3,4-dichlorophenylhydroxylamine). Work-up of the mother liquor yielded more product. Recrystallization from aqueous methanol gave off-white crystals, m.p. 149–150° dec., in 89% yield (based on weight of isocyanate).

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A Simple Method for Predicting the Carcinogenic Properties of Polycyclic Aromatic Molecules[1]

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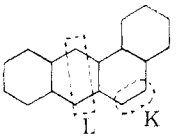
There have been numerous attempts to predict theoretically the carcinogenic activity of aromatic, substituted aromatic, and heteroaromatic compounds. As early as 1938 it was postulated that the electron density in the mesophenanthrenic bond (the K region) of such molecules could be correlated with carcinogenic activity.[2] Later work has employed calculations of free valence, localization energies,[3,4] energies of the various molecular orbitals of the carcinogen,[5,6] and other theoretical quantities. All of these workers have been more or less successful in finding a usable correlation between the theoretical property under consideration and carcinogenic activity. The complexity of these methods, however, has prevented their use by those who are not experienced in the application of molecular orbital (MO) theory.

It is the purpose of the present work to illustrate the application of one of the simplest MO approximations, the Dewar localization energy approximation,[7] to the prediction of the carcinogenic activity of aromatic systems.

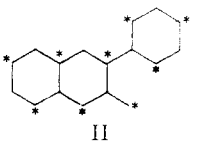
Method.—It has been shown that in many polycyclic aromatic systems the presence of a highly reactive K region is favorable for carcinogenic activity[8] while the presence of a reactive L region is unfavorable.[9]

One of the theoretical indices which has been used successfully to approximate the reactivity of a π-electronic system is the localization energy, the energy required to localize the appropriate number of π-electrons in the area undergoing attack.[10] The localization energies for the K region and the L region should thus lead to a prediction of the carcinogenic activity of various compounds. Dewar's implementation[7] of the Coulson, Longuet-Higgins perturbation theory[11,12] leads to an extremely simple method which involves no matrix diagonalization or other matrix manipulation for calculating the required localization energies.

As an example of the application of the method, consider 1,2-benzanthracene (I), a molecule which has both an active K region and an active L region.

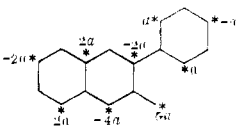


I



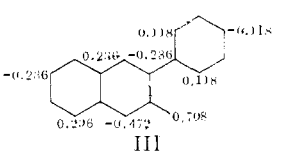
II

If a single position is removed from conjugation in an alternant aromatic hydrocarbon, an odd-alternant system results. For example, if position 3 in I is removed, the remaining system, II, has 17 centers which may be designated as active (starred) and inactive (unstarred). These are assigned such that more centers are starred than not, and no two adjacent centers are either starred or unstarred. The highest occupied MO in an odd-alternant system with n centers and n or $n + 1$ electrons is a nonbonding molecular orbital (NBMO). Coulson and Rushbrooke[11] have shown that such a NBMO has nodes (*i.e.*, no electron density) at the unstarred centers. They have further shown that the algebraic sums of the MO coefficients around a given unstarred center must be equal to zero. This, coupled with the normalizing condition that the sum of the squares of the coefficients for any given MO must equal unity allows a simple calculation of the MO coefficients for the starred centers. For example, by arbitrarily assigning the value of a to the coefficient at position 1′ in II, the following values fulfill the first criterion. The second criterion leads to eq. 1.

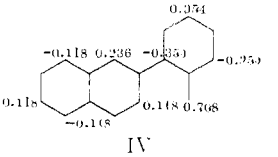


$$a^2 + (-a)^2 + (a)^2 + (-2a)^2 + (2a)^2 + (-2a)^2 + 12a)^2 + (-4a)^2 + (6a)^2 = 1 \quad (1)$$

or $71a^2 = 1$ and $a = 1/\sqrt{71} = 0.118$. This gives the following NBMO coefficients for II. For isolation of

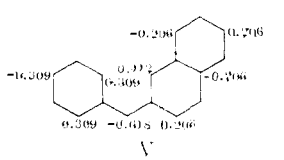


III

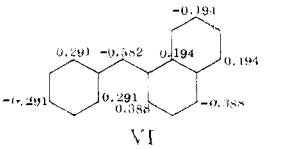


IV

the 4 position in I, the NBMO coefficients are shown in IV. For isolation of the 9 and 10 positions, the NBMO coefficients are shown in V and VI, respectively.



V



VI

(1) Presented before the Division of Medicinal Chemistry at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963.

(2) O. Schmidt, *Z. physik. Chem.*, **39**, 39 (1938).

(3) O. Chalvet, R. Daudel, and C. Moser, *Compt. rend.*, **246**, 3457 (1958).

(4) A. Pullman and B. Pullman, "Cancerisation par les Substances Chemique et Structure Moleculaire," Masson et Cie., Paris, 1955.

(5) O. Chalvet and R. Mason, *Nature*, **192**, 1070 (1961).

(6) A. Pullman and B. Pullman, *ibid.*, **196**, 228 (1962).

(7) M. J. S. Dewar, *J. Am. Chem. Soc.*, **74**, 3341 (1952).

(8) P. Daudel and R. Daudel, *Bull. soc. chim. biol.*, **31**, 353 (1949).

(9) A. Pullman, *Bull. soc. chim. France*, 394 (1954).

(10) A. Streitwieser, Jr., "Molecular Orbital Theory," John Wiley and Sons, Inc., New York, N. Y., 1961, p. 335.

(11) C. A. Coulson and G. S. Rushbrooke, *Proc. Cambridge Phil. Soc.*, **36**, 193 (1940).

(12) H. C. Longuet-Higgins, *J. Chem. Phys.*, **18**, 275 (1950).

TABLE I

ortho AND *para* LOCALIZATION ENERGIES[a]

Hydrocarbon	O.L.E. This work	O.L.E. H.M.O.[c]	P.L.E. This work	P.L.E. H.M.O.[c]	Index[b]
Anthanthrene	1.64	1.03	...	...	−
Pentacene	1.65	1.18	1.60[d]	3.18	−
3,4-Benzopyrene	1.72	1.03	...	...	++++
3,4-9,10-Dibenzopyrene	1.72	...	...	...	++++
Pentaphene	1.74[d]	1.01	2.83[d]	3.45	−
1,2-7,8-Dibenzonaphthacene	1.80	1.03	2.55	3.37	−
2′,3′-3,4-Naphthopyrene	1.81	1.00	2.50	3.34	−
1,2-Benzonaphthacene	1.84[d]	1.01	2.21[d]	3.27	−
3,4-8,9-Dibenzopyrene	1.86	1.07	...	...	++++
Naphthacene	1.86	1.19	2.26[d]	3.25	−
1,2-Benzanthracene	1.90[d]	1.03	2.79[d]	3.42	−
2,3-7,8-Dibenzophenanthrene	1.91	1.09	2.66	3.39	−
1,2-3,4-Dibenzopyrene	1.92	1.02	...	...	+++
1,2-5,6-Dibenzanthracene	1.94[d]	1.04	3.02[d]	3.51	++
1,2-5,6-Dibenzophenanthrene	1.96	1.09	...	...	+
2,3-5,6-Dibenzophenanthrene	1.98	1.07	2.66	3.39	−
1,2-7,8-Dibenzanthracene	1.99[d]	1.04	3.04[d]	3.51	+
1,2-Benzopyrene	2.02[d]	1.08	...	...	−
Chrysene	2.08[d]	1.12	...	...	−
Phenanthrene	2.15[d]	1.06	...	...	−
1,2-4,5-Dibenzopyrene	2.15	1.13	...	...	−
3,4-5,6-Dibenzophenanthrene	2.20	1.09	...	...	−
3,4-Benzophenanthrene	2.23[d]	1.10	...	...	+
Perylene	2.30	...	...	...	−
1,2-3,4-Dibenzophenanthrene	2.34	1.15	...	...	+
Pyrene	2.35[d]	1.06	...	...	−
1,2-3,4-Dibenzanthracene	2.42	1.24	3.00	3.49	−
Picene	2.72	1.11	...	...	−
1,2-6,7-Dibenzopyrene	2.95	...	...	...	−
Triphenylene	2.99[d]	1.38	...	...	−

[a] In units of β. [b] From ref. 13, 14, and 15 following the convention of A. Lacassagne, F. Zajdela, N. P. Buu-Hoi, and H. Chalvet, *Compt. rend.*, **224**, 273 (1957). The experimental conditions were skin painting of a benzene solution of the compound under study. Any tumor activity was considered as positive. [c] Hückel molecular orbital calculations from ref. 4. [d] From ref. 7.

In Dewar's approximation, the energy required to localize a single center is

$$\delta E_\pi = 2(a_r + a_s)\beta$$

where a_r and a_s are the NBMO coefficients for the positions on either side of the localized center. Thus for I, the localization energies for the positions under consideration are

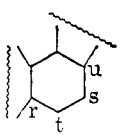
position 3 = position 4 $\quad \delta E_\pi = 1.65\beta$
position 9 $\quad \delta E_\pi = 1.44\beta$
position 10 $\quad \delta E_\pi = 1.35\beta$

For simultaneous localization of two *para* positions, the energy required is the sum of the individual energies. Thus the *para* localization energy for the L region of I is 2.79β.

For the simultaneous localization of two *ortho* positions, the energy formula is

$$\delta E_\pi = (2a_r + a_s + b_t + 2b_u)\beta$$

where the a and b values are the NBMO coefficients for the two odd-alternant systems which result on localization of the individual positions (structures III and IV in the example) with the subscripts indicating the positions as follows



VII

The *ortho* localization energy for the K region of I is then 1.90β.

Results and Discussion

Table I presents the calculated *ortho* and *para* localization energies (O.L.E. and P.L.E.) and the experimental carcinogenic activity for a number of aromatic systems. For consistency in experimental conditions, the experimental data are those of Badger and co-workers where possible.[13] Where Badger's data were not available, data which most closely paralleled his experimental conditions were used.[14,15] The conditions under consideration are twice weekly painting the skin of stock mice with a 0.3% solution of the studied compound in benzene. Any type of induced tumor activity is considered as a positive result. It is quite likely that under other conditions, many of the compounds which are reported as inactive would be carcinogenic. For example, under the conditions cited, 1,2-benzanthracene is inactive; however, it is slightly active if administered subcutaneously,[16] intramuscularly,[17] or orally.[18]

(13) G. M. Badger and J. W. Cook, *J. Chem. Soc.*, 409 (1940); G. M. Badger, J. W. Cook, C. L. Hewitt, E. L. Kennaway, N. M. Kennaway, R. H. Martin, and A. M. Robinson, *Proc. Roy. Soc.* (London), **B129**, 439 (1944); G. M. Badger, J. W. Cook, C. L. Hewitt, E. L. Kennaway, N. M. Kennaway, and R. H. Martin, *ibid.*, **B131**, 170 (1945).

(14) J. Hartwell, "Survey of Compounds which have been tested for Carcinogenic Activity," U. S. Public Health Service, 1955.

(15) J. C. Arcos and M. Arcos, *Progr. Drug. Res.*, **4**, 407 (1963).

(16) P. E. Steiner and J. H. Edgecomb, *Cancer Res.*, **12**, 657 (1952).

(17) M. Klein, *J. Natl. Cancer Inst.*, **13**, 333 (1952).

(18) F. R. White and A. B. Eschenbrenner, *Cancer Res.*, **5**, 594 (1945).

It is seen that there is good correlation between the localization energies and the experimental values. All compounds having an O.L.E. of less than 2.00β for their K region are carcinogenic unless they have an L region with a P.L.E. of less than approximately 2.93. The correlation is considerably better than that obtained using *ortho* and *para* localization energies obtained from the mathematically more complicated Hückel molecular orbital method. The quantitative correlation is, in fact, somewhat better than that obtained in many of the more sophisticated calculations.[3,4] This is quite possibly due to the fact that Dewar's approximation is more closely related to those reaction indices which involve charge-transfer mechanisms[19] than it is to true localization energies.

When working with substituted aromatic systems and heteroaromatic systems, the approximations which were used in the derivation of the Coulson, Longuet-Higgins perturbation theory are no longer valid; therefore, as might be expected, correlations based on Dewar's approximate localization energies are no longer as close as in the nonsubstituted aromatic cases. Certain generalizations can be made, however.

Substitution by methyl groups will result in compounds which are at least as active as the parent compound and usually more active, due to hyperconjugation, unless such substitution sterically blocks the K region. Activity is particularly enhanced if the L region is blocked. For example, 1,2-benzanthracene is inactive under the cited conditions due to a highly reactive L region. 9,10-Dimethyl-1,2-benzanthracene, on the other hand, has high activity.

(19) K. Fukui, T. Yonezawa, and C. Nagata, *Bull. Chem. Soc. Japan*, **27**, 423 (1954).

Cyano Analogs of Phenolic Nitrogen Mustards[1]

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Cyano derivatives of both antimetabolites and alkylating agents have recently shown promise as anticancer agents in preliminary studies. For example, 6-(cyanomethylthio)purine exhibited marked inhibition of Adenocarcinoma 755[3] in mice, and *p*-[bis(2-chloroethyl)]aminobenzylidenemalononitrile and related compounds were active against Dunning leukemia[4] in rats. In addition, a series of bicyclic nitriles and related compounds were recently prepared by the Diels–Alder reaction for evaluation as antitumor agents.[5]

Work in this laboratory[6] has utilized the Mannich reaction as a route to phenolic nitrogen mustards. One of these compounds, 2,5-bis{[bis(2-chloroethyl)amino]-methyl}hydroquinone, was effective against Carcinoma 755, Dunning leukemia, Lymphoma 8, Ehrlich EF, Yoshida hepatoma, and Walker 256 ascites in preliminary tests and is currently being evaluated clinically.[7] Just recently Kuehne and Konopka[8] have shown that related phenolic Mannich bases devoid of 2-chloroethylamino groups also possess antitumor activity.

In view of these results and the unusually low toxicity of the hydroquinone mustard in comparison with other agents of this type, the synthesis of cyano analogs of phenolic nitrogen mustards was undertaken. Condensation of hydroquinone with formaldehyde and bis(2-cyanoethyl)amine in the required proportions in refluxing methanol gave a 33% yield of the desired 2,5-bis-{[bis(2-cyanoethyl)amino]methyl}hydroquinone (XII, Table I). Somewhat higher yields were obtained in the synthesis of analogous disubstituted compounds from resorcinol (74%) and 2-methylresorcinol (83%) by a similar procedure at 5°. Efforts to prepare a monosubstitution derivative of resorcinol by condensation of equimolar proportions of the reactants led to the isolation of only the same disubstituted product. The infrared spectrum showed bands at 11.4 and 11.6 μ, which is characteristic[9] of isolated ring hydrogens. In view of this and the presence of a moderate band at 13.4 μ, which is not generally shown by aromatic compounds with two adjacent hydrogens, it was assumed that the [bis(2-cyanoethyl)amino]methyl groups entered the 4 and 6 positions of resorcinol. This assignment was consistent with the observation that two substituents were introduced into 2-methylresorcinol while only a monosubstituted derivative was obtained from 4-chlororesorcinol, even when the reactant ratio was that required for disubstitution.

As indicated in Table I, several *ortho*-monosubstituted [bis(2-cyanoethyl)amino]methyl derivatives of monohydric phenols were also prepared. Under the conditions used in the condensation of 4-substituted phenols with two free *ortho* positions, only a monosubstituted product was isolated even when sufficient amine and formaldehyde were present to give a disubstituted product. Further treatment of 2-{[bis(2-cyanoethyl)-amino]methyl}-4-chlorophenol (I) with formaldehyde and bis(2-cyanoethyl)amine resulted in the recovery of the original Mannich base in high yield.

A 2,6-disubstituted product XV was obtained readily and in good yield, however, by the reaction of 2,6-bis-(chloromethyl)-4-chlorophenol with excess bis(2-cyanoethyl)amine in benzene at 65°. In a similar manner 2-{[bis(2-cyanoethyl)amino]methyl}-4,6-dichlorophenol (IX) was prepared in 87% yield from 2-chloromethyl-4,6-dichlorophenol. The compound was also prepared directly from 2,4-dichlorophenol by the Mannich reaction but in lower yield.

(1) Supported in part by Research Grant CY-5211 from the National Cancer Institute of the Public Health Service.

(2) Author to whom inquiries should be addressed, Department of Chemistry, Arizona State University, Tempe, Ariz.

(3) T. P. Johnston, L. B. Holum, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958); H. E. Skipper, J. A. Montgomery, J. R. Thomson, and F. M. Schabel, *Cancer Res.*, **19**, 287 (1959); L. R. Duvall, *Cancer Chemotherapy Rept.*, **11**, 232 (1961).

(4) F. D. Popp, *J. Org. Chem.*, **25**, 646 (1960); **26**, 3019 (1961).

(5) P. Scheiner and W. R. Vaughan, *ibid.*, **26**, 1923 (1961).

(6) C. Weatherbee, R. Temple, and W. J. Burke, *ibid.*, **21**, 1138 (1956).

(7) W. L. Wilson, R. B. Green, C. Labra, and E. Parrist, *Cancer Chemotherapy Rept.*, **12**, 199 (1961).

(8) M. E. Kuehne and E. A. Konopka, *J. Med. Pharm. Chem.*, **5**, 257 (1962).

(9) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen and Co., Ltd., London, 1958, pp. 78, 79.