]

The presence of Me, OH, and MeO (EtO) substituents was found to confer an electropositive charge onto the substituent-bearing skeletal position, the effect of the MeO group being the most marked and that of the Me the least. At the same time, the positions neighboring the substituent-bearing skeletal positions were found to become more electronegative. It is generally recognized that the extended Hückel theory calculations tend to exaggerate the net atomic charges.[7,27] However, the close similarity of the compounds in this study was thought to warrant a comparison which in any case stresses relative rather than absolute values.

The R_m values, the energies of the lowest empty molecular orbital (LEMO), the highest occupied molecular orbital (HOMO), the first electronic transition (EE, taken to be the difference between the HOMO and

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TABLE III
CORRELATIVE PARAMETERS OF VARIOUS COUMARINS

Compound	R_m	LEMO energy, eV	HOMO energy, eV	First excitation energy, EE, eV	Induced enzyme activity[a] Substrate: Coumarin	Substrate: 4-Methylcoumarin
Coumarin	0.75	−9.385	−12.080	2.695	94 ± 12.0	86 ± 19.4
Coumarin substituents						
4-Me	0.94	−9.158	−11.995	2.838	419 ± 13.7	848 ± 16.4
4-OH	−0.38	−9.077	−12.061	2.984	132 ± 18.5	95 ± 11.3
3-Me	1.24	−9.316	−12.062	2.646	227 ± 8.3	150 ± 15.5
3-OH	0.68	−9.303	−12.118	2.815	96 ± 2.8	98 ± 3.9
7-OH	0.63	−9.310	−12.152	2.842	92 ± 23.3	103 ± 12.6
4-MeO	0.90	−9.085	−12.041	2.956	154 ± 2.1	170 ± 12.1
3-MeO	0.90	−9.299	−12.112	2.813	129 ± 9.3	130 ± 11.3
4-Me-7-OH	0.78	−9.080	−12.144	3.064	185 ± 5.7	228 ± 10.5
4-Me-7-EtO	1.45	−9.091	−12.162	3.071	317 ± 11.7	301 ± 13.2

[a] Measured as coumarin 3-hydroxylase, expressed as per cent of control and given as mean ± S.E.

TABLE IV
CORRELATIVE REGRESSION EQUATIONS

Substrate[a]	Equation[b]	n[c]	F[d]	r[e]	r^2[f]	Equation No.
Coumarin	$\log BA = 0.231\pi + 0.199$ (1.591)	10	2.530	0.490	0.240	4
	$\log BA = 0.265\pi + 1.845\text{HOMO} + 22.514$ (1.916) (1.452) + 22.850	10	2.494	0.645	0.416	5
	$\log BA = 0.160\pi - 17.716(\text{NET } 2) + 22.850$ (1.466) (−2.831)†	10	6.382†	0.803	0.646	6
	$\log BA = 0.211\pi - 11.259(\text{NET } 1 + 2) + 6.650$ (2.298) (−3.633)†	10	9.795‡	0.852	0.737	7
	$\log BA = 0.239\pi + 1.514\text{HOMO} - 10.710(\text{NET } 1 + 2) + 24.644$ (3.279)† (2.318)† (−4.385)†	10	12.399‡	0.928	0.861	8
	$\log BA = 0.244\pi + 1.820\text{HOMO} - 11.094(\text{NET } 11 + 2) + 23.171$ (3.337)† (2.715)† (−4.379)‡	10	12.374‡	0.928	0.861	9
	$\log BA = 0.263\pi + 1.587\text{HOMO} - 7.124(\text{NET } 1 + 2 + 11) + 14.988$ (3.256)† (2.132) (−3.818)‡	10	9.747†	0.911	0.830	10
	$\log BA = 0.145\pi - 1.175\text{EE} - 21.046(\text{NET } 1 + 2) + 15.632$ (2.381) (−3.408)† (−6.060)‡	10	20.306‡	0.954	0.910	11
4-Methylcoumarin	$\log BA = 0.286\pi + 0.209$ (1.436)	10	2.058	0.452	0.205	12
	$\log BA = 0.331\pi + 2.498\text{HOMO} + 30.416$ (1.742) (1.429)	10	2.246	0.654	0.428	13
	$\log BA = 0.195\pi - 22.525(\text{NET } 2) + 29.009$ (1.212) (−2.439)†	10	4.639	0.755	0.570	14
	$\log BA = 0.260\pi - 14.426(\text{NET } 1 + 2) + 8.475$ (1.856) (−3.053)†	10	6.759†	0.812	0.629	15
	$\log BA = 0.300\pi + 2.120\text{HOMO} - 13.658(\text{NET } 1 + 2) + 33.669$ (2.520)† (1.993) (−3.433)†	10	7.742†	0.891	0.795	16
	$\log BA = 0.303\pi + 2.464\text{HOMO} - 14.700(\text{NET } 2 + 11) + 31.287$ (2.721)† (2.409) (−3.802)†	10	9.074†	0.905	0.819	17
	$\log BA = 0.328\pi + 2.159\text{HOMO} - 9.342(\text{NET } 1 + 2 + 11) + 20.547$ (2.678)† (1.908) (−3.293)†	10	7.116†	0.884	0.781	18
	$\log BA = 0.187\pi - 1.269\text{EE} - 24.818(\text{NET } 1 + 2) + 18.076$ (1.451) (−1.776) (−3.464)†	10	6.944†	0.881	0.776	19

[a] Substrate used for the determination of coumarin 3-hydroxylase activity (1). [b] Variables of these equations: log BA = common logarithm of the biological activity; HOMO = energy of highest molecular orbital; EE = first excitation energy; $\pi = \Delta R_m = R_x - R_h$, where R_x, R_h = partition coefficients of substituted coumarins and coumarin, resp; NET 2 = net atomic charge at position 2 in the coumarin structure; NET 1 + 2 = sum of net atomic charges at positions 1 and 2; NET 11 + 2 = sum of net atomic charges at positions 11 and 2; NET 1 + 2 + 11 = sum of net atomic charges at positions 1, 2, and 11. Quantity in parentheses below each variable represents the t value for the significance of the nonzero hypothesis applied to the respective regression coefficient. Daggers represent the significance levels of the statistical quantity to which they refer, † $p<0.05$; ‡ $p<0.01$. [c] Number of compounds used in each regression. [d] F value for the significance of the correlation. [e] Multiple correlation coefficient of each fit. [f] Index of determination, representing the proportion of variance in the dependent variable accounted for by the regression.

LEMO energies), and the activity of drug-metabolizing enzymes induced by these compounds are listed in Table III.

The inducing capability represented by the log of the induced enzyme activity compared to control (log BA) was regressed against various combinations of nonlinearly related parameters listed in Tables II and III. Some of the resulting regression equations are listed in Table IV. Variables not significantly contributing to the overall fit were eliminated with the aid of the Student's t test of the null hypothesis applied to their coefficient.

In general, slightly better correlations were found in the case of hydroxylase activities measured using coumarin as a substrate (eq 4–11). The best fits using 3 variables were those involving π, HOMO or EE, and the net charges at atomic positions O-1, C-2, and O-11. Equations 8 and 9 account for 86%, eq 11 for 91% of the variance in log BA ($p < 0.01$). No significant correlations were demonstrated with any of the other elec-

tronic parameters listed in Tables II and III. Furthermore on inclusion of a π^2 term, its coefficient was found to be not significantly different from zero. It seems therefore, that, within its range in this study, the second-order contribution of π is minimal.

The significant partial correlation to π indicates an influence of the hydrophobic–hydrophilic character on the relative activities of the coumarins.

The partial correlation to the HOMO energy, an index of electron donor ability,[26] may be interpreted[6,13] as an indication that charge-transfer complex formation may occur at the site of the critical reaction.

The excitation energy of a molecule is a parameter of an analogous nature to a free energy of reaction or activation, thus substituent effects on the former may well parallel these effects on the latter.[14] Hence, it is not too surprising to find the EE to be in significant partial correlation with log *BA*. It is also interesting to note that in eq 11 and 19 the π term coefficient was no longer significant at the $p < 0.05$ level. This seems to indicate that the EE term incorporates part of the variance in π relevant to its effect on log *BA*.

Finally, the partial correlation to the sum of the net charges on the C-2/O-11 (NET 2 + 11) and C-2/O-1 (NET 1 + 2) groups seems to implicate these positions as possible sites of H bonding[26] or of charge transfer. Ease of electron donation in any case would increase sensibly as the net charge at these points becomes more negative, as reflected in eq 6–11 and 14–19.

The data presented here suggest that the induction of drug metabolism by coumarins may be associated with the carbonyl (C-2/O-11) and the α-pyran (O-1/C-2) moieties of the molecules. It should be clarified that in fact the absolute need for the carbonyl or the α-pyran moieties for this biological activity has not been established. This study has simply delineated a pattern of charge distribution that seems to favour it. Such activity might well be achieved by reproducing this pattern with the use of any other fortuitous combination of substituents on this or other molecules exhibiting a similar electronic geography. However, the electronic properties on the carbonyl and α-pyran moieties of the lactone ring seem to be salient to the capability of the various coumarins to induce drug-metabolizing enzymes in the liver of the rat to varying degrees. The stabilization of this structure by 4-Me substitution[28] may also play a role in the structure–activity relationship. The degree of resolution in the present study does not allow more specific conclusions to be drawn concerning the drug–receptor site interaction involved. The mechanism by which these compounds exert their action on the liver cell remains to be elucidated.

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Interaction of Ergothioneine with Metal Ions and Metalloenzymes[1]

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The interaction of ergothioneine (I) with a number of divalent metal ions was determined using a pH titration method. Formation constants for complexes containing 2 moles of ligand and 1 mole of metal ion were found to be $10^{18.2}$ (Cu^{2+}), $10^{11.6}$ (Zn^{2+}), $10^{8.2}$ (Ni^{2+}), and $10^{8.2}$ (Co^{2+}). A study of the inhibitory power of I on selected Zn and Cu metalloenzymes was made. None of the 4 Zn-containing enzymes, including 3 dehydrogenases and alkaline phosphatase, were inhibited even if preincubated for 6 hr with 10^{-2} *M* I. Four different Cu enzyme systems were investigated, including uricase, ascorbate oxidase, MAO, and a number of polyphenol oxidases. Of these, only the polyphenol oxidases were inhibited by I. Kinetic measurements using *Psalliota campestris* (mushroom) polyphenol oxidase showed that inhibition by I does not involve removal of Cu from the enzyme, but is reversible and displays characteristics of both competitive and noncompetitive inhibition. This type of inhibition may result from the presence of Cu at noncatalytic as well as catalytic sites of mushroom polyphenol oxidase.

Ergothioneine, the betaine of thiolhistidine, was first described by Tanret over 50 years ago.[2] This fungal alkaloid has often engaged the interest of investigators because of its obvious potential as a metabolically important compound; however, most studies in this regard have proven to be disappointing. Melville[3] has extensively reviewed this and other aspects of ergothioneine chemistry and biochemistry.

A previously unexplored approach to the elucidation of a possible metabolic role for ergothioneine lies in its metal ion binding potential. Qualitative information regarding metal binding is supplied by the fact that purification of ergothioneine from natural sources includes a precipitation step using Cu_2O.[4] Also, Mann and Leone[5] showed that an unusually high concn of ergothioneine in boar serum prevented Cu^{2+} inhibition of sperm motility and fructolysis.

This paper reports findings on the interaction of

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