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Studies on the antibacterial and antifungal properties of 1, 4-naphthoquinones

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Summary

1. New halogenated 1,4-naphthoquinones were synthesized and together with other known 1,4-naphthoquinones, were screened for antibacterial activity by a turbidimetric method, and for antifungal activity by the diffusion method on agar plates.

2. The half-wave potentials and the influence on the oxidative phosphorylation of some of these compounds were determined.

3. 2-chloro-3,2′-chloro-ethyl-1,4-naphthoquinone (half-wave potential = −187 mV) was the most active compound, completely inhibiting cell respiration.

4. While the natural active naphthoquinones, vitamin K and ubiquinones, possess, as substituent, the electron repelling methyl group, the microbiologically active 1,4-naphthoquinones are substituted, in the quinone moiety, with electron attracting groups such as OH or Cl.

5. The half-wave potentials can give only an initial indication of the activity of the compounds studied ; a good correlation, on the contrary, can be found between the ultraviolet spectra of such compounds and their activity which seems to depend on the ability of active compounds to exist in an extensively conjugated structure and to form hydrogen bonds.

Introduction

Quinones, in particular benzoquinone and naphthoquinone derivatives, have been repeatedly isolated from lower as well as higher species of plants, and are found frequently in animals.

Among them are biologically important compounds, such as the vitamin K group and the Q coenzyme, the ubiquinones. These compounds play an important role in cell metabolism, especially as electron carriers (Martius, 1961 ; Green, Warton, Tzagoloff, Riske & Brierley, 1965 ; Redfearn & Burgos, 1966). In addition to quinones possessing a biological function, other compounds of this class have been found, showing an antibiotic activity.

Some of them are present in nature, but the largest number are obtained synthetically, among them 2-hydroxy-3-alkyl-1,4-naphthoquinones, which are active against malaria parasites (Fieser & Leffler, 1948) and as coccidiostats (Bullock, 1968).

Other derivatives are active against bacteria and fungi (Thalhimer & Palmer, 1911; Colwell & McCall, 1946; Oster & Golden, 1948; Akiya, 1956; Holmes, Currie, Maltman, Silver, Lough & Lesky, 1964), their activity being increased through halogenation in the nucleus (Jouin & Buu-Hoi, 1946).

The activity of these compounds has been explained as a short circuiting of the cell electron transfer normally executed by quinones (Holmes *et al.*, 1964). We have resumed the study of naphthoquinones and found that compounds substituted with chlorine in the nucleus and side chain show a particularly marked activity against bacteria and fungi.

Methods

Chemical syntheses

In Table 1 new halogenated naphthoquinones are shown and their physical properties described.

Compounds Nos. 1 and 3 were prepared from the corresponding alkyl derivatives: the acetic solutions were saturated with chlorine, and the 2,3-dichloro-1,4-dioxotetralins obtained were precipitated with water. The substances were then carefully dehydrohalogenated in concentrated sulphuric acid.

Similarly, compound No. 2 was obtained by bromination in the dark, with a bromine solution in glacial acetic acid: dehydrohalogenation was performed by gently warming the acetic acid solution with dry sodium acetate (Fries & Lohmann, 1921). Compound No. 6 was obtained from 2-*tert*-butyl-1,4-naphthoquinone, prepared after Bromby, Peters & Rowe (1943), by chlorination and successive dehydrohalogenation as stated above.

Compounds Nos. 4, 5, 7 and 8 were prepared from 2-chloro-1,4-naphthoquinone by alkylation, both with appropriate diacyl peroxide in warm acetic acid according to Fieser *et al.* (1948), and with esters of tetravalent lead in the presence of a promoter, as described by Fieser & Chang (1942) for alkyl naphthoquinones. Table 2 contains known compounds to be screened, prepared according to standard methods.

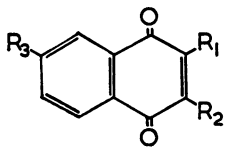
Compound No. 13 was prepared by oxidation of 2,7-dimethyl-naphthalene (Kruber & Weissgerber, 1919) while the intermediate hydrocarbon was obtained following Baker's method (Baker, McOnie & Warburton, 1952); however, the cyclization of α-methyl-γ-*p*-tolybutyric acid to 2,7-dimethyl-tetralone was effected by using concentrated sulphuric acid and then dehydrogenating the 2,7-dimethyl-tetralin to 2,7-dimethyl-naphthalene in the liquid phase at 280°, for 90 min in the presence of Pd 10% on carbon and in flowing CO_2.

Microbiological determination

The antimicrobial activity of these compounds was investigated as follows:

1. Antibacterial activity by turbidimetric method.
2. Antifungal activity by diffusion method on agar plates.
3. Bactericidal activity of the most interesting compound.
4. Antagonism of glutathione or cysteine for the antibacterial activity.
5. Study of a possible adaptation of a strain of a staphylococcus to these compounds.

TABLE 1. *Preparation of new naphthoquinones substituted with halogen*



No.	R_1	R_2	R_3	Name	Melting point (°C)	Formula	Calculated % C	Calculated % H	Found % C	Found % H
1	CH_3	Cl	CH_3	2,7-Dimethyl-3-chloro-1,4-naphthoquinone	104–106	$CH_{12}H_9ClO_2$	65·35	4·12	65·46	4·22
2	CH_3	Br	CH_3	2,7-Dimethyl-3-bromo-1,4-naphthoquinone	116–117	$C_{12}H_9BrO_2$	54·40	3·43	54·11	3·40
3	C_2H_5	Cl	CH_3	2-Ethyl-7-methyl-3-chloro-1,4-naphthoquinone	81	$C_{13}H_{11}ClO_2$	66·60	4·73	66·55	4·71
4	Cl	$CH(CH_3)_2$	H	2-Chloro-3-isopropyl-1,4-naphthoquinone	50	$C_{13}H_{11}ClO_2$	66·80	4·75	66·63	4·80
5	Cl	$CH_2CH(CH_3)_2$	H	2-Chloro-3-isobutyl-1,4-naphthoquinone	106–108	$C_{14}H_{13}ClO_2$	67·60	5·27	67·41	5·31
6	Cl	$C(CH_3)_3$	H	2-Chloro-3-tert.butyl-1,4-naphthoquinone	67–69	$C_{14}H_{13}ClO_2$	67·60	5·27	67·51	5·30
7	Cl	CH_2CH_2Cl	H	2-Chloro-3,2′-chloro-ethyl-1,4-naphthoquinone	113–115	$C_{12}H_8Cl_2O_2$	56·50	3·13	56·70	3·20
8	Cl	$CH_2CH_2CH_2Cl$	H	2-Choloro-3,3′-chloropropyl-1,4-naphthoquinone	86–88	$C_{13}H_{10}ClO_2$	58·00	3·75	58·22	3·77

Determination of antibacterial activity by turbidimetric method

The activities of the test compounds were assayed against *Staphylococcus aureus* ATCC 6538P, *Shigella sonnei* ATCC 11060 and *Escherichia coli* 120 by a turbidimetric method, similar to that used by USP XVII (1967) for the assay of tetracyclines. Peptone broth (F.D.A. 1965 141 c.20(i)), was used for *Staphylococcus aureus*, and heart brain infusion for the other two bacterial species. All the experiments were repeated in the same medium with the addition of 10% horse serum.

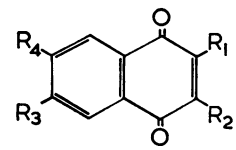
Vitamin K_3 was used as a standard at the following final concentrations in broth: 0·5, 1 and 2 μg/ml in the absence of serum, and 1·5, 3 and 6 μg/ml in the presence of 10% serum with *Staphylococcus aureus*; 1·5, 3 and 6 μg/ml in the absence of serum, and 6, 12 and 24 μg/ml in the presence of 10% serum with the two Gram negative bacteria. The concentrations of the samples were adjusted so that at the end of the assay, transmittance of the broth cultures was close to that of the standards.

All the solutions were prepared in dimethylformamide and diluted in broth in order to obtain a final solvent concentration of 1% which had no significant activity on bacteria. The readings were made after about 3 h incubation at 37° C.

Determination of antifungal activity by the diffusion method on agar plates

The antifungal activity of the test compounds was assayed against *Candida albicans* ATCC 2091 on Sabouraud agar plates, a 2+2 biological assay, where the response (diameter of growth inhibition zone) was measured at two dose levels both for the standard and for the sample.

TABLE 2. *Naphthoquinones prepared for the screening programme*



No.	R_1	R_2	R_3	R_4	Name
9	CH_3	H	H	H	2-Methyl-1,4-naphthoquinone
10	C_2H_5	H	H	H	2-Ethyl-1,4-naphthoquinone
11	n-C_6H_{13}	H	H	H	2-Hexyl-1,4-naphthoquinone
12	H	H	CH_3	H	6-Methyl-1,4-naphthoquinone
13	CH_3	H	H	CH_3	2,7-Dimethyl-1,4-naphthoquinone
14	CH_3	H	CH_3	H	2-6-Dimethyl-1,4-naphthoquinone
15	C_2H_5	H	H	CH_3	2-Ethyl-7-methyl-1,4-naphthoquinone
16	Cl	CH_3	H	H	2-Chloro-3-methyl-1,4-naphthoquinone
17	Br	CH_3	H	H	2-Bromo-3-methyl-1,4-naphthoquinone
18	Cl	C_2H_5	H	H	2-Chloro-3-ethyl-1,4-naphthoquinone
19	Cl	n-C_3H_7	H	H	2-Chloro-3-propyl-1,4-naphthoquinone
20	Br	n-C_3H_7	H	H	2-Bromo-3-propyl-1,4-naphthoquinone
21	Cl	n-C_5H_{11}	H	H	2-Chloro-3-pentyl-1,4-naphthoquinone
22	Br	n-C_5H_{11}	H	H	2-Bromo-3-pentyl-1,4-naphthoquinone
23	CH_3	CH_2Cl	H	H	2-Methyl-3-chloromethyl-1,4-naphthoquinone
24	Cl	CH_2Cl	H	H	2-Chloro-3-chloromethyl-1,4-naphthoquinone
25	CH_2Cl	CH_2Cl	H	H	2,3-Di-chloromethyl-1,4-naphthoquinone
26	CH_3	$CH(Cl)CH_3$	H	H	3-1′-Chloroethyl-2-methyl-1,4-naphthoquinone
27	CH_3	$S.CH_2CH_3$	H	H	2-Methyl-3-thioethyl-1,4-naphthoquinone
28	Cl	$S.CH_2CH_3$	H	H	2-Chloro-3-thioethyl-1,4-naphthoquinone
29	C_2H_5	$S.CH_2COOH$	H	H	S-(2-Ethyl-1,4-naphthoquinonyl-3)-thioglycollic acid
30	CH_3	NO_2	H	H	2-Methyl-3-nitro-1,4-naphthoquinone
31	CH_3	NH_2	H	H	2-Methyl-3-amino-1,4-naphthoquinone
32	H	H	OH	H	6-Hydroxy-1,4-naphthoquinone
33	Br	H	OH	H	2-Bromo-6-hydroxy-1,4-naphthoquinone

The substances were dissolved in dimethylformamide and then diluted in phosphate buffer at pH 6, until a final 10% solvent concentration was obtained. At this concentration the dimethylformamide does not produce growth inhibition zones.

Vitamin K_3 was used as a standard in concentrations of 60 and 120 μg/ml. The concentrations of the samples were adjusted so as to have growth inhibition zones equivalent to those obtained from the standard.

The measurements of zone diameters were made after 18 h incubation at 37° C.

Determination of bactericidal activity

This determination was carried out only on compound No. 7 of Table 1 (2-chloro-3,2′-chloroethyl-1-4-naphthoquinone), considered the most interesting of the series, in comparison with vitamin K_3. As test organism *Staphylococcus aureus* Smith, *Bacillus subtilis* ATCC 9466, *Streptococcus faecalis* ATCC 6057, *Shigella sonnei* ATCC 11060 and *Candida albicans* ATCC 2091 were employed.

The compounds were dissolved in dimethylformamide and diluted in water to give concentrations of 25 and 50 μg/ml in the absence of serum, and of 50 and 100 μg/ml in the presence of 10% serum (final dimethylformamide concentration=2·5%); the solutions were then inoculated with bacteria 1×10^7/ml or *C. albicans* 2×10^7/ml. Subcultures were made after 1, 2, 5, 24, 36, and 48 h of contact in heart brain infusion for bacteria, and in Sabouraud broth for *C. albicans*.

Antagonism of glutathione or cysteine for the antibacterial activity

The activity of compound No. 7 was also assayed in media containing glutathione or cysteine at a concentration of $333\cdot10^{-5}$M in order to detect any possible inhibitory effect of the SH-groups on such naphthoquinone derivatives. The activity was determined by the turbidimetric method against *S. aureus* ATCC 6538 P, *Shigella sonnei* ATCC 11060 and *E. coli* 120 in peptone broth.

Study on the metabolic adaptation of microorganisms

The study was also limited to the 2-chloro-3,2′-chloroethyl-1,4-naphthoquinone. Suspensions of *S. aureus* in peptone broth containing 0·5 μg/ml of this substance were incubated at 37° C. At this concentration, only a temporary inhibition was obtained.

At the stationary phase, the bacteria were transferred in fresh medium containing the antibacterial compound at the same concentration and, as control, in a fresh medium without the drug.

The activity of the naphthoquinone derivatives on the original strain was then compared to the activity on the pre-adapted bacteria, by periodic turbidimetric readings.

Biological tests

Uncoupling of oxidative phosphorylation

The method described by Lardy & Wellman (1952) was used with some modification. The oxidative phosphorylation was observed with rat liver mitochondria

using potassium glutamate substrate. Oxygen consumption was determined by Warburg manometry; for each sample a 0·5 ml suspension of mitochondria was taken, corresponding to 250 mg fresh liver; the substance was then dissolved in 0·025 ml alcohol. The concentration of inorganic phosphorus in the filtrate which had been deproteinized with trichloroacetic acid, was determined by the method of Fiske & Subbarow (1925).

Polarographic methods

A controlled potential three electrode polarograph (mod. 463 Amel, Milan, Italy) was employed in all measurements, with a scanning rate of 50 mV/min and a chart speed of 2·54 cm/minute. The half-wave values are referred to the saturated calomel electrode (Mod. 303 NS, Ingold, Swiss) and are automatically corrected for *iR* drop across the cell; no correction was made for the liquid junction potential.

The *m* value of the capillary was 2·45 mg s^{-1} and the drop time 3·6+3·8 s (E=0, closed circuit). The half-wave potential determinations were made at 25° ±0·1° C on 1·4 naphthoquinones dissolved in 75% v/v ethanolic aqueous buffer solution 0·1 M acetic acid and sodium acetate pH 6·6 ±0·05 as measured with glass electrode; methylene blue thiocyanate (0·001%) was employed as maximum suppressor. This solution corresponds to the acid buffer employed by Currie & Holmes (1966).

Preliminary measurements showed some instability of certain 1,4-naphthoquinones, either in stock solution or in polarographic conditions, as shown by the time dependence of polarographic characteristics. Therefore, in an attempt to minimize this decomposition, no stock solutions were prepared, but the solid depolarizers were directly dissolved in the deaerated buffer solution. After stirring for exactly 5 min, the first polarogram was recorded; a second one was recorded after 30 min standing. The weight of depolarizers was chosen so as to permit a maximum concentration of 8×10^{-4}M to be obtained. No attempt was made to obtain total solution of different samples: as indicated by rough measurement all compounds were 60–100% dissolved—that is, the concentration of depolarizers was in the range of $5–8\times10^{-4}$M.

From the two polarograms we obtained two $E_{\frac{1}{2}}$ values, which did not differ by more than 3 mV: the mean values correct to the nearest integer are reported in Table 3.

Results

The antibacterial activity was determined by the turbidimetric method. It was not possible to obtain valid data by determining the minimum inhibitory concentration, since almost all the substances examined were relatively insoluble in water, and liable to precipitate at a concentration of 20–30 μg/ml, which is near to the microbiologically effective values. In order to obviate this inconvenience, the compounds were compared with 2-methyl-1,4-naphthoquinone (vitamin K_3) the activity of which was taken as equivalent to 100. Vitamin K_3 was used as standard, since it is well known as a good preservative and is chemically similar to this series of compounds. Readings were taken after 3 h at a concentration below the limit of solubility. The results are reported in Table 3.

In the series of compounds tested, the halogen derivatives showed most increase in the activity against *S. aureus*; chloro-derivatives showing more increase than the equivalent bromo-derivatives. Maximum activity was obtained with 2-chloro-3,2′-

chloroethyl-1,4-naphthoquinone (compound No. 7, Table 3). On *S. aureus* it was six times more active than vitamin K_3 and displayed a partial inhibitory action at a concentration of 0·25 g/ml; it caused a longer delay in bringing the bacteria to the log phase of growth, but by prolonging the incubation period of the cultures it was noted that the number of micro-organisms at the final stationary phase of growth was practically the same for both this compound and vitamin K_3. In order to interpret these results, we presumed that the bacteria might have become resistant to the compound; however, the test carried out for this purpose did not confirm our hypothesis.

2-Chloro-3-chloromethyl-1,4-naphthoquinone (compound 24, Table 3) is nearly inactive, due to its poor stability as shown by chromatographic analysis. Other 2-chloroderivatives of 1,4-naphthoquinone (Nos. 16 and 28, Table 3), which have a short side chain, show good activity against *S. aureus*. A longer chain causes the activity to fall, as in compound No. 21 (Table 3). This contrasts with the antimalarial activity of the 2-hydroxy-3-alkyl-naphthoquinone which increases to a peak value for a C_9 group and then falls off (Fieser & Richardson (1948)).

TABLE 3. In vitro *activity of* 1,4-*naphthoquinones* (*% of vitamin* K_3) *and half-wave potentials of some* 1,4-*naphthoquinones*

	Turbidimetric method						Diffusion on agar plates	
	Staphylococcus aureus ATCC 6538 P		*Shigella sonnei* ATCC 11060		*Escherichia coli* 120		*Candida albicans* ATCC 2091	$-E_{\frac{1}{2}}$ (mV)
	*	†	*	†	*	†	*	
1	165	60	60	<10	50	25	190	
2	200	70	90	<10	70	<10	65	
3	200	70	75	<10	70	<10	200	
4	320	75	55	20	100	25	330	226
5	400	70	70	20	80	20	‡	
6	340	80	55	50	90	45	270	
7	620	230	120	125	200	130	550	180
8	340	150	120	90	120	55	350	199
9	100	100	100	100	100	100	100	213
10	90	90	50	50	50	60	100	
11	90	25	<10	<10	<10	<10	‡	
12	100	100	30	<10	80	<10	100	
13	90	90	45	50	70	70	75	235
14	55	50	30	25	40	40	35	
15	40	50	50	30	50	25	60	
16	500	110	100	30	130	50	250	207
17	380	60	100	30	100	30	230	
18	380	120	95	55	110	55	250	
19	350	105	85	30	100	25	180	
20	330	100	60	<10	70	<10	170	
21	320	30	30	<10	60	15	‡	
22	270	30	40	<10	45	15	‡	218
23	130	30	50	<10	50	<10	250	
24	200	50	30	<10	30	<10	85	(≤0)§
25	100	<10	25	<10	25	<10	65	(≤0)§
26	160	70	50	<10	50	<10	250	
27	150	50	40	<10	50	<10	‡	
28	420	100	100	<10	100	<10	300	152
29	<10	<10	<10	<10	<10	<10	‡	
30	25	25	<10	<10	<10	<10	<10	(100)§
31	<10	<10	<10	<10	<10	<10	<10	410
32	80	50	20	<10	<10	<10	25	
33	100	30	25	<10	25	<10	20	

*Medium without serum
†Medium with 10% horse serum
‡The compound precipitates
§First wave approximate values, owing to interactions with the solvent of the buffer or mercury.

If chlorine is substituted by a nitro-group (compound No. 30, Table 3), a relatively inactive compound is produced, in contrast to chloramphenicol, nitrofuran and nitro-imidazone derivatives, in which compounds the nitro group is responsible for the activity.

Against the two Gram negative bacteria, *Shigella sonnei* and *Escherichia coli* only compound No. 7 (Table 3) displayed any appreciable activity in absence of serum. In the presence of serum the halogen derivatives showed a greater reduction of activity than vitamin K_3: the activity ratio between compound No. 7 and vitamin K_3 on *S. aureus* is 6:2 in the absence of serum, and decreases to 2:3 by adding 10% serum. Compound No. 7 showed bactericidal and fungicidal activities against *S. aureus* and *C. albicans* only at high concentration and after prolonged contact periods (25–50 μg/ml for 36 h). It was inactive against *Bacillus subtilis, Staphylococcus faecalis,* and *Shigella sonnei.* Vitamin K_3 was also inactive against all the five strains tested.

In the test for antibacterial activity in the presence of glutathione or cysteine, the 2-chloro-3,2′-chloroethyl-1,4-naphthoquinone was relatively inactive. With all the three micro-organisms tested, the activity was reduced to less than 10% by either inhibitor; bacterial growth, however, was partially inhibited by glutathione and cysteine in a control experiment; as observed by Colwell & McCall (1946) and Holmes *et al.* (1964) on other 2- or 2,3-substituted naphthoquinones. Under these experimental conditions sulphydryl compounds react with the naphthoquinones to give inactive compounds.

Since many difficulties arose in carrying out the turbidimetric tests, the diffusion method on agar plates was used to determine the antifungal activity. *Candida albicans* ATCC 2091 (Table 3) was used as test organism. The results are parallel to those obtained from *S. aureus* using the turbidimetric method. In particular, the 2-chloro-3,2′-chloroethyl-1,4-naphthoquinone which proved to be the most active on bacteria, was also 5·5 times more active than vitamin K_3; it produces an inhibition zone on *C. albicans* at a concentration of 1·5 μg/ml. Table 3 also shows the polarographic results obtained from the test compounds examined.

Discussion

The naphthoquinone derivatives can act as bacterial growth inhibitors by participating competitively in electron transport with the cell components, possibly with the endogenous vitamin K_3 or ubiquinone (Holmes *et al.*, 1964). According to the authors, this may occur when the $-E_{\frac{1}{2}}$ value of the naphthoquinones is between 163 and 307 or between 314 and 536 mV.

If naphthoquinones are able to react with a bacterial protein, the $-E_{\frac{1}{2}}$ value of the derivative must fall within these ranges. Thus taking into account the difference of about −25 mV between the results recorded by Currie & Holmes (1966) and our data, due to the correction for the fall in resistance, all the compounds in Table 3 displaying some bacteriological activity have an $-E_{\frac{1}{2}}$ value lying between 193 and 307. This is consistent with the above hypothesis if we consider that our compounds do not react with protein: If, on the contrary, we consider that they do react (as in fact they do), the comparison must be on the basis of the $-E_{\frac{1}{2}}$ values of the corresponding naphthoquinone protein-derivatives; these new values, which are

easily calculated for standard derivatives based on the empirical shifts of Currie & Holmes (1966), lie within the two potential levels of the active compounds.

The polarographic method gives evidence of the scanty stability of compounds Nos, 24, 25 and 30 in Table 3, the $-E_{\frac{1}{2}}$ values lie 100 mV out of the range of values expected. The only inconsistent result is of 2-methyl-3-amino-1,4-naphthoquinone (Table 3, compound 31) which, in spite of its $-E_{\frac{1}{2}}$ value, displayed a negligible antibacterial activity. The half-wave potentials appear to give a first indication for the activity of most of the compounds studied in this paper but do not explain why some of our compounds, for example, compounds 7 and 16, have an outstanding activity in comparison with the others. There must be other unrelated factors responsible for this difference.

We think that the activity of the compounds which we studied depends on their electronic structure and their ability to form hydrogen bonds ; we attach no importance to the distance between active sites as these distances have little correlation with the activity of compounds. On the contrary, we have noticed that the ultraviolet spectra of every active compound shows a strong band at 270 nm, as well as the bands at 246 and 262 nm, typical of a *p*-quinone structure. Since every active compound is characterized by the presence of a $-Cl$ in the quinone moiety, we relate its activity to the ability of the $-Cl$ to increase the degree of conjugation in the quinone structure (band at 270 nm) and give rise to molecular forms involving a greater separation of charges. On the other hand, the $-Cl$ substituted quinone retains its ability to form hydrogen bonds.

We explain the inactivity of the compound 30 by the increased electron attractive character of the NO_2 group which prevents the conjugation ; consequently the ultraviolet spectrum of this compound does not show the band at 270 nm. Compound 31 has a band at 280 nm, which indicates extended conjugation ; it has no activity, however, which can be explained by a decreased ability of the conjugated $-NH_2$ group to form hydrogen bonds. It is a striking fact that quinones which are present in nature, such as vitamin K and ubiquinone, possess—as substituents—not only a longer side-chain, but also a methyl group, which has electron repelling capacity. Should this group, as in the case of vitamin K_1, be replaced with an OH group or a Cl atom—which are both electron attracting groups—inactive vitamin compounds result (Loewental & McFarland, 1961).

The naphthoquinones which are active against malaria, possess at position 2 an electron attracting group (OH group). These anti-malarials are powerful inhibitors of respiration and act in the region of cytochrome C/cytochrome B in the respiratory enzyme chain. The succinate oxidase system is completely inhibited by some of these compounds (Ball, Anfinsen & Cooper, 1947). We have tested some of our compounds (reported in Tables 1 and 2) on the uncoupling of oxidative phosphorylation ; the 2-chloro-3,2-chloroethyl-1,4-naphthoquinone (Table 1, compound 7) completely inhibits respiration at a 10^{-4}M concentration. The same applies to 2-chloro-3-ethyl-1,4-naphthoquinone (Table 1, compound 18). Natural vitamin K_1 on the other hand neither inhibits respiration nor uncouples oxidative phosphorylation nor possesses any anti-bacterial activity. Between these two cases, there is a whole range of compounds—for example, 3-methyl-1,4-naphthoquinone, vitamin K_3—which even if it does not possess all the biological properties of vitamin K_1, uncouples the oxidative phosphorylation at $2{\cdot}5 \times 10^{-4}$M concentration and has a slight inhibitory

effect on respiration. Analogous experiments have also been carried out on 1,4-naphthoquinones by Martius & Nitz-Litzow (1953) and Howland *et al.* (1963).

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