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Influence of the electron-donating properties on the psychotropic activity of phenothiazine derivatives

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The electron-donating properties of substituted phenothiazine derivatives and related cyclic compounds have been determined by measuring the maximum of the lowest-energy absorption band of the corresponding charge transfer complexes with tetracyanoethylene and other electron acceptors. Phenothiazine displays a high electron-donating power. The presence and the nature of the side-chain in the 10-position do not significantly influence this property, which is much modified according to the substituent on the 2-position of the ring. The electron donor ability of phenothiazine progressively falls as the Hammet σ *para* constant of this substituent rises. The decrease parallels increase of psychotropic activity. The observations are consistent with the view that an essential part in the mechanism of psychotropic activity of the phenothiazine drugs is played by a positive radical ion.

Much attention has been paid to the electron-donating properties of phenothiazine neuroleptic drugs in relation to their pharmacological and therapeutic activities. Re-evaluation of the initial molecular-orbital treatments indicated that phenothiazines, without being the outstanding electron donors as previously described (Karreman, Isenberg & Szent-Gyorgyi, 1959; Pullman & Pullman, 1959; Malrieu, 1967; Bodea & Silberg, 1968), still display good donor ability (Orloff & Fitts, 1961; Bloor, Gilson & others, 1970). This conclusion correlates well with the low value of their ionization potential calculated from photoelectric measurements (Lyons & Mackie 1963) and from the maximum of the charge transfer absorption band with tetracyanobenzene (Bloor & others, 1970). Furthermore, the chlorpromazine free radical has been demonstrated to be capable of inhibiting microsomal ($Na^+ + K^+$)-ATPase activity (Akera & Brody, 1968, 1970) as well as other enzyme systems (Levy & Burbridge, 1967) and recently it has been shown *in vivo* that pharmacological effects of this drug depend at least in part on this free radical form (Gooley, Keyzer & Setchell, 1969).

However, in the understanding of phenothiazine's actions it is essential to take into account the part played by the side-chain in the 10-position mainly responsible for the appearance of a psychotropic activity, by the S atom whose transformation into sulphoxide strongly reduces this activity (Courvoisier, Ducrot & others, 1962) and by the substituent at position 2, which markedly influences the potency. The aim of the present investigation was to search for a possible relation between the structural specificity and the electron donor ability shown by these drugs. To this end, the electron-donating properties of substituted phenothiazine derivatives and related cyclic compounds have been determined by measuring the maximum of the lowest-

energy absorption band of the corresponding charge transfer complexes with tetracyanoethylene and other electron acceptors.

MATERIALS AND METHODS

Materials

Tetracyanoethylene TCNE (FLUKA Chemicals, purum grade) was purified by recrystallization from chlorobenzene then sublimation under vacuum (10 mm Hg; 135°). The product was stable for months in a stoppered vial. s-Trinitrobenzene TNB (FLUKA Chemicals, purum grade) was crystallized twice from chloroform. Chloranil CHL (B.D.H., purissimum grade) was used without further purification.

The phenothiazine derivatives were obtained as a gift from the Rhone-Poulenc Laboratories (France). They were crystallized twice from benzene and kept in the dark. The base form of *N*-aminoalkylphenothiazine drugs was prepared by dissolving the corresponding salt in dilute NaOH (pH 12) and extracting the solution twice with an equal volume of dichloromethane. The combined dichloromethane phases were rinsed with distilled water, dried over anhydrous Na_2SO_4 and evaporated at room temperature in the dark.

All other drugs were of purissimum grade and used without further purification.

Anhydrous dichloromethane of spectroscopic grade was used as a solvent for all wavelength measurements.

Apparatus and methods

Absorption spectra were recorded on a Zeiss Spektralphotometer PMQ_2 II, with fused silica cells of optical path 1 cm. The measurements were performed at an ambient temperature of 20° ± 2°. Wavelength maxima for charge transfer complexes were assigned by determining the midpoint on the broad peak of the complex.

RESULTS AND DISCUSSION

Charge transfer (CT) bands and ionization potential I^D of unsubstituted phenothiazine

When an electron acceptor compound e.g.: tetracyanoethylene (TCNE), s-trinitrobenzene or chloranil, is added to a solution of phenothiazine in dichloromethane two new and well separated bands of unequal intensity are formed in a spectral region where both donor and TCNE acceptor have no absorption and which can be ascribed to the formation of an intermolecular charge transfer (CT) complex (Mercier & Dumont, 1971). From the position of the lowest-energy or first CT band, it is possible to estimate the ionization potential I^D of the donor by use of the equation:

$$h\nu_{CT_1} = I^D - C_a + \frac{C_b}{I^D - C_a}$$ (Hastings, Franklin & others, 1953)

where $h\nu_{CT_1}$ is the CT transition energy and C_a, C_b are two constants characteristic of the electron affinity of the given acceptor. The results are presented in Table 1. It can be seen that, allowing for the various uncertainties inherent in the procedure, consistent values of I^D are obtained for phenothiazine, whatever the electron acceptor used. These appear in good agreement with the values $I^D = 6{\cdot}7$ eV, calculated by Lyons & Mackie (1963) from direct photoelectric measurements and $I^D = 7{\cdot}02$ eV estimated by Bloor & others (1970) from the position of the CT band with tetracyanobenzene.

Table 1. *Ionization potential (I^D) of phenothiazine calculated from the position of the first charge transfer (CT_1) absorption band with different electron acceptors.* The wavelength (λ) and frequency (ν) measurements were made using solutions of phenothiazine and electron acceptor in dichloromethane.

Electron acceptor	λ (nm) λ_1	λ_2	C* (eV) C_a	C_b	$h\nu_{CT_1}$ (eV)	I^D (eV)
Tetracyanoethylene ..	865	485	6·10	0·54	1·433	6·82
Chloranil	775	445	5·70	0·44	1·599	6·95
s-Trinitrobenzene ..	510	405	5·00	0·70	2·430	7·10

* Taken from Briegleb (1961).

Charge transfer bands and ionization potential of cyclic compounds structurally related to phenothiazine

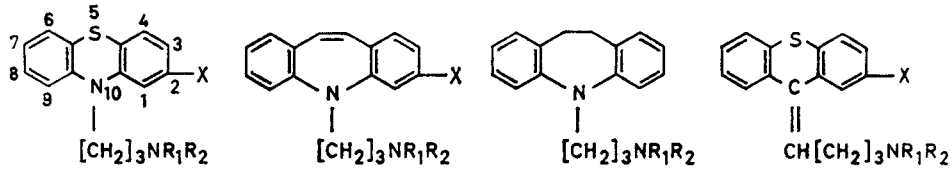
As shown in the previous section, the procedure on which the calculation of phenothiazine I^P is based, gives successful results and confirms the electron-donating ability of the molecule. To bring out the meaning of this property in relation to the pharmacological actions of the phenothiazine nucleus, the work was extended to other cyclic structures bearing some relation to the present ring. For these measurements, tetracyanoethylene was chosen as it does not absorb in the near ultraviolet and visible regions and gives CT bands with a minimum of overlapping.

From Table 2 it will appear that phenothiazine displays the highest electron donor power of all the molecules investigated. This ability does not seem to be specially dependent on the influence of any one of the elements of the ring but rather on their joint participation. More particularly, comparison between phenothiazine, iminodibenzyl, iminostilbene and thioxanthene indicates that the S atom does not play an essential part in the electron-donating properties.

Table 2. *Ionization potential (I^D) of cyclic compounds related to phenothiazine, calculated from the position of the first charge transfer (CT_1) absorption band on complexation with tetracyanoethylene.* The wavelength (λ) and frequency measurements were made using solutions of the compound and tetracyanoethylene solutions in dichloromethane. The values of C_a : 6·10 eV and C_b : 0·54 eV are identical with those shown in Table 1.

Compounds	λ (nm) 1	2	$h\nu_{CT_1}$ (eV)	I^D (eV)
Phenothiazine	865	485	1·433	6·82*
Iminodibenzyl	840	410	1·475	6·905
Iminostilbene	787	474	1·575	7·17
Anthracene	740	465	1·675	7·34
Thioxanthene	613	380	2·022	7·805†
Carbazole	600	385	2·066	7·86
Fluorene	571	420	2·171	7·98
Indole	555	—	2·233	8·06
Pyrrole	526	—	2·356	8·20
Acridane	520	357	2·384	8·23‡
Dihydroanthracene	425	—	2·917	8·82
Thiophene	392	—	3·162	9·08

* 7·02; † 7·84; ‡ 7·08 (Bloor & others, 1970).



I II III IV

General formula of psychotropic derivatives of phenothiazine (I), iminostilbene (II), iminodibenzyl (III) and thioxanthene (IV).

In addition to phenothiazine, both iminodibenzyl (III) and iminostilbene (II) rings, on which two series of psychoactive drugs are based also possess a remarkable electron donor ability. This feature is explicable for iminostilbene which differs from phenothiazine only in the replacement of the S atom by a carbon-carbon double bond. On the other hand, replacement of the same hetero-atom by a saturated bond as in iminodibenzyl would have been expected to bring about a large reduction of the electron-donating properties.

Thioxanthene (IV) as such appears to be a moderate electron-donating molecule. However a psychotropic action specifically depends on the introduction of a double bond between the corresponding C10 atom of the ring and the adjacent carbon of the lateral chain. This structural requirement is likely to restore an electronic distribution similar to that of phenothiazine derivatives and so to enhance the electron donor ability of the thioxanthene nucleus.

Electron-donating properties of phenothiazine substituted derivatives

Among the derivatives of phenothiazine, only those with *N*-propyl at the 10 position display a psychotropic activity. This disappears when the three carbon chain is either shortened or lengthened and is modified according to the nature of the side-chain nitrogen group. Moreover the pharmacological potency is related to the nature and the location of the ring substituents. It increases in the approximate order: $SCH_3 < OCH_3 < CH_3 < H < CO[CH_2]_2CH_3 < COCH_3 < Cl < CF_3 < CN < SO_2N(CH_3)_2$, and is strongly reduced when the substituents, even those most active like Cl, CF_3, CN . . . , are removed from position 2 to positions 1, 3 or 4. (Janssen, Niemegeers & Schellekens, 1965a, b; 1966, 1967).

To study the possible influence of the side-chain at the 10 position on the electron-donating properties of phenothiazine drugs, preliminary measurements were made with different acceptors: chloranil, trinitrobenzene and tetracyanoethylene. From these results, trinitrobenzene was chosen as it forms with the derivatives CT complexes, having double absorption bands and that remain stable for days (Mercier & Dumont, 1971). Comparative determinations show that the two broad peaks occur in the same region as for unsubstituted phenothiazine. Replacement of a 3′-dimethylamino-1′-propyl chain by a 1″-methyl-4″-piperazinyl-3′-propyl-1′, a 4″-hydroxy-*N*,3′-piperidinopropyl-1′ or a 2″, N^1-hydroxyethyl-N^2, 3′-piperazinyl-1′-propyl chain progressively improves the psychotropic activity, but does not affect the maxima of the CT bands of the complexes with trinitrobenzene, located respectively at 515 and 410 nm. Moreover, these maxima are identical with those characterizing the CT complex between trinitrobenzene and 10-CH_3 phenothiazine. It may thus be concluded that electron donor ability of the phenothiazine ring is not related to the

presence and the nature of the *N*-propyl side chain. The essential role played by this chain in the appearance of a psychotropic activity rests on the control of a hydrophilic-hydrophobic balance, as shown by measurements of the partition coefficients (Mercier & Dumont, 1969).

In contrast with these findings, the position of the maxima of the CT absorption bands on complexation between phenothiazine, unsubstituted on the 10 position and trinitrobenzene as well as other acceptors is dependent on the nature of the substituent at 2-C atom of the ring.

Examination of Table 3 indicates that, whatever the acceptor, the energy of the CT absorption band progressively increases as the substituent becomes more and more electron-withdrawing. Excepting the SCH_3 and $COCH_3$ groups, a linear relation can be found with a high degree of correlation ($r = 0{\cdot}963 - 0{\cdot}998$) between $h\nu_{CT_1}$ and the Hammet σ *para* constants (Fig. 1).

Previously, Chatten & Semaka (1970) have pointed out that the 2-position substituent plays an important role in determining the absorption maximum on the free radical formed by treating the phenothiazine derivatives in nitromethane with perchloric acid, while no or little influence is observed with the 10 position substituent. Tozer, Tuck & Craig (1969) measuring the activity of phenothiazine anthelmintics have shown that the reduction potentials yield a linear Hammet plot when the *para* substituent constants are assigned to the 3 and 7 positions and the *meta* to the 2 and 4 positions. On the other hand, the present observations on the psychotropic phenothiazine drugs suggest that the 2-position substituent should exert its main influence on the *para* position and so, on the S atom. Consequently the electronic impoverish-

Table 3. *Effect of the substituent at the 2-position of the phenothiazine ring, unsubstituted on the 10-position, on the transition energy (eV) of charge transfer complexes with different electron acceptors.* The position of the highest-energy or second CT_2 band of complexes with chloranil and trinitrobenzene has not been reported because of the lack of accuracy in the wavelength determinations, due to the strong absorption of the two components in the region below 450 nm.

Substituent at the 2-position	Tetracyanoethylene $h\nu_{CT_1}$	Tetracyanoethylene $h\nu_{CT_2}$	Chloranil $h\nu_{CT_1}$	Trinitrobenzene $h\nu_{CT_1}$	Hammet para substituent constant σ^*para
SCH_3	1·408	2·454	1·569	2·402	(−0·047)
OCH_3	1·416	2·479	1·599	2·407	−0·268
CH_3	1·424	2·519	1·599	2·407	−0·170
H	1·433	2·556	1·599	2·430	0·000
$CO[CH_2]_2CH_3$	1·441	2·556	1·599	2·459	
$COCH_3$	1·449	2·582	1·620	2·459	(+0·516)
Cl	1·458	2·593	1·629	2·469	+0·227
CF_3	1·493	2·660	1·668	2·509	+0·551
CN	1·502	2·666	1·689	2·509	+0·628
$SO_2N(CH_3)_2$	1·511	2·666	1·698	2·530	

* Taken from Jaffe (1953).

Least-square calculation gives the following equations:

$h\nu_{CT_1}$ (TCNE) $= 1{\cdot}4386 + 0{\cdot}09725\ \sigma$ $\quad r = 0{\cdot}998$

$h\nu_{CT_2}$ (TCNE) $= 2{\cdot}5464 + 0{\cdot}20113\ \sigma$ $\quad = 0{\cdot}994$

$h\nu_{CT_1}$ (CHL) $= 1{\cdot}6140 + 0{\cdot}10207\ \sigma$ $\quad = 0{\cdot}963$

$h\nu_{CT_1}$ (TNB) $= 2{\cdot}4348 + 0{\cdot}12635\ \sigma$ $\quad = 0{\cdot}993$

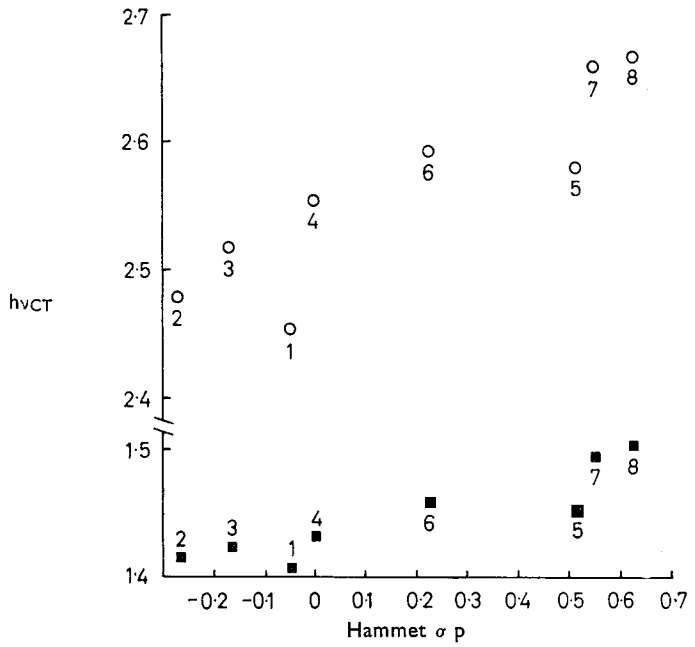


FIG. 1. Plot of energies of the first (■) and second (○) charge transfer transitions for TCNE complexes with C_2 substituted phenothiazines, where the substituent is (1) - SCH_3; (2) - OCH_3; (3) - CH_3; (4) - H; (5) - $COCH_3$; (6) - Cl; (7) - CF_3; (8) - CN.

ment induced by the electron-withdrawing substituents should preferably affect the lone pair of the S atom. The progressive decrease of the electron donor ability is parallel to an improvement of the psychotropic potency of the corresponding derivatives.

All these observations favour the view that the psychotropic activity is related to the establishment of a delicate balance between the electron-donating power of the phenothiazine ring and the electron-withdrawing effect of the substituent at the 2-position. This high donor ability makes one electron loss extremely easy and is responsible for the formation of a positive radical ion to which an essential role in the mechanism of the psychotropic activity has been assigned by other authors (see above).

An essential requirement for psychotropic activity is the existence of a three carbon chain between the N-atom of the phenothiazine ring (or the corresponding C atom of thioxanthene) and the terminal N of the side-chain. This strictly fixed distance leads to the consideration that both the phenothiazine ring and the side chain N-atom might play the main role in the attachment of the molecule to the pharmacological receptor, by means of their different chemical reactivity.

The cation radical from phenothiazine ring should be electron deficient whereas the N-atom belonging to the lateral chain should retain its nucleophilic character.

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