Quantitative Structure-Activity Relationships for Dicoumarol Antivitamins K in the Uncoupling of Mitochondrial Oxidative Phosphorylation†

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The dynamic structure of dicoumarols substituted on the methylene bridge has been studied by nuclear magnetic resonance (nmr) spectroscopy. These molecules may be considered as dimers with restricted rotation around the methylene bridge, held by intramolecular hydrogen bonds; the presence of the substituent R modifies this dynamic process, mainly the facility of exchange of the two hydroxyl protons. These compounds have been compared in respect to their potency in uncoupling oxidative phosphorylation in pig heart mitochondria; the data have been correlated with constants used to characterize the substituent R; to make a potent uncoupler, R should be as small and as hydrophilic as possible. These results are discussed in consonance with the postulated mechanisms of action of the uncouplers, but no simple conclusion can be drawn, especially concerning the role of the dissociable protons.

Many hypotheses have been advanced to explain the mechanism by which compounds can uncouple the energy conservation reactions from the oxidoreduction reactions in mitochondria. All those compounds are characterized by being lipid soluble and by having a dissociable proton and a structure permitting a large charge delocalization.

Although it is actually generally accepted that the uncouplers are proton conductors,[1-3] the precise mechanism of proton conduction is still disputed.[4-6] On the other hand, after Harold's works,[7] it is still a matter of argument to decide whether uncoupling results from passage of protons *across* the membrane (discharging the pH gradient and/or the membrane potential[8]) or *into* the membrane (allowing for the hydrolysis of a chemical intermediate by general acid or base catalysis[9,10]). It should also be important to decide whether the protons are conducted in[8] or out[11] of the mitochondria.

A detailed knowledge of the behavior of the uncoupling molecule in the mitochondrial membrane environment would help understand the mechanism of uncoupling. One way to approach it has been to study the behavior of the molecule in a model system and relate the findings in the mitochondria to this model.[3,9,10,12]

Another approach for studying these problems is to follow the effects of modification of the physicochemical properties of uncoupling molecules upon this activity.[13-24] Having synthesized many derivatives of dicoumarol [3,3′-methylenebis(4-hydroxycoumarin)] of which the dynamic structure had been well studied,[25,26] the present work was undertaken to find out which of the physicochemical parameters correlate best with the biological activity. The results presented in this paper emphasize the role played by the lipophilic and proton-carrier characters of these molecules in uncoupling, but nothing definite can be reasonably said concerning the mechanism of the proton conduction and of the uncoupling.

Results

Dynamic Nmr.[25,26] The dicoumarol derivatives substituted at the bridge carbon exhibit a double hindered rotation around the bonds connecting this carbon. The restricted rotation is due to intramolecular hydrogen bonds, represented in Figure 1.

Variable temperature nmr indicates four equivalent sites for each pair, due to symmetry for compounds with R ≠ H (hence, two signals are visible for the hydroxyl protons at low temperature).

The free activation energies calculated at 37° (ΔG^*_{37}) are dependent on (1) steric effect of substituent R (the barrier to rotation increases with steric factor E_s); (2) intermolecular bonds which may exist between substituent R and the hydroxyl groups; and (3) solvent effect (an increasingly electron-donating solvent will lower the barrier through the formation of intermolecular bonds between the hydroxyl groups and solvent molecules). The qualitative dynamic nmr study (Figure 2) which proves that one hydroxyl site is easily exchangeable in the presence of trace amounts of water, as, for example, upon addition of methanol.

The nuclear Overhauser effect indicates that the site concerned is the one opposed to the substituent R (Figure 1).[25]

Table I indicates a decrease of $pK_{a(1)}$, with the bulk of R, which is in consonance with nmr results (see Results and Correlation).

Finally, the nmr investigation shows that the site opposed to the substituent R is the one which is preferentially solvated by polar solvents (chemical shift variations of the bridge protons as a function of the solvent medium).[25,26]

Dicoumarol itself represents a particular case. The rotation and exchange may be too rapid on the dynamic nmr time scale as far down as −60°. By all means, the intramolecular bonds are the strongest in the series, and this is further verified by the fact that dicoumarol is both water-insoluble and insoluble in organic solvents. This behavior may be nullified if the molecule exists in solution as a monoion $(UH)^-$ (see Discussion).

Uncoupling Activity of the Dicoumarol Derivatives. The results of a typical experiment are presented in Figure 3. The phosphorylation produces both a stimulation of the respiration rate (state 3) and an alkalinization of the medium. When the added ADP has been phosphorylated (state 4), the uncoupler is added; the respiration is more or less stimulated, but no acidication of the medium is observed (due to activation of the ATPase). Upon a second addition of ADP (1 min after the uncoupler), the respiration increased, but the alkalinization is more or less pronounced, to be nil (same slight basal acidification as with substrate alone) in case of complete uncoupling. If an excess of dicoumarol is added, ATPase activation and inhibition of respiration both occur, as shown[27] previously. This concentration in excess depends on the dicoumarol derivative used; there is no correlation between the

† Preliminary reports were presented at the meeting of the French Biochemical Society, Grenoble, France, Feb 7–9, 1973, and at the meeting on Structure-Activity Relationship (Program Chairman, C. Hansch), held by the Société Droit et Pharmacie, Paris, March 25–26, 1974. This work was supported by grants from the Centre Nationnal de la Recherche Scientifique (E.R.A. No. 172) and from the Universities VI and VII of Paris.

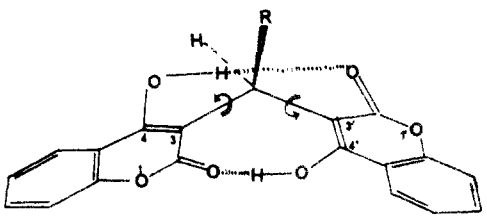


Figure 1. Dynamic structure of substituted dicoumarols.

concentration for complete uncoupling and for activation of the ATPase, indicating that two phenomena are probably not related.[20] The ratio of the two state-4 respirations, before and after addition of dicoumarol, gives the degree of uncoupling. But since the respiratory control may be often lost although some phosphorylation activity remains, the uncoupling is best evaluated by the ratio of the two rates of alkalinization during the phosphorylation, in the presence or not of uncoupler (a correction is introduced for the slight acidity observed). If this ratio is plotted as a function of at least five concentrations of uncoupler, straight lines are obtained. Each analysis was performed at least twice. The potency of the uncoupling activity (I_{50}) is expressed as the concentration required to produce 50% of uncoupling. This concentration is expressed per milligram of mitochondrial protein, since the uncoupling activity was found to be dependent on the protein concentration, and is calculated with an error of about 15%.

The data, listed in Table I, point out that dicoumarol is the most potent uncoupler of all the derivatives tested (as reported by Martius, *et al.*,[28] for a few derivatives); the bulkier the substituent R, the weaker the uncoupling activity. An exception is apparent for compounds **11**, **14**, and **15** where the substituent is respectively a benzene, thiophene, and furan ring. These results may be explained, however, if one admits that these rings are in a "perpendicular position of least hindrance" according to Kutter and Hansch hypotheses.[29] Further, the small thiophene and furan rings are more hydrophilic than benzene. The substitution of the two mobile protons of dicoumarol by methyl groups (not shown in Table I) nullifies the uncoupling capacity of the molecule.

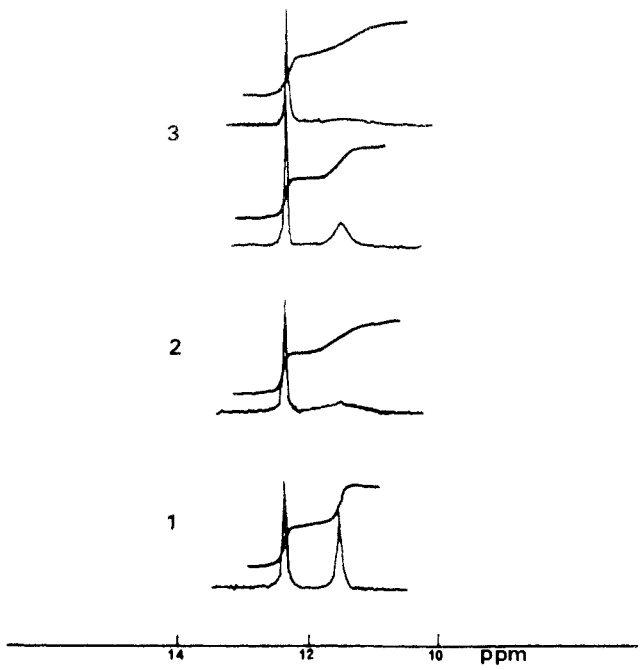


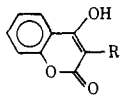
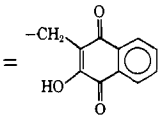
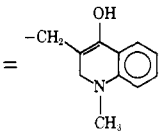
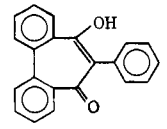
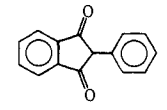
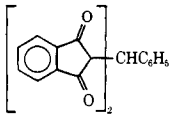
Figure 2. Nmr spectrum of the dicoumarol, R = Pr, in the region of hydroxyl protons resonances 10–14 ppm: (1) solvent $CDCl_3$ at −60°; (2) after addition of 3 drops of methyl alcohol at −60°; (3) same solvent dried over 4 Å molecular sieves after addition of 1 drop of methyl alcohol at −60°; after addition of 3 drops of alcohol at −60°.

Table I. Uncoupling Activity and Physicochemical Parameters of Substituted Dicoumarols

Substituent R	No.	I_{50},[a] %	π[b]	σ^*[b]	E_s[b]	ΔG^*_{37}[c] $DCCl_3$	ΔG^*_{37}[c] C_6H_5Cl	pK_a[d] 1	pK_a[d] 2	Mp, °C
H	1	2.35	0.0	0.49	1.24			3.7–4.1	8.01 ± 0.05	288–289
CH_3	2	8.0	0.50	0.0	0.0	15.06	15.21	2.40	9.16	176–178
C_2H_5	3	18.5	1.0	−0.10	−0.07		16.39			143–144
n-C_3H_7	4	25.0	1.50	−0.11	−0.36		16.35			124–125
n-C_4H_9	5	72.0	2.0	−0.13	−0.39		16.66			113
i-C_3H_7	6	53.0	1.37	−0.19	−0.47		19.56			209–210
$C_6H_5CH_2$	7	96.0	2.69	0.22	−0.38	16.88				190–192
CH_3OCH_2	8	17.0	0.03[e]	0.52	−0.19					156–157
$CH_3S(CH_2)_2$	9	23.0	1.62[e]		−0.39[f]	15.63	16.25			154–155
COOEt	10	26.5						1.5	7.58 ± 0.03	154–157
										178–182
C_6H_5	11	13.2	2.13	0.60	0.23[g]			2.40 ± 0.10	9.11 ± 0.05	228–229
p-ClC_6H_4	12	22.5	2.84[e]	0.75[h]	0.23[g]	14.61				256
p-$NO_2C_6H_4$	13	17.5	1.85[e]	1.14[h]	0.23[g]					234–235
Thienyl	14	3.1	1.81							224–226
Furyl	15	6.0								Dec

[a] Concentration required to produce 50% uncoupling, expressed as mole × 10^{-8}/mg of mitochondrial protein (*cf.* Experimental Section). [b] π, σ^*, and E_s, respectively the hydrophobic, electronic, and steric constants, are described in the text. [c] Free energy of activation of the dynamic process at 37° (*cf.* the text), expressed in kcal/mol. [d] Values for the first and second ionization are taken from J. A. Tomlinson, Ph.D. Thesis, University J. Warwick, Coventry, England, 1968, p 114. [e] Calculated by additivity. Example: $\pi(CH_3OCH_2) = \pi(CH_3O) + \pi(CH_2) = -0.47 + 0.50 = 0.03$. [f] Value estimated equivalent to *n*-butyl. [g] The benzene cycles are supposed in a "perpendicular position" (*cf.* the text). [h] Y. Nagai, *et al.*, *Bull. Chem. Soc. Jap.*, **45**, 2560 (1972).

Table II. Uncoupling Activity and Physicochemical Parameters of Compounds Related to Dicoumarols

	No.	I_{50}, %[a]	pK_a[a] 1	pK_a[a] 2	Mp, °C
					
R = −H	**16**	500[b]	4.20 ± 0.02		
R = $-C_6H_5$	**17**	25	3.76 ± 0.02		265
R = 	**18**	3.2			243
R = 	**19**	21.0			252
	**20**	20.0			182–183
	**21**	53.0			149
	**22**	105			162

[a] See footnote *a*, Table I. [b] Dissolved in ethanol.

Table II shows that hydroxycoumarin **16** is a very poor uncoupler; the addition of a benzene group **17** increases considerably its potency probably by shielding the charge, increasing thus the lipophilicity of the molecule. Compounds **21** and **22** show a small activity which may be due to the low probability of existing in the enolic forms.

Correlations. Equation 3 in Table III shows that the steric effect is decisive in explaining the variation of this activity and highly significant, as indicated by the value of the F test: $F_{1,9} = 25.55$; $F_{1,9;\alpha\ 0.005} = 13.61$.

The combination of the two independent variables π and E_s (as indicated in Table IV) gives a good correlation for the substituents for which σ^*, E_s, and π constants are known (eq 4, $F_{2,8} = 32.88$; $F_{2,8;\ \alpha\ 0.005} = 11.04$). The other combinations are less significant (σ^* and π, and E_s). Equation 6 includes compound **9** for which we do not have the σ^* value and for which E_s is estimated to be equivalent to that of *n*-butyl. The results have varied little in comparison to eq 4 (Table V).

The equation in π, π^2, E_s cannot be maintained because of the insignificant values of the coefficients of the π and π^2 terms (eq 5), in respect to 95% confidence intervals.

The biological results for the other compounds, **10**, **14**, and **15**, have not been included, since at least one of the parameters is unknown.

The value of the steric constant E_s, in the case when R = *p*-X-C_6H_4-, X = H, NO_2, compounds **11–13**, has been estimated at 0.23 in the hypothesis of a position at least hindrance, according to Kutter and Hansch.[29]

We have noted that the values for the free energy of activation, ΔG^*_{37}, were a good measure of the steric effect,[25,26] hence, the idea of replacing E_s by ΔG^*_{37}. Unfortunately, because of experimental limitations,[25,26] we could not determine ΔG^*_{37} values for all the compounds. However, it is noteworthy that a result equivalent to eq 4 is obtained with only six compounds.‡

Finally, the interpretation of eq 4 and 6 indicates that in order to make good uncouplers, the bridge substituents must be as small and as hydrophilic as possible. Dicoumarol (**1**) appears to be the limit of the series.

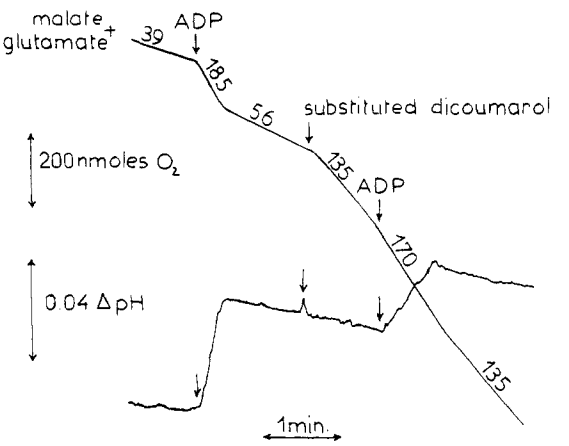


Figure 3. Uncoupling of pig heart mitochondria by substituted dicoumarols. The numbers next to the oxygen trace indicate the rate of oxygen uptake in nmol of O_2/min/mg of protein. ADP and substituted dicoumarol, here 86 × 10^{-8} mol/mg of protein of the compound **5** (R = C_4H_9), were added as indicated by the arrows. The reaction was carried out as described in the text (*cf.* Experimental Section).

Table III. Correlations between Uncoupling Activity and the Constants π, E_s, and σ^*[a]

	n	r[b]	s[b]	Eq no.
pI_{50}[e] = 4.12 (±0.45) − 0.30 (±0.26) π	11[c]	0.65	0.36	1
pI_{50} = 3.58 (±0.37) + 0.35 (±0.72) σ^*	11	0.34	0.44	2
pI_{50} = 3.68 (±0.16) + 0.79 (±0.35) E_s	11	0.86	0.24	3
pI_{50} = 3.96 (±0.22) − 0.19 (±0.13) π + 0.67 (±0.26) E_s	11	0.94	0.16	4
pI_{50} = 3.89 (±0.31) − 0.03 (±0.50) π − 0.05 (±0.17) π^2 + 0.71 (±0.31) E_s	11	0.95	0.17	5
pI_{50} = 3.98 (±0.22) − 0.19 (±0.13) π + 0.63 (±0.26) E_s	12[d]	0.93	0.17	6

[a] *Cf.* the text for the definition of the different parameters and Table I for their values. [b] *Cf.* the Experimental Section. [c] Compounds 1–8 and 11–13. [d] Compounds 1–9 and 11–13 (*cf.* the text). [e] $pI_{50} = \log 1/ID_{50}$.

Table IV. Squared Correlation Coefficient for 11 Substituents (Eq 1–5)

	π	E_s	σ^*
π	1.00	0.11	0.03
E_s	0.11	1.00	0.29
σ^*	0.03	0.29	1.00

‡ For instance, with ΔG^*_{37} values measured in chlorobenzene (six points only indicated in Table I), we obtain the correlation pI_{50} = 0.94 (±1.97) − 0.10 (±0.10) ΔG^*_{37} − 0.46 (±0.35) π: n = 6; r = 0.96; s = 0.12. *This equation is given* only for indication.

Table V. Calculated Biological Results

No.	pI_{50} (obsd)	pI_{50} (calcd)[a]	pI_{50} (calcd)[b]
1	4.63	4.78	4.76
2	4.09	3.86	3.88
3	3.73	3.72	3.74
4	3.60	3.43	3.46
5	3.14	3.32	3.35
6	3.27	3.38	3.42
7	3.01	3.19	3.22
8	3.77	3.82	3.85
9	3.64		3.77
11	3.87	3.70	3.71
12	3.64	3.57	3.58
13	3.75	3.76	3.77

[a] From eq 4. [b] From eq 6.

Since we have seen from the dynamic study of the structure of these compounds in a nonpolar environment that the presence of R modifies the mobility of the two protons (making the first H more exchangeable, whereas the second becomes less mobile), we may conclude that the steric effect of R upon the activity reflects the importance of the mobility of the two protons in the uncoupling. But, one has now to try understand why the dicoumarol is the most potent uncoupler, when it is the most stable and less soluble molecule; in other words, if these molecules act by conducting protons through the membrane, how is this performed?

It can be noted that it is probable that a certain overlapping could exist between $pK_{a(1)}$ and E_s (or ΔG^*_{37}) as it has been assumed by Hansch, *et al.*,[30] for dimeric benzoic acid in toluene. In our case, the instability of the substituted dicoumarol to uv, notably at acidic and basic pH, renders difficult the pK_a measurement (and partition coefficient measurement) and we could not verify this assumption.

Discussion

Many compounds have been tested in order to find out which of their properties might be involved in the uncoupling of mitochondrial oxidative phosphorylation. It has been found that their uncoupling potency generally paralleled their lipophilic character;[13-17,19-23,31] the pK_a of their acidic group has been shown to be[17,19,22,23,32] or not[13-16,20] an important factor in the rate-limiting process of uncoupling, although steric effects[13,15] or the electron-withdrawing character of the substituents[24,31] have also been suggested as playing a role in the modulation of their activity.

The data reported in this paper for the dicoumarols substituted on the methylene bridge show the importance for uncoupling efficiency of both the lipophilic character of R and its steric parameter affecting mainly the facility of exchange of the two protons; the nmr results show that increase of the bulkiness of R renders the first H^+ more exchangeable and the second one less exchangeable; this results paradoxically in a decrease of the activity which is then best correlated with the properties of the second H^+.

That the *lipophilic* character of the molecule is important for the activity is evident from all these studies. It seems reasonable to state that the uncoupling potency is determined not by the known amount of reagent added in the medium but by that unknown dissolved in the membrane. Indeed, whatever the mechanism of uncoupling involved,[3,8,10,11] the uncoupler must migrate from the solvent in which it is dissolved, through the assay aqueous medium, and then go into the membrane in an unknown solvated and ionized from. Thus, although the lipophilicity has been taken as an argument for the presence and the action of the molecule in the lipid moiety of the membrane, mitochondrial protein binding has been shown,[14,18,33] or suggested,[23,24] to occur also. As an example it was found[34] from the changes of its fluorescence properties that the ostruthin (6-geranyl-7-hydroxycoumarin) with uncoupling potency was bound through hydrophobic interaction to mitochondrial fragments, the number of binding sites corresponding to the amount needed for uncoupling. We tried to evaluate, in a rather rough calculation based on their partition coefficients at pH 7.3 between water and octanol, the concentration of dicoumarol and substituted compounds 2–5 in the mitochondria after partitioning from chloroform into the medium and then into the mitochondria (representing about 0.1% of the total assay volume); we found that, for the same amount added initially, compounds 2–5 would be three to five times more concentrated in the membrane than dicoumarol. Consequently, the difference of their activity cannot be accounted for by a decreased concentration in the membrane.

Indeed, up to now we have neglected in this discussion the fact that we are dealing with charged molecules and charged membranes; in the case of the substituted dicoumarols, there are one or two negative delocalized charges, and it is documented now that energization is associated with electrical charge changes of the membrane, leading to an electrical field across the mitochondrial inner membrane. *These electrical properties* may effect (1) the binding of the uncouplers, (2) their pK_a, and (3) the mobility of the charged species, three parameters which in turn determine the efficacity of uncoupling.

If the substituted dicoumarol anions bind to the surface of the membrane, they may modify the interfacial charge density, therefore the surface potential, as shown for the 2,4-dinitrophenol adsorbed to the surface of a phospholipid bilayer.[6] It is known also that the surface charge of the membrane strongly influences the efficiency of uncouplers in inducing proton permeability of phospholipid bilayers.[35] On the other hand, the behavior of the ionized molecules may depend on the surface charge existing at the interfaces, as shown for the 7-hydroxycoumarin adsorbed on phospholipid micelles.[36] Accordingly, the pK_a of the uncoupler, which is supposed to pick up (and release) a proton at the interfaces, should depend on this surface potential.

Such interactions might explain the diversity of the often conflicting relationship between pK_a and uncoupling or proton conduction found in the literature for a wide variety of molecules studied in different experimental conditions; in some cases the anion, and in others the undissociated molecules, seems to be the active uncoupling agent. So although *a dissociable proton* is necessary for uncoupling, its role and the mechanism of its conduction is far from being completely understood. It has been proposed[4-6] that, at least for some compounds such as substituted monophenols and benzimidazoles, the permeant species is a negatively charged dimer complex formed between the undissociated uncoupler and its anion, U_2H^-. In the case of dicoumarols, which may be thought of as constrained dimers, the results would suggest that the second ionization is concerned and that the proton carrier species is the diionized form of the molecule.

However, since we ignore the concentration and the ionization behavior of these substituted dicoumarols both inside the membrane and at the interfaces, and their relative mobility in the membrane which is considered to be

Table VI. New Dicoumarol Analogs Prepared for This Study

No.	Formula	Mp, °C	Crystn solvent	Analyses[a]
7	$C_{26}H_{18}O_6$	190–192	EtOH–Me_2CO	C, H
14	$C_{23}H_{14}O_6S$	224–226	EtOH–$HCCl_3$	C, H
15	$C_{23}H_4O_7$	Dec[b]	EtOH–$HCCl_3$	*b*
18	$C_{20}H_{12}O_6$	243	C_6H_6	C, H
19	$C_{20}H_{15}O_5N$	252	AcOH	C, H, N

[a] Analyses within ±0.4%. [b] This compound is unstable and the melting point cannot be correctly measured.

the rate-limiting step in the activity, it is difficult to decide whether these molecules uncouple by driving protons in or across the membrane.

Experimental Section

Measurement of Uncoupling Activity. Pig heart mitochondria were prepared by the method of Crane, *et al.*,[37] and stored at 0° until used in KCl 0.16 *M*, tris buffer 10 m*M*, pH 7.2, at a concentration of 40–50 mg of protein/ml. Protein determination was performed by the Folin method[38] using lysozyme as standard. All experiments were run at 25°.

The oxygen consumption was measured polarographically using a Clark oxygen electrode (with a Gilson oxygraph recorder). Simultaneously, the pH of the reaction medium was monitored with a combined Ingold pH electrode, Model LOT 402-50-NS, connected to a pH meter Tacussel Model ISIS 4000 and recorded with the Gilson oxygraph recorder. The reaction medium (4.5 ml) consisted of sucrose 0.225 *M*, $MgCl_2$ 1 m*M*, KCl 10 m*M*, potassium phosphate 5 m*M* at pH 7.4, and mitochondria (0.5–1.5 mg/ml). Substrate, glutamate + malate, was added to give a final concentration of 5 m*M*; ADP was in 80 μM final concentration. The dicoumarol derivatives were added as small aliquots (0.002–0.015 ml) of concentrated solution in chloroform. Appropriate checks with equivalent volumes of chloroform were carried out. The extent of reduction of cytochromes was determined with an Aminco-Chance dual wavelength spectrophotometer, using the following wavelengths pairs: 605–630 nm for cytochrome a + a_3; 562–575 nm for cytochrome b, and 550–540 nm for cytochrome c + c_1. Optical difference spectra were recorded with the same spectrophotometer working in the split-beam mode. Cytochrome a + a_3 content was estimated from the 605–630-nm difference spectrum, dithionite reduced minus oxidized, assuming $\Delta\epsilon_{mM} = 14.0$. The concentration of cytochrome a + a_3 in the assay was around 1 nmol/mg of protein. All chemicals used were of reagent grade.

Preparation of Substituted Dicoumarols. The compounds **1**, **8–10**, **16**, and **21** (*cf.* Tables I and II) were of commercial origin (anticoagulants). Compounds **2–7** and **11–15** were synthesized by the method described by Sullivan, *et al.*,[39] and **17** according to Stahman, *et al.*[40] Compounds **18** and **19** were prepared by the methods described by Robertson and Link[41] and Abramovitch and Gear,[42] and compounds **20** and **22** according to Aleksiev, *et al.*,[53] and Vanags, *et al.*[43]

After recrystallization, each compound was subsmitted to elemental analysis and examined by infrared, ultraviolet, and nmr spectroscopy.[25] The new compounds are compilated in Table VI.

Nmr Spectroscopy. Nmr analysis is described in more detail by Laruelle and Godfroid.[25,26] The variable temperature studies were carried out on a Varian A-60 instrument and the nuclear Overhauser effect (NOE) assured on a Varian 100D. The methods described in the reviews[25,44,45] were utilized for the determination of the free energy of activation at the temperature of coalescence, ΔG^*_{Tc}. The activation parameters at 37° were calculated using the formula given by Gutowsky, *et al.*[46]

Correlations. Correlation between uncoupling activity and physicochemical properties of the substituted dicoumarols was sought as described by Hansch.[32,47] The constants used to characterize the substituent R carried by the methylene bridge of the molecule were (1) π "aliphatic," a factor quantifying the hydrophobic character of R (the values were taken from the literature;[48,49] for compounds 1–5, the partition coefficient between octanol and water at pH 7.3 and 11 has been estimated by determining spectroscopically the ratio of the concentrations of the compound in both phases); (2) σ^*, the polar electronic constant, describing the electronic effect of R;[50] and (3) E_s, the steric factor, either after Taft[51] or Kutter and Hansch.[29]

The regression analyses were performed on a CII 10.070 computer; r is the correlation coefficient and s the standard deviation; the test F was calculated according to ref 52. Each regression coefficient has its 95% confidence intervals in parentheses.

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Amidines and Related Compounds. 6.[1] Studies on Structure–Activity Relationships of Antihypertensive and Antisecretory Agents Related to Clonidine

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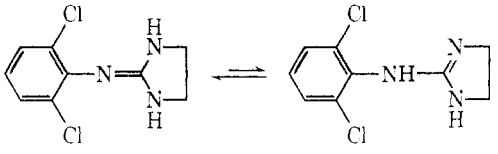
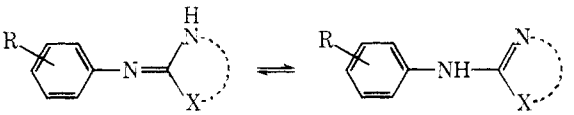
Correlations of antihypertensive and antisecretory activities with various structural modifications of the antihypertensive agent clonidine [2-(2,6-dichlorophenylimino)imidazolidine] are described. Eleven chemical classes of compounds containing an "amidine" moiety were prepared in this study. The antihypertensive activity of these compounds was evaluated in metacorticoid hypertensive rats and unanesthetized neurogenic hypertensive dogs following oral administration. Antisecretory activity was evaluated in fistula rats by measuring pH and volume of gastric secretion. Two compounds, 2-(2,6-dimethylphenylimino)imidazolidine and 2-(2,6-dichlorophenylimino)pyrrolidine, are particularly effective antisecretory agents with minimal antihypertensive activity.

Many cyclic amidines possess interesting biological properties particularly as antihypertensive agents.[2,3] The discovery of clonidine [2-(2,6-dichlorophenylimino)imidazolidine] as a centrally acting antihypertensive agent[4,5] with antisecretory activity[6] (reduction of gastric acidity) prompted us to initiate a broad investigation of structures containing an "amidine" moiety [the term "amidine" is used here to include the system -NHC(X)=N- in which X = C, N, O, or S]. In previous papers we reported the antihypertensive activity of a series of 1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolines[7,8] and certain thioureas.[9] We now describe the structure–activity relationships (SAR) of other "amidines" related to clonidine. A particular objective of the present study was to develop selective antisecretory agents having minimal antihypertensive activity.



clonidine

Chemistry. The majority of compounds prepared for this study may be represented by the general structure I in which the dotted line denotes the variations involving either cyclic or open forms, ring size, or unsaturation. These compounds may exist in two tautomeric forms. ^1H and ^{13}C nmr spectral studies on representative examples suggest that except for 2-aminoimidazoles, -oxazoles, and -thiazoles the imino form is the predominant tautomer in cases where potential tautomerism exists.[10] For the convenience of SAR discussion, the compounds are grouped into five general structural types: (1) cyclic guanidines (Table I), (2) cyclic amidines (Table II), (3) 2-aminoimidazoles (Table III), (4) guanidines and amidines (Table IV), and (5) cyclic isoureas and isothioureas (Table V). Each type may be divided into different chemical classes.



I, imino form I, amino form

X = C, N, O, or S
R = H, Cl, CH_3, or CH_3O (mono or disubstituted)

With the exception of **1b**, **1i**, and **1j**, the imidazolidines and tetrahydropyrimidine (**1**, Table I) were prepared by method A. The appropriate *S*-methylphenylisothiuronium iodide was heated with the appropriate diaminoalkane. Treatment of **1a** with acetic anhydride under different conditions gave selectively the mono- or diacetylated products **1i** or **1j**. The structures of **1i** and **1j** are supported by nmr spectral data. The triplets at δ 3.42 and 3.96 in the spectrum of **1i** are due to the nonequivalent C-4 and C-5 protons, respectively. The alternative structure having the acetyl group on the exocyclic nitrogen atom is excluded by the absence of a four-proton singlet. The chemical shifts of the C-4 and the para aromatic protons (δ 6.80 q) also support the imino form.[10] In the spectrum of **1j**, the proton signals of the methyls (δ 2.30, s, 6 H) and the methylenes (δ 3.95, s, 4 H) are consistent with the assignment of the two symmetrical acetyl groups.

The pyrrolidines and piperidines (**2**, Table II) were prepared by method B: treatment of 2-pyrrolidinone or 2-piperidinone with $POCl_3$ followed by the appropriate aniline. Acetylation of **2d** gave **2i**. The position of the acetyl group in **2i** is assigned on the basis of nmr data: the chemical shift of the C-5 protons (δ 3.90 t) is similar to that of **1i** (δ 3.96) and **1j** (δ 3.95).

The pyrroline **3** was prepared by treatment of **2d** with MeI. The position of the *N*-methyl group in **3** was deduced from its different physical and spectroscopic properties in comparison with those of **2h** in which the methyl group is on the endocyclic nitrogen atom. In the nmr