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STRUCTURE–ACTIVITY RELATIONSHIPS FOR *N,N'*-BIS(DICHLOROACETYL) DIAMINES AND SUBSTITUTED NAPHTHOQUINONES IN THE INHIBITION OF MITOCHONDRIAL ELECTRON TRANSPORT*

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Abstract—The inhibition of mitochondrial electron transport was measured with four homologous series of *N,N'*-bis(dichloroacetyl) diamines and one homologous series of substituted naphthoquinones. Partition coefficients between acetone–water and silicone for these compounds were estimated by their R_m values in reversed-phase thin-layer chromatography. Parabolic correlations between a log function of biological activity and R_m were obtained with all series. A statistical analysis suggested that a homologous series of secondary amide derivatives of the *N,N'*-bis(dichloroacetyl) diamines differed from 3 homologous series of tertiary amide derivatives of the *N,N'*-bis(dichloroacetyl) diamines. Although *N,N'*-bis(dichloroacetyl) diamines and substituted napthoquinones affected the respiratory chain at different points and the substituted naphthoquinones were more effective inhibitors of electron transport, R_m values for maximum inhibition differed by only two methylene elements between the most widely separated series. Similarities between R_m values for maximum inhibition suggest that penetration of the mitochondrial membrane is a highly significant determinant for structure–activity relationships.

IN 1961 Surrey and Mayer[1] reported the synthesis of a series of *N,N'*-bis(dichloroacetyl) diamines. These compounds were subsequently shown to possess several interesting pharmacological properties. They are antispermatogenic,[2] inhibit alcohol metabolism probably through an inhibition of acetaldehyde dehydrogenase,[3] and are potent inhibitors of a number of microsomal reactions involving mixed-function oxidase activity.[4] Recently, Merola and Brierley[5] reported that these compounds affect mitochondrial function through an inhibition of electron transport at the level of flavoprotein oxidation of pyridine nucleotide linked substrates and that these compounds did not inhibit succinoxidase activity. Secondary amide derivatives of the *N,N'*-bis(dichloroacetyl) diamines also uncouple oxidative phosphorylation while tertiary amide derivatives have little effect on this activity.[6] A homologous series of naphthoquinone derivatives with antimalarial activity were synthesized by Fieser *et al.*[7] Tappel[8] reported that two of these naphthoquinones blocked the reduction of coenzyme Q by succinate, while Hatefi *et al.*[9] later reported that one derivative in this homologous series inhibited the reduction of cytochrome c by reduced coenzyme Q.

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The secondary and tertiary amide derivatives of the *N,N'*-bis(dichloroacetyl) diamines and the substituted naphthoquinones provide compounds for the investigation of structure–activity relationships in inhibitors which act at different points on the respiratory chain. It is well established that, as the structure of members of a homologous series of compounds is altered, the quantitative change in biological activity is correlated with the oil–water partition coefficient.[10] In liquid–liquid partition chromatography, the partition coefficient, α, is a function of R_f:[10]

$$\alpha = k(1/R_f - 1)$$

and log α varies directly with R_m since:

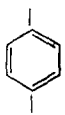
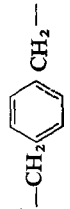
$$R_m = \log(1/R_f - 1).$$

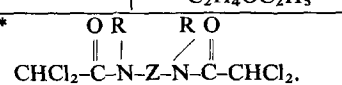
This relationship was used by Boyce and Milborrow[12] in their studies on the molluscicidal activities of a homologous series of *N,N*-alkyltrityl amines. In the present investigation we have compared a log function of biological activity and R_m for each homologous series of *N,N'*-bis(dichloroacetyl) diamines and the substituted naphthoquinones.

MATERIALS AND METHODS

Compounds. All *N,N'*-bis (dichloroacetyl) diamine derivatives, except compounds A_6 and A_7 (Table 1), and the 2-hydroxy-3-alkyl-1,4-naphthoquinone derivatives (Table 2) were kindly supplied by the Sterling–Winthrop Research Institute (Rensselaer, N.Y.). Compounds A_6 and A_7 were synthesized by the method described by Surrey and Mayer.[1] All compounds showed only one spot on reversed-phase TLC with the mobile phases used in this study (Tables 1 and 2).

Reversed-phase TLC. Silica gel G (Brinkmann Instruments, Westbury, N.Y.) was applied to 20 × 20 cm plates and the plates were impregnated with silicone DC 200 (Applied Science Laboratories, State College, Pa.) by developing two plates in a chromatographic chamber containing 100 ml of 5% (v/v) silicone in ether. The plates were removed as soon as the solution reached the top edge of the silica gel G layer. Plates were dried in air and stored in a desiccator. Edge effects were minimized by marking the coated plate with a vertical line 2·5 cm from each side of the plate. The coated plate was also marked by a line 13·5 cm from and parallel to the bottom of the plate. All mobile phases were allowed to migrate to this parallel line. Compounds were dissolved in acetone at a concentration of 3 μg/μl and 1-μl aliquots were applied to the plate at 1·5 cm intervals in a line 3·5 cm from the bottom edge of the plate. Chromatograms were developed with mobile phases which contained from 20 to 70% (v/v) acetone in water. The mobile phases were equilibrated overnight with silicone. Two hundred ml of a specific mobile phase was placed in a chamber lined with filter paper and allowed to equilibrate for 30 min before development. Two plates were developed simultaneously in the chamber. Systematic errors were avoided by randomizing the experiments. Compounds were applied to the plate in a random manner and plates and mobile phases were chosen in a random manner for each experimental series. Compounds were visualized as yellow spots on a red background when the plates were sprayed with a solution which contained 0·01 N potassium permanganate in 0·1 N sodium carbonate and then heated at 200° for several min. Migration from the origin to the center of the spot was used to calculate the R_f value.

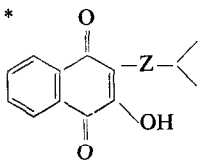
Compound* No.	Z	R	Volume fraction acetone 0·20	0·30	0·40	0·50	0·60	log (1/C)†
				R_m‡				
A_1	$(CH_2)_5$	H	+0·164 ± 0·022	−0·071 ± 0·010	−0·351 ± 0·016	−0·680 ± 0·018	−0·956 ± 0·040	2·925
A_2	$(CH_2)_6$	H	+0·414 ± 0·031	+0·097 ± 0·009	−0·207 ± 0·012	−0·515 ± 0·030	−0·847 ± 0·047	3·625
A_3	$(CH_2)_7$	H	+0·669 ± 0·033	+0·279 ± 0·009	−0·048 ± 0·010	−0·424 ± 0·023	−0·807 ± 0·058	3·925
A_4	$(CH_2)_8$	H	+0·909 ± 0·021	+0·508 ± 0·010	+0·122 ± 0·016	−0·331 ± 0·026	−0·873 ± 0·058	4·425
A_5	$(CH_2)_9$	H	+1·210 ± 0·016	+0·759 ± 0·008	+0·311 ± 0·010	−0·169 ± 0·021	−0·642 ± 0·048	4·925
A_6	$(CH_2)_{10}$	H		+1·034 ± 0·027	+0·445 ± 0·022	−0·041 ± 0·021	−0·548 ± 0·060	5·300
A_7	$(CH_2)_{12}$	H		+1·481 ± 0·033	+0·829 ± 0·018	+0·267 ± 0·010	−0·321 ± 0·073	6·000
B_1	$(CH_2)_5$	C_2H_5		+0·447 ± 0·014	+0·126 ± 0·013	−0·262 ± 0·038	−0·630 ± 0·045	3·760
B_2	$(CH_2)_6$	C_2H_5		+0·632 ± 0·021	+0·261 ± 0·013	−0·159 ± 0·026	−0·487 ± 0·034	4·390
B_3	$(CH_2)_7$	C_2H_5		+0·832 ± 0·028	+0·460 ± 0·035	+0·026 ± 0·047	−0·411 ± 0·041	4·520
B_4	$(CH_2)_8$	C_2H_5		+1·168 ± 0·025	+0·706 ± 0·031	+0·100 ± 0·039	−0·439 ± 0·028	5·060
B_5	$(CH_2)_9$	C_2H_5		+1·399 ± 0·021	+0·846 ± 0·012	+0·258 ± 0·033	−0·294 ± 0·043	5·133
B_6	$(CH_2)_{10}$	C_2H_5		+1·900 ± 0·026	+1·082 ± 0·025	+0·440 ± 0·019	−0·194 ± 0·016	5·500
B_7	$(CH_2)_{12}$	C_2H_5		+1·500 ± 0·024	+1·435 + 0·038	+0·752 ± 0·022	+0·030 ± 0·002	5·500
C_1	$(CH_2)_6$	C_2H_5		+0·632 ± 0·021	+0·261 ± 0·013	−0·159 ± 0·026	−0·487 ± 0·034	4·390
C_2	$(CH_2)_6$	C_3H_7		+1·104 ± 0·032	+0·638 ± 0·020	+0·099 ± 0·034	−0·379 ± 0·046	5·060
C_3	$(CH_2)_6$	C_4H_9		+1·529 ± 0·037	+1·187 ± 0·032	+0·547 ± 0·042	−0·098 ± 0·035	5·160
C_4	$(CH_2)_6$	C_6H_{13}		+1·407 ± 0·030	+ 1·513 ± 0·013	+ 1·205 ± 0·025	+0·342 ⊥ 0·032	3·425
D_1		H		+0·377 ± 0·021	−0·161 ± 0·029	−0·481 ± 0·078	−0·810 ± 0·129	3·397
D_2		CH_3		+0·365 ± 0·023	−0·044 ± 0·038	−0·349 ± 0·029	−0·693 ± 0·055	4·000
D_3		C_2H_5		+0·711 ± 0·028	+0·261 ± 0·028	−0·160 ± 0·032	−0·517 ± 0·027	3·886
D_4		$CH(CH_3)_2$		+1·058 ± 0·044	+0·465 ± 0·037	+0·042 ± 0·035	−0·355 ± 0·044	
D_5		$CH(CH_3)C_2H_5$		+1·404 ± 0·016	+0·798 ± 0·022	+0·297 ± 0·042	−0·112 ± 0·031	
E_1				+0·182 ± 0·034	−0·320 ± 0·040	−0·626 ± 0·076	−0·809 ± 0·066	3·699
E_2		CH_3		+0·453 ± 0·020	+0·003 ± 0·028	−0·304 ± 0·022	−0·712 ± 0·025	4·356
E_3		C_2H_5		+0·708 ± 0·016	+0·225 ± 0·029	−0·144 ± 0·013	−0·596 ± 0·056	4·585
E_4		C_3H_7		+1·165 ± 0·019	+0·612 ± 0·023	+0·114 ± 0·015	−0·344 ± 0·026	5·096
E_5		C_4H_9		+1·607 ± 0·062	+1·064 ± 0·062	+0·433 ± 0·024	−0·082 ± 0·023	4·523
E_6		$CH(CH_3)_2$		+1·030 ± 0·026	+0·421 ± 0·019	−0·010 ± 0·010	−0·364 ± 0·047	
E_7		$C_2H_4OC_2H_5$		+1·274 ± 0·010	+0·661 ± 0·025	+0·146 ± 0·044	−0·292 ± 0·047	

* 

† Concentration required for 30 per cent inhibition of oxygen uptake.
‡ Mean ± S.E.

Table 2. Biological activities of substituted naphthoquinone derivatives and their R_m values in several acetone–water binary mobile phases

Compound*		Volume fraction acetone				
no.	Z	0·40	0·50	0·60	0·70	(log 1/C)†
			R_m‡			
F_1		−0·381 ± 0·016	−0·528 ± 0·025	−0·682 ± 0·016	−0·906 ± 0·037	5·301
F_2	$(CH_2)_2$	−0·170 ± 0·020	−0·418 ± 0·031	−0·594 ± 0·059	−0·900 ± 0·067	5·523
F_3	$(CH_2)_3$	+0·006 ± 0·001	−0·295 ± 0·032	−0·457 ± 0·041	−0·828 ± 0·027	5·657
F_4	$(CH_2)_4$	+0·262 ± 0·023	−0·215 ± 0·035	−0·413 ± 0·065	−0·741 ± 0·022	6·096
F_5	$(CH_2)_5$	+0·576 ± 0·025	+0·013 ± 0·023	−0·308 ± 0·015	−0·694 ± 0·020	6·398
F_6	$(CH_2)_6$	+0·828 ± 0·020	+0·199 ± 0·020	−0·226 ± 0·035	−0·580 ± 0·025	6·657
F_7	$(CH_2)_7$	+1·079 ± 0·005	+0·355 ± 0·028	−0·131 ± 0·042	−0·499 ± 0·061	6·585
F_8	$(CH_2)_8$	+1·313 ± 0·023	+0·654 ± 0·002	−0·109 ± 0·015	−0·496 ± 0·047	6·301
F_9	$(CH_2)_9$	+1·459 ± 0·016	+0·702 ± 0·012	+0·167 ± 0·031	−0·489 ± 0·020	6·000
F_{10}	$(CH_2)_{10}$	+1·512 ± 0·018	+0·926 ± 0·026	+0·210 ± 0·035	−0·488 ± 0·030	5·301

* 

† Concentration required for 50 per cent inhibition of oxygen uptake.
‡ Mean ± S.E.

Electron transport particles (ETP). Bovine heart mitochondria were isolated by the method described by Brierley.[13] ETP were prepared from the heavy mitochondrial fraction by fragmentation at maximum power for 30 sec with a Fisher BP-2 ultrasonic probe. Unbroken mitochondria and large mitochondrial fragments were sedimented by centrifugation at 17,000 *g* (max) for 30 min. The ETP were collected by centrifugation at 104,000 *g* for 30 min and suspended in 0·25 M sucrose at a protein concentration of 15 mg/ml. Protein was determined by a biuret method.[14]

Biological activity. The inhibition of electron transport in ETP was used as a measure of biological activity. The incubation mixture contained 4 mM phosphate buffer at pH 7·4, 10 mM magnesium chloride, 0·25 M sucrose, and 1·5 mg of ETP protein diluted to a final volume of 5 ml. Oxygen uptake was followed with a Beckman or Yellow Springs polarograph. Compounds were dissolved in 10 μl of dimethylsulfoxide and added to the incubation mixture 2 min before the addition of 1 μmole of NADH. The dimethylsulfoxide had no effect on oxygen uptake. Dose–response (inhibition) curves were measured for each compound. Molar concentrations necessary for 30 per cent inhibition, *N,N'*-bis(dichloroacetyl) diamines, and 50 per cent inhibition, substituted naphthoquinones, of oxygen uptake were used in the correlations between biological activity and R_m.

RESULTS AND DISCUSSION

The *N,N'*-bis(dichloroacetyl) diamine derivatives were placed in five groups with related structural formulas (Table 1). Compounds in groups A, B and C formed homologous series. Only compounds E_3, E_4 and E_5 in group E were members of a homologous series. Compounds in group D did not form a homologous series. All substituted naphthoquinone derivatives (Table 2) were members of a homologous series. The methylene group was the common structural element in the five homologous series which were investigated.

Experimental R_m values were obtained with several mobile phases and these data are summarized in Tables 1 and 2. Interplate variability in the R_m value was indicated by standard error data. A mobile binary phase where the volume fraction of acetone was 0·5 was chosen for subsequent calculations since this mobile phase yielded R_m values between 0·954 and −0·368 for most compounds studied. Franc and Jokl[15] have shown that the composition of the mobile phase is constant in the R_f region which corresponds to the R_m range which we have specified for this study.

R_m values for several compounds, particularly compounds in the A series, were outside the specified R_m range. The validity of these data and other R_m data was confirmed by extrapolating the R_m value for the binary mobile phase where the volume fraction of acetone was 0·5 from R_m data with other binary mobile phases where R_m values were within the specified R_m range. The extrapolation was based on the studies of Soczewinski and Wachtmeister[16] which showed that R_m values for a compound in certain ternary two-phase systems are a linear function of the volume composition of the binary mobile phase. R_m and phase composition are related by the equation:

$$R_m = \mu_1 R_{m_1} + \mu_2 R_{m_2}$$

where μ_1 and μ_2 are the volume fractions of the components in the mixed solvent phase and R_{m_2} and R_{m_1} are values for the compound chromatographed with pure component$_1$ and pure component$_2$ respectively. Linear regression equations were

calculated for all compounds where at least three binary mobile phases yielded R_m values between 0·954 and −0·368. For example, a linear regression equation was not calculated from data in Table 1 for compound A_6 since only two experimental R_m values for this compound were within the specified R_m range. Extrapolated R_m values obtained from linear regression equations and experimental R_m values are compared in Table 3. Extrapolated R_m data were within one standard error of experimental R_m data for all compounds examined except A_1. Linear regression equations were not calculated for substituted naphthoquinones (Table 3) since only a few compounds in this series had three experimental R_m values within the specified R_m range.

TABLE 3. COMPARISON OF EXPERIMENTAL R_m VALUES AND EXTRAPOLATED R_m VALUES FOR N,N'-BIS(DICHLOROACETYL) DIAMINES

Compound*	R_m†	
	Experimental	Extrapolated‡
A_1	−0·680	−0·600
A_2	−0·515	−0·519
A_3	−0·424	−0·416
A_4	−0·331	−0·315
A_5	−0·169	−0·164
A_7	+0·267	+0·258
B_1	−0·262	−0·250
B_2	−0·159	−0·150
B_3	+0·026	+0·036
B_5	+0·285	+0·270
C_1	−0·159	−0·150
C_2	+0·099	+0·119
D_2	−0·349	−0·366
D_3	−0·160	−0·164
E_2	−0·304	−0·327
E_3	−0·144	−0·163
E_4	+0·114	+0·127

* See Table 1 for structures.

† Data for a binary phase which contained 50% (v/v) acetone in water.

‡ Calculated from regression equations obtained from R_m data in Table 1.

Correlations between biological activity and R_m were examined by regression analysis. Extrapolated R_m values (Table 3) were used where available and experimental R_m values (Tables 1 and 2) were used for other compounds. Biological activity was expressed as log $(1/C)$ where C was the molar concentration of a compound required for a specified inhibition of oxygen uptake (Tables 1 and 2). A logarithmic function for biological activity was chosen since Hansch[17] has described the theoretical basis for a logarithmic relationship between biological activity and structure and since logarithmic relationships have yielded good correlations in many studies. Regression equations gave a parabolic relationship between log (1/C) and R_m for every series

examined in our study (Table 4). The quadratic term was significant at the 0·05 level in all experimental series and at the 0·01 level in 5 of the 9 experimental series.

TABLE 4. REGRESSION EQUATIONS FOR THE PARABOLIC RELATIONSHIP BETWEEN BIOLOGICAL ACTIVITY AND R_m

Series	log (1/C)=	n*	σ†	Significance of R_m^2‡	Equality of parabolas§
A	$5{\cdot}450+2{\cdot}642\,R_m-2{\cdot}285\,R_m^2$	7	0·106	<0·01	
B	$4{\cdot}650+2{\cdot}678\,R_m-2{\cdot}038\,R_m^2$	7	0·182	<0·05	
C	$4{\cdot}802+2{\cdot}221\,R_m-2{\cdot}795\,R_m^2$	4	0·043	<0·05	
D	$2{\cdot}694-10{\cdot}275\,R_m-18{\cdot}321\,R_m^2$	3			
E	$4{\cdot}849+0{\cdot}480\,R_m-2{\cdot}400\,R_m^2$	5	0·203	<0·05	
F	$6{\cdot}450+1{\cdot}164\,R_m-2{\cdot}491\,R_m^2$	10	0·141	<0·01	
A+B	$4{\cdot}972+2{\cdot}060\,R_m-1{\cdot}755\,R_m^2$	14	0·379	<0·05	<0·01
B+C	$4{\cdot}746+2{\cdot}922\,R_m-3{\cdot}253\,R_m^2$	11	0·227	<0·01	>0·10
C+E	$4{\cdot}831+0{\cdot}921\,R_m-1{\cdot}705\,R_m^2$	11	0·256	<0·01	>0·10
A+B+C+D+E	$4{\cdot}910+1{\cdot}559\,R_m-2{\cdot}082\,R_m^2$	26	0·405	<0·01	<0·01

* n is the number of compounds used.
† Square root of the error mean square.
‡ t-test.
§ Significance of F ratio from analysis of variance.

Merola *et al.*[6] have shown that N,N'-bis(dichloroacetyl) diamines have a marked structural specificity in their ability to uncouple oxidative phosphorylation. The tertiary amide members of this group of compounds show only slight activity, while the secondary amides, beginning with an 8 carbon polymethylene chain, strongly uncouple oxidative phosphorylation. Since secondary and tertiary amides differed in their ability to uncouple oxidative phosphorylation, these compounds were examined in greater detail for differences in their abilities to inhibit electron transport. In this respect, when all N,N'-bis(dichloroacetyl) diamines were considered as 1 population, a parabolic relationship between biological activity and R_m was obtained (series A+B+C+D+E in Table 4). However, an analysis of variance showed that the parabolas formed from series A, B, C, D and E, respectively, were not identical at the 0·01 level of significance. When the secondary amides in series A and the tertiary amides in series B were compared, an analysis of variance showed that these parabolas were not identical at the 0·01 level of significance (series A+B in Table 4). Similar statistical analyses with the tertiary amides B and C and the tertiary amides C and E showed that these parabolas did not differ at the 0·10 level of significance. It was apparent from these statistical analyses that the tertiary amides were more closely related to each other than to the secondary amides in the inhibition of electron transport.

The structure–activity relationships described in Table 4 are presented graphically in Fig. 1. Since log (1/C) for substituted naphthoquinones was obtained for 50 per cent inhibition of oxygen uptake and log (1/C) for N,N'-bis(dichloroacetyl) diamines was obtained for 30 per cent inhibition of oxygen uptake, it was apparent that substituted naphthoquinones were more effective inhibitors of electron transport than N,N'-bis (dichloracetyl) diamines. However, the R_m for maximum biological activity,

R_{m_0}, was similar in each experimental series. The R_{m_0} value is analogous to the log α_0 value which was used by other investigators[10] to estimate the partition coefficient, α, for maximum biological response. The R_{m_0} values for the most widely separated series, the F and A series (Fig. 1), were calculated as 0·234 and 0·540, respectively, from the regression equations in Table 4. The difference between R_{m_0} values, ΔR_{m_0}, may be expressed as methylene elements for the different homologous series since the methylene group was the common structural element in each series. Regression analysis of R_m data (Tables 1 and 2) showed that R_m was a linear function of the number of methylene elements, n, in the series:

$$R_m = a + \Delta R_m n.$$

The ΔR_m values were 0·131 and 0·174 for methylene groups in the A and F series respectively. Since ΔR_{m_0} between the A and F series was 0·306, R_{m_0} values for these

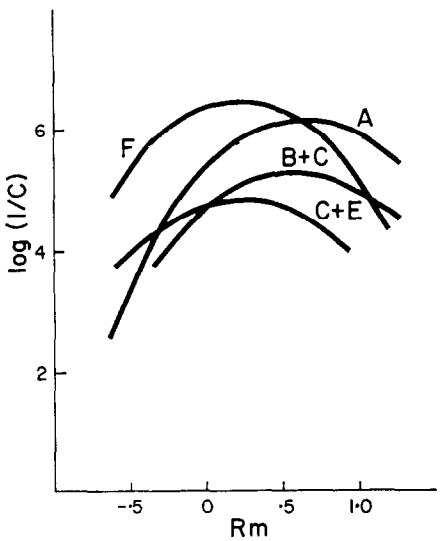


FIG. 1. Relationships between R_m and biological activity calculated from the regression equations in Table 4 for secondary N,N'-bis (dichloroacetyl) diamines, A, tertiary N,N'-bis(dichloroacetyl) diamines, B + C and C + E, and substituted naphthoquinones, F.

two series differed by only two methylene elements. Similar calculations showed that the A and C+E series differed by two methylene elements while the A and B+C series differed by only one methylene element. These calculations indicate that partition coefficients have similar effects on mitochondrial electron transport inhibitors even though these inhibitors have different structures and act at different points on the respiratory chain. The data suggest that penetration of the mitochondrial membrane is a highly significant determinant for structure–activity relationships with these compounds. Furthermore, these data demonstrate the superiority of the regression analysis, emphasized by Hansch,[17] when regression analyses are compared to the simple inspection of data. For example, the subtle differences between secondary and tertiary N,N'-bis(dichloroacetyl) diamines are not apparent from the inspection of data. These analyses show further that the absence of uncoupling activity in tertiary N,N'-

bis(dichloroacetyl) diamines is not the result of an inappropriate partition coefficient since there is considerable overlap between the secondary amide and tertiary amide series. The uncoupling effect, therefore, depends on other properties of these compounds.

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REFERENCES

1. A. R. Surrey and J. R. Mayer, *J. med. pharm. Chem.* **3,** 409 (1961).
2. A. L. Beyler, G. O. Potts, F. Coulston and A. R. Surrey, *Endocrinology* **69,** 819 (1961).
3. R. A. Dietrich and L. Hellerman, *J. biol. Chem.* **238,** 1683 (1963).
4. A. J. Merola and J. D. Turnbull, *Biochem. Pharmac.* **16,** 211 (1967).
5. A. J. Merola and G. P. Brierley, *Biochem. Pharmac.* **19,** 1429 (1970).
6. A. J. Merola, K. M. Hwang, M. Jurkowitz and G. P. Brierley, *Biochem. Pharmac.* **20,** 1393 (1971).
7. L. F. Fieser, E. Berliner, F. J. Bondhus, F. C. Chang, W. G. Dauben, M. G. Ettlinger, G. Fawaz, M. Fields, M. Fieser, C. Heidelberger, H. Heymann, A. M. Seligman, W. R. Vaughan, A. G. Wilson, E. Wilson, M. Wu, M. T. Leffler, K. E. Hamlin, R. J. Hathaway, E. J. Matson, E. E. Moore, M. B. Moore, R. T. Rapala and H. E. Zaugg, *J. Am. chem. Soc.* **70,** 3151 (1948).
8. A. L. Tappel, *Biochem. Pharmac.* **3,** 289 (1960).
9. Y. Hatefi, A. G. Haavik and D. E. Griffiths, *J. biol. Chem.* **237,** 1681 (1962).
10. J. T. Penniston, L. Beckett, D. L. Bentley and C. Hansch, *Molec. Pharmac.* **5,** 333 (1969).
11. I. E. Bush, *Meth. biochem. Anal.* **13,** 357 (1965).
12. C. B. C. Boyce and B. V. Milborrow, *Nature, Lond.* **208,** 537 (1965).
13. G. P. Brierley, *J. biol. Chem.* **242,** 1115 (1967).
14. A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **177,** 251 (1949).
15. J. Franc and J. Jokl, *Coll. Czech. Chem. Commun.* **21,** 1161 (1956).
16. E. Soczewinski and C. A. Wachtmeister, *J. Chromat.* **7,** 311 (1962).
17. C. Hansch, *Farmaco, Ed. sci.* **23,** 293 (1968).