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Structure–Activity Studies on Mutagenicity of Nitrosamines Using Molecular Connectivity

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Abstract □ The structure–activity relationship of a series of nitrosamines was evaluated for mutagenic potency as measured in the Ames test. The structural description was made using molecular connectivity. A good correlation was found.

Keyphrases □ Nitrosamines, various—mutagenic potency related to molecular connectivity indexes □ Mutagenicity—various nitrosamines, related to molecular connectivity indexes □ Molecular connectivity indexes—various nitrosamines, related to mutagenic potency □ Structure–activity relationships—various nitrosamines, mutagenic potency related to molecular connectivity indexes

The mutagenicity of a molecule is important in assembling a profile of its toxicity and environmental effects. It was stated (1) that there is a correlation between mutagenicity and carcinogenicity, high enough to warrant the routine testing for this property. Such a test is currently used in some laboratories (2).

The compounds are tested on petri plates with mutants of *Salmonella typhimurium*. Homogenates of rat or human liver are added to the plates. The number of revertants per nanomole is determined from dose–response curves and is an indication of the compound's mutagenicity. McCann *et al.* (1), using the Ames test, recently evaluated 300 compounds of various types.

The nitrosamines, known to occur in cigarette smoke, nitrate pickled meat, and smoked fish (3), were studied (1). It was demonstrated (4) with experimental animals that nitrosamines are potent carcinogens.

In this study, previous data (1) on mutagenicity were used as a measure of potency to examine the structural influences on this activity. Fifteen nitrosamines (Table I), tested under identical conditions and having precisely reported data, were considered.

EXPERIMENTAL

To evaluate the structure–activity relationships in this series, a recently developed method, molecular connectivity (5), was used. This method for describing the structure of a molecule has its roots in topology. A series of indexes reflect a weighted count of subgraphs which, from simple calculations, lead to values encoding considerable structural information. The information makes possible the evaluation of structural features influencing physical property values (6) and biological activity (7–9). The use of extended terms (10) and the application of molecular connectivity to heteroatoms (11) make the method ideally suited for a study of the structure–activity relationships of biologically important molecules.

RESULTS

A systematic search of the connectivity indexes for the molecules in Table I revealed two relationships with nearly equal quality. By expressing the activity as the natural log number of revertants per nanomole, the following equations were found:

$$\ln R = 2.398\ (\pm 0.032)\ {}^{0}\chi - 4.095\ (\pm 0.132)\ {}^{1}\chi^{v} - 5.590\ (\pm 1.158) \quad \text{(Eq. 1)}$$

$$r = 0.964 \quad s = 1.09 \quad n = 15 \quad F = 78.8\ (p < 0.02)$$

$$\ln R = 2.946\ (\pm 0.066)\ {}^{2}\chi - 9.090\ (\pm 0.729)\ {}^{4}\chi_{p}{}^{v} - 4.662\ (\pm 1.052) \quad \text{(Eq. 2)}$$

$$r = 0.967 \quad s = 1.05 \quad n = 15 \quad F = 85.2\ (p < 0.02)$$

Both equations predict the ln R to less than 10% of the log range of experimental values in this set. The common structural feature in the set is the nitrosamine group and must be considered to be necessary, but not sufficient, for potency. Subsidiary structural features, quantified by

Table I—Mutagenicity/Carcinogenicity of Nitrosamines

	Compound	ln R[a]	Equation 1, Calc. ln R	Equation 2, Calc. ln R
1	Dipropyl-*N*-nitrosamine	−2.526	−2.573	−2.711
2	Dibutyl-*N*-nitrosamine	−1.897	−3.276	−2.128
3	Dipentyl-*N*-nitrosamine	−2.996	−3.979	−3.747
4	*N*-Nitrosopyrrolidine	−3.912	−3.626	−4.232
5	*N*-Nitrosomorpholine	−2.813	−2.247	−1.857
6	*N*-Nitrosopiperidine	−4.605	−3.977	−4.798
7	*N*-Methyl-*N*-nitroso-*N*′-nitroguanidine	7.226	5.176	5.287
8	*N*-Ethyl-*N*-nitroso-*N*′-nitroguanidine	5.858	4.513	4.020
9	*N*-Propyl-*N*-nitroso-*N*′-nitroguanidine	3.689	4.161	3.791
10	*N*-Butyl-*N*-nitroso-*N*′-nitroguanidine	3.892	3.811	3.959
11	*N*-Isobutyl-*N*-nitroso-*N*′-nitroguanidine	4.344	4.792	5.304
12	*N*-Pentyl-*N*-nitroso-*N*′-nitroguanidine	3.091	3.669	3.264
13	*N*-Hexyl-*N*-nitroso-*N*′-nitroguanidine	1.668	3.317	2.699
14	*N*-Nitrosomethylurea	1.482	1.716	2.676
15	*N*-Nitrosoethylurea	0.095	1.117	1.069

[a] Natural log of revertants per nanomole.

the connectivity indexes, influence the degree of potency as manifested in this test.

DISCUSSION

The set contains three subclasses of nitrosamines: alkyl, guanidino, and ureido derivatives. Within the alkyl subset are three cyclic derivatives. The equations treat these subsets equally well for the correlation with potency.

The activities exhibited by the entire set range over five natural log units (nearly 12 orders of magnitude). The equations thus can discriminate between quite potent and virtually inactive molecules. On this basis, the structural analysis derived from this admittedly limited set of nitrosamines may have utility as a theoretical screen for other untested nitrosamines.

A negative test is a result less than 0.01 revertant/nmole ($\ln R < -4.61$) (1). Results greater than 1.0 revertant/nmole ($\ln R > 0.00$) are positive to the degree of the value. McCann *et al.* (1) claimed a 90% correspondence between carcinogenicity and mutagenicity.

An examination of the equations reveals certain structure–activity relationships. The ability of the equations to discriminate between the subclasses is illustrated by a comparison of Compounds 2 and 8. Both have the same number of nonhydrogen atoms but vastly different activities. Both equations account for this difference. The activity of Compound 5 is correctly predicted to be less than its isostere, Compound 6. In Eq. 1, the $^0\chi$ values are the same for both; however, the $^1\chi^v$ value is less for Compound 5 because the valence connectivity delta assignment for oxygen in the ring is greater than the ring methylene delta in Compound 6. The cyclic molecules generally have a larger $^1\chi^v$ value than the noncyclic molecules and, therefore, are correctly predicted to be less active than cyclic molecules of about the same size.

This limited set of data provided a good relationship between a molecular connectivity description of structure and mutagenic potency (1) in the Ames test. This result may be a useful beginning to the development of theoretical prediction of mutagenic potential in discrete chemical classes of molecules. Examination of additional nitrosamines, similarly tested, should afford a constructive challenge to this approach. The intention is to examine other classes of mutagenic molecules using molecular connectivity.

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Determination of Plasma Hydrochlorothiazide Levels in Humans

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Abstract □ A method for determining plasma hydrochlorothiazide levels was developed with a sensitivity of 5 ng/ml. Accuracy and precision were demonstrated over the 5–648-ng/ml range by an overall recovery of 95 ± 8%. The detector response was linear for the 5–250-ng/ml range. The method was sufficiently sensitive for hydrochlorothiazide bioavailability studies and also was applicable for the determination of whole blood drug levels. Plasma levels in two subjects reached peak levels of 428 and 450 ng/ml at 2.5 and 2 hr, respectively, after a 50-mg dose. Whole blood levels at 3 hr after the same dose were 547 and 851 ng/ml and were approximately 2.5 times the 3-hr plasma levels.

Keyphrases □ Hydrochlorothiazide—GLC analysis in plasma □ GLC—analysis, hydrochlorothiazide in plasma □ Diuretics—hydrochlorothiazide, GLC analysis in plasma

The diuretic hydrochlorothiazide has been used clinically for a number of years. In spite of the availability of GLC (1, 2) and liquid chromatographic (3, 4) methods, only limited human plasma level data have been published. Plasma levels were reported (1) in four subjects, but levels past 4 hr (6 and 24 hr) were noted for only one subject. Plasma level–time curves were reported for a single subject (5), as was an average curve from eight subjects covering 9 hr following administration (6).

This paper reports individual plasma level data obtained in a study with two male volunteers. Plasma hydrochlorothiazide levels were determined using a modification of the GLC method reported previously (2).

EXPERIMENTAL

Procedure—Plasma (2 ml) was pipetted into a 12-ml polytef[1]-lined screw-capped centrifuge tube. The internal standard, the bromo analog of hydrochlorothiazide (6-bromo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide), was added (200 μl of a 1-μg/ml solution in methanol), and the sample was mixed thoroughly on a vortex mixer[2]. Then methyl isobutyl ketone[3] (5 ml) was added, and the tightly closed tube was vigorously shaken horizontally on a platform shaker[4] for 20 min.

Following centrifugation for 20 min, 4 ml of the organic layer was transferred to a clean 12-ml centrifuge tube. Sodium hydroxide (2.5 ml, 0.1 *M*) was added, and the tightly closed tube was vigorously shaken for 20 min. Following centrifugation for 15 min, 2 ml of the aqueous layer was transferred to a clean 12-ml centrifuge tube. A solution of tetrahex-

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[2] Scientific Industries Inc., Springfield, Mass.
[3] J. T. Baker, Phillipsburg, N.J.
[4] Eberbach Corp., Ann Arbor, Mich.