(25) I. P. Dirkx and Th. J. de Boer, *Recl. Trav. Chim. Pays-Bas*, **83**, 535 (1964).
(26) H. E. Smith, J. R. Neergaard, E. P. Burrows, and F.-M. Chen, *J. Am. Chem. Soc.*, **96**, 2908 (1974).
(27) J. B. Blumberg, R. E. Taylor, and F. Sulser, *J. Pharm. Pharmacol.*, **27**, 125 (1975).
(28) Used as the hydrochloride.
(29) Used as the acid D-tartrate.
(30) Used as the sulfate.
(31) "Merck Index", 8th ed, Merck & Co., Inc., Rahway, N.J., 1968.
(32) H. E. Smith, S. L. Cook, and M. E. Warren, Jr., *J. Org. Chem.*, **29**, 2265 (1964).
(33) O. H. Lowry, N. L. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
(34) A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.*, **67**, 305 (1970).

Differences in Antischistosomal and Mutagenic Properties between an Isothiocyano- and an Isocyanonitrodiphenylamine

A. W. Coulter, E. Bueding,* and R. P. Batzinger

Department of Pharmacology and Experimental Therapeutics, School of Medicine, and Department of Pathobiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205. *Received January 3, 1977*

While 4-isothiocyano-4′-nitrodiphenylamine has high schistosomicidal activity in vivo and is devoid of mutagenic properties in vitro, the reverse is true for the isocyano analogue of this compound; i.e., replacement of the sulfur by oxygen results in a compound that has no demonstrable antischistosomal effects and exhibits significant mutagenic activity.

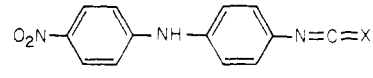
Recently it has been reported that 4-isothiocyano-4′-nitrodiphenylamine (1) (CGP 4540) has high chemotherapeutic activity when administered to animals experimentally infected with schistosomes including *Schistosoma japonicum*.[1,2] It is noteworthy that this compound, in contrast to other schistosomicidal agents, exhibited no mutagenic effects on the sensitive bacterial tester strains of Ames et al.[3] In order to obtain information about the structural characteristics conferring antischistosomal activity, the effect of replacing sulfur by oxygen in the side chain, i.e., the biological activity of the corresponding isocyanate derivative **2**, was determined.

Results and Discussion

Antischistosomal Activity. The antischistosomal effectiveness of **1** is greatly increased when the particle size is reduced to an average diameter of 0.5 μ by ball milling.[2] Alternatively, suspension of larger particle size of the compound in Emulphor EL was nearly as effective. When administered as a single oral dose in the same vehicle, up to 50 times higher doses of the isocyanate analogue **2** had no demonstrable schistosomicidal activity (Table I). In fact, even at the highest dose tested, there was no temporary shift of the worms from the mesenteric veins to the liver sinuses. This phenomenon is considered as a manifestation of minimal antischistosomal activity.

In contrast to the isothiocyanate **1**, the oxygen analogue **2** exhibited mutagenic activity; the latter was fairly substantial with one tester strain (TA 100) but low with the other (TA 98). With neither strain was there any activation by the microsomal preparation (see Table I).

The lack of schistosomicidal activity of **2** may be due to its more rapid rate of hydrolysis when compared to the hydrolysis rate of **1**,[4] thus rendering the former inactive in in vivo studies. In any case, it is evident that the sulfur atom is critical for conferring both schistosomicidal activity and a lack of mutagenic activity. This is reversed when the sulfur atom is replaced by oxygen. This results in a loss of schistosomicidal activity and confers mutagenic properties. Previous studies have shown that antischistosomal and mutagenic activities are not necessarily associated with each other. In fact, investigations with hycanthone analogues have demonstrated that structural modifications can bring about dissociation of these two activities.[5,6] The properties of the two nitrodiphenylamines provide further support for the lack of obligatory association between antischistosomal and mutagenic properties.



1, X = S
2, X = O

Table I. Antischistosomal and Mutagenic Activities of 4-Isothiocyano- and 4-Isocyano-4′-nitrodiphenylamine

	Antischistosomal activity				Mutagenic activity				
						No. of mutants in excess of controls			
						TA-100		TA-98	
Compd	Single oral dose, mg/kg	No. of mice	% redn in no. of worms	% mice with parasital cures	nmol per plate	(−) S_9	(+) S_9	(−) S_9	(+) S_9
1	5	7	68	14	5	0	0	0	0
	7	8	82	25	16	0	0	0	0
	10	12	99	92	53	0	0	0	0
	20	8	100	100	158	0	0	0	0
2	50	6	0	0	17	42 (2.47)	42 (2.42)	6 (0.35)	6 (0.35)
	125	7	0	0	60	100 (1.80)	104 (1.73)	20 (0.35)	18 (0.30)
	250	6	0	0					
	500	12	0	0					

[a] Numbers in parentheses indicate the number of revertants per nanomole.

Experimental Section

Melting points were taken on a Kofler hot stage. IR spectra were obtained on a Perkin-Elmer 137 spectrophotometer. Mass spectra were measured on a CEC 21-110 double-focusing instrument. Where elemental analyses are indicated by symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical value. Antischistosomal effects in vivo were determined as described previously.[7] Mutagenic activity was assayed according to Ames et al.[3] using *Salmonella typhimurium* strains TA-98 and TA-100 in the presence and absence of a preparation of rat liver microsomes (fraction S_9). The two compounds were dissolved first in Me_2SO, the final Me_2SO concentration of all plates (including that of controls) being adjusted to 0.1 mL per 15 mL of medium. The highest concentrations of each of the two compounds tested were close to the one producing 10% growth inhibition.

4-Amino-4′-nitrodiphenylamine. This compound was synthesized by the method of Morgan and Micklethwait.[8]

4-Isocyano-4′-nitrodiphenylamine (2). A solution of 229 mg (1 mmol) of 4-amino-4′-nitrodiphenylamine in 25 mL of dry ethyl acetate was added dropwise via a syringe over 1 h to an excess of phosgene in 150 mL of dry ethyl acetate. Phosgene was bubbled into the reaction during the addition. The solution was stirred for an additional hour, allowed to stand overnight, then evaporated to 10 mL under nitrogen, and filtered to remove a small amount of dark brown material, and 500 mL of petroleum ether (bp 30–60 °C) was added. The precipitated product (112 mg, 44% yield) was collected: mp 134–135 °C; IR (KBr) 2250 cm^{-1} (NCO); MS, molecular ion at *m/e* 255. Anal. ($C_{13}H_9N_3O_3$) C, H, N.

Acknowledgment. The authors gratefully acknowledge the support of this project by National Institutes of Health Grant GM-16492, Training Grant AI-149, and the Agency for International Development Contract AID/ta-C-1312.

References and Notes

(1) H. P. Striebel, *Experientia*, **32**, 457 (1976).
(2) E. Bueding, R. Batzinger, and G. Petterson, *Experientia*, **32**, 604 (1976).
(3) B. N. Ames, J. McCann, and E. Zamusaki, *Mutat. Res.*, **31**, 347 (1975).
(4) S. Petersen, A. Mitowsky, and A. Dolans, *Methoden Org. Chem. (Houben-Weyl), 4th ed, 1952*, 8, 119 (1952); M. Bagemann, S. Petersen, O.-E. Schultz, and H. Soell, *ibid.*, **9**, 867 (1955).
(5) P. B. Hulbert, E. Bueding, and P. E. Hartman, *Science*, **186**, 647 (1974).
(6) E. Bueding, *J. Toxicol. Environ. Health*, 1, 329 (1975).
(7) E. Bueding, C. Naquira, S. Bouwman, and G. Rose, *J. Pharmacol. Exp. Ther.*, **178**, 402 (1971).
(8) G. T. Morgan and F. M. G. Micklethwait, *J. Chem. Soc.*, **93**, 602 (1908).

Book Reviews

Quantitative Structure–Activity Relationships. Edited by Milon Tichy. Birkhauser Verlag, Basel and Stuttgart. 1976. 265 pp. 16 × 24 cm. SwF 58 ($23.50).

The book is a compilation of papers presented at the conference on "Chemical Structure–Biological Activity: Quantitative Approaches" held in Prague, Czechoslovakia, in June 27–29, 1973. The articles cover a wide variety of groups of compounds possessing varied pharmacological activity. The authors have used different physicochemical parameters in estimating the quantitative characteristics of a given biological activity. A total of 35 presentations have been divided under five major portions.

After a brief review of different methods used in quantum mechanics in co-relating the structural and electronic features to biological activity, the first part deals with discussions on the possibility of hydrophobic interactions on the receptor protein dominating over the transport system; the importance of the inclusion of the change in charge density values along with the stereochemical changes in the drug molecule; the contributions of dissolution rate; substructural components and the positive inductive effect of the substituents of a drug molecule as a controlling factor in the biologic response; the improvement in the relationship between the partition coefficient and paper chromatographic R_m values after incorporation of pK_a values in the mathematical equation; lipophilicity and the contribution of inorganic cations (Cu, Cd, Mi, Mn) in inhibiting the mobility of Tubifex worms. The second part has one article explaining the advantages of a modified cluster analysis technique in interpreting the chemical structure–activity relationships. The third part deals with calculation methods used in the Soviet Union to determine the toxicohygienic index—the maximum permissible concentration of chemicals in air in industrial areas; the comparative effect of lipophilicity to deacylation rates in co-relating the β-adrenergic blocking activity of Trimepranol; the dominance of steric effects over electronic effects in substituted isonicotinic acid hydrazides as antitubercular agents; substituent effects on the algicidal activity of phenylthiolacetates; the action of foreign compounds with hemiproteins and isolated ferriprotoheme (here an attempt has been made to study the ligand interaction of methemoglobin with a set of foreign compounds to cause a significant spectral change); structure–activity relationships of 1-ethylpiperidine derivatives in which the calculation for the less toxic *N*-oxides in contrast to the *N*-ethyl derivatives is explained; a study of the regression analysis on some 4*H*-pyridopyrimidine analogues wherein a co-relation between toxicity, the hot-plate test, the algolytic test, and the narcotic potentiating effect has been shown; and finally the lipophilicity paralleling the epileptogenic action of aliphatic penicillins is discussed. Part IV reports on the indices obtained from molecular orbital calculations in co-relating the biologic response. The topics discussed and inferences drawn include the preferred conformations of the pharmacophore in β-adrenergic agents, PGE, serotonin, and clofibrate. Some of these studies have appeared in the literature before. In addition, the antiradiation activity of the thiazolidine molecule as a function of an increase in the density on the S and N atoms of the parent molecule is discussed; the antirheumatic activity of hydroxybenzoic acids as influenced by the acidity and dipole moment is explained; the rationalization of the most stable planar form among the tricyclic antidepressants being the most potent; the ionic defect in an α-helical polypeptide causing a change in the geometry in the helix; calculations of electronic characteristics of the indole and the benzofuran nuclei from LCAO–SCF–MO which run parallel to the approximate methods; and the antituberculotic activity of substituted benzenecarboxylic acid thioamides is dependent on the electron density of the thioamide group. The final section deals with the calculation of the absolute values of Henry constants and partition coefficients for a given compound in different solvent as investigated by gas–liquid chromatography. Using water as the stationary phase, Henry constants in the system blood/air have been calculated. In the next paper, a model is presented of Sephadex-G-10 possibly resembling the biologically active macromolecule and the steric effects involved in binding of small molecules to the surface are calculated. The last study describes the dipole moment playing a part in the activity of cardiotonic steroids.