authors showed that 6 times as much chlorthalidone was present in the red cell fraction of rat blood as in the plasma. The present study reports similar findings in humans where from 8 to 10 times as much chlorthalidone is found in the red cells as in the plasma. On the basis of the results described in this paper, approximately 90% of a given dose of chlorthalidone is bound in the tissues. It is this tissue binding that probably accounts for the long half-life (54 hr) noted in the normal human subject and for the prolonged diuretic action of chlorthalidone in humans (3).

REFERENCES

(1) R. Pulver, H. Wirz, and E. G. Stenger, *Schweiz. Med. Wochenschr.*, **89**, 1130(1959).

(2) G. Beisenherz, F. W. Koss, L. Klatt, and B. Binder, *Arch. Int. Pharmacodyn. Ther.*, **161**, 76(1966).

(3) R. V. Ford, *Tex. Med.*, **56**, 343(1960).

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Dependence of Toxicity on Molecular Structure: Group Theory Analysis

J. L. COHEN*, W. LEE, and E. J. LIEN ˣ

Abstract □ Group theory was applied to a wide variety of toxic substances, drugs, and endogenous organic compounds to test the apparent empirical relationship between toxicity of a molecule and its degree of molecular symmetry. Many compounds in each classification were analyzed and separated into individual symmetry groups using "space-filling" molecular models. With few exceptions, an apparent relationship was noted between each symmetry group and the relative toxicities. A brief introduction to group theory is presented as well as the rationale and implications of these observations.

Keyphrases □ Structure-activity relationships—group theory analysis, toxicity-molecular symmetry □ Toxicity—dependence on molecular structure, group theory analysis of various substances □ Molecular structure—role in toxicity, group theory analysis of various substances

Numerous reports in the literature attempt to correlate the activities or toxicities of drugs and chemicals with various structural or physical-chemical parameters of the molecules (1-3). These approaches, in general, have shown the greatest applicability for structurally related series of compounds, but there has been no satisfactory general correlation which explains the activities of molecules with significantly different structures and physical-chemical properties. The reason for this may be that fundamental parameters, such as molecular symmetry, or other relevant properties of molecules have not been included among the physical-chemical parameters used in existing quantitative structure-activity correlations. Because the overall objective of all correlations of this type is to design drugs with very specific activities and limited toxicities, the approach along specific drug classification lines or specific disease lines is clearly appropriate. Therefore, the implication is that a fundamental explanation of the general type stated may direct future research to include additional specific parameters required to design drugs of specific pharmacological activity.

Table I—Mean LD_{50} Values for Oral Doses in Rats of Different Symmetry Groups

Group	Number of Compounds	LD_{50}[a]	*df*	*t*	Significance Level, %
C_1	29	17.60 ± 2.59	46	4.26	99.5
C_{2v}	19	3.57 ± 0.91	—	—	—
C_{1h}	5	5.21 ± 2.58	32	1.95	95
D_{2h}	5	3.21 ± 1.54	32	2.27	97.5

[a] LD_{50} values in rats, millimoles per kilogram, mean ± standard error ($S\bar{x}$, where $S\bar{x} = S/\sqrt{n}$).

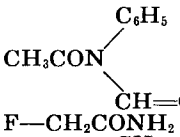
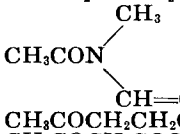
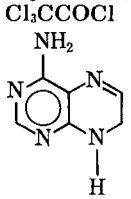
In this paper the empirical relationship between molecular symmetry and toxicity is explored with various drugs, toxic chemicals, and endogenous organic compounds.

GROUP THEORY

Group theory invariably differentiates between all possible geometric isomers and places them into their respective symmetry groups. Once the compounds are classified into their respective symmetry groups and subgroups, the similarities and contrasts within the groups can be compared. An extensive mathematical discussion of group theory will not be presented here; however, several references (5, 6) discuss the theoretical details of group theory analysis. Most appropriate to this discussion is the actual application of some principles to the compounds analyzed in the present study.

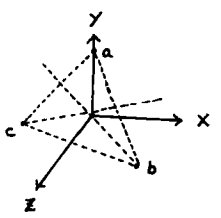
Group symmetry operations are mathematical operators which perform linear transformations on molecular orbital wave func-

Table II—Oral Toxicity in Rats: C_1 Group Symmetry

Number	Compound	LD_{50}, mg/kg	Molecular Weight	LD_{50}, mmoles/kg
1		2460	113	21.77
2	$F—CH_2CONH_2$	85	77	1.10
3		2830	99	28.59
4	$CH_3COCH_2CH_2CON(C_2H_5)_2$	4760	172	43.37
5	$CH_3COCH_2COOC_2H_5$	3980	130	30.62
6	$HOCH_2COCH_3$	2200	74	29.73
7	$FCl_2CCOCClF_2$	277	214	1.29
8	$4\text{-}NH_2—C_6H_4COCH_3$	1870	135	13.85
9	$3\text{-}CH_3,4\text{-}CH_3O—C_6H_3COCH_3$	1500	164	9.15
10	$3\text{-}NO_2—C_6H_4—COCH_3$	3250	165	19.70
11	$Cl_2HC—COCl$	2460	134	18.36
12	Cl_3CCOCl	600	180	3.33
13		745	133	5.60
14	$CH_3CHOH—CH_2CHO$	2180	88	24.77
15	$3\text{-}Cl—C_6H_4NH_2$	880	127	6.93
16	$2,4,5\text{-}(CH_3)_3—C_6H_2NH_2$	1585	135	11.74
17	$n\text{-}C_3H_7—C_6H_4—CHO$	1600	164	9.76
18	$C_6H_5CH_2OH$	1600	108	14.81
19	$CH_3—CHOH—C_6H_5$	400	122	3.28
20	$4\text{-}Cl—2\text{-}(C_6H_5CH_2)—C_6H_3OH$	1700	218	7.80
21	$(C_2H_5)_2CHCHO$	3900	100	39.00
22	$(C_2H_5)_2NH$	649	73	8.89
23	$(C_3H_7)_2NH$	930	101	9.21
24	$H_2N—CH_2CH_2OH$	3320	61	54.43
25	$CH_3NHCH_2CH_2OH$	2340	75	31.20
26	$n\text{-}C_6H_{13}OH$	2380	116	20.52
27	$C_2H_5OCH_2CH_2OH$	3000	90	33.33
28	$CH_2=CH—CH_2—OCHCH_2$	550	84	6.55
29	$C_2H_5O—(n\text{-}C_4H_9)$	1870	114	1.84

tions. The set of operators that sends a symmetrical figure into itself is said to form a "group." For example, given a triatomic molecule placed in three-dimensional (orthogonal) space, the operators that send the molecule into itself are:

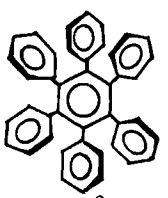
1. The identity operation $\hat{E}$, which leaves each point unchanged.
2. $\hat{\sigma}xy$, reflection in the plane passing through point b and perpendicular to the line joining a and c.
3. σyz, reflection in the yz plane passing through point a and perpendicular to bc.
4. $\hat{\sigma}d$, reflection in the plane passing through point b and perpendicular to ac.
5. $\hat{C}_3^1$, clockwise rotation through 120°.
6. $\hat{C}_3^{-1}$, counterclockwise rotation through 120°.



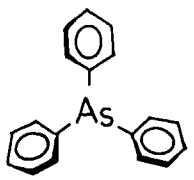
Other symmetry operations are possible; however, they turn out to be equivalent to one of these six operations. For example, a clockwise rotation through 240° is an operation identical to $\hat{C}_3^{-1}$, and a rotation through 180° about the y-axis is identical to $\hat{\sigma}_d$. The successive application of any two of the operations discussed here is equivalent to some other single operation.

C_1 Symmetry Group—This is the simplest point group possible, consisting of only the identity operation $\hat{E}$. Examples of this point group that have no symmetry at all are adenosine, glucose, cholesterol, and many other endogenous and exogenous compounds.

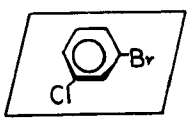
C_n Symmetry Group—This group is associated with an n-fold rotational axis and consists of the operations $\hat{E}$, $\hat{C}_n^1$, $\hat{C}_n^2$, . . ., $\hat{C}_n^{n-1}$; there is no limit to the possible value for n in these compounds. Examples of compounds in this symmetry group are:



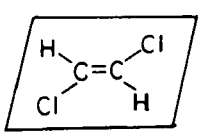
$n = 6$



C_{nh} Symmetry Group—This group is classified by two independent operations, C_n and σ_h, which add to a horizontal plane to the molecule. Examples of compounds in this group are:



C_{1h}



C_{2h}

Table III—Oral Toxicity in Rats: C_{2v} Group Symmetry

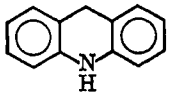
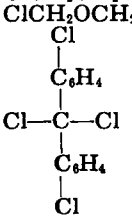
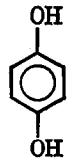
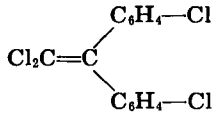
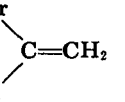
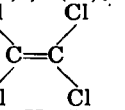
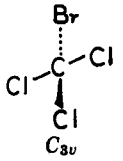
Number	Compound	LD_{50}, mg/kg	Molecular Weight	LD_{50}, mmoles/kg
1	$ClCF_2COCF_2Cl$	61	198	0.31
2		2140	179	11.96
3	$CH_2{=}CHCH_2{-}O{-}CH_2CH{=}CH_2$	320	98	3.27
4	$(ClCH_2CH_2)_2{-}N{-}C_6H_5$	123	213	0.58
5	$4\text{-}Cl{-}C_6H_4{-}NH_2$	300	127	2.36
6	$(CH_3)_2N{-}C_6H_5$	1410	121	11.65
7	$2,4,6\text{-}(CH_3)_3{-}C_6H_2{-}NH_2$	506	135	3.75
8	$n\text{-}C_4H_{10}$	658	58	11.34
9	$BrCH_2CH{=}CH{-}CH_2Br$	75	214	0.35
10	$ClCH_2CH{=}CH{-}CH_2Cl$	89	126	0.71
11	$(CH_2{=}CH{-}CH_2)_2NH$	650	97	6.70
12	$(CH_3)_3CNHC(CH_3)_3$	550	129	4.26
13	$Cl(CH_2)_2O(CH_2)_2{-}Cl$	105	142	0.74
14	$CH_3CHCl{-}O{-}CHCl{-}CH_3$	105	142	0.74
15	$ClCH_2CH_2OH$	89	80	1.11
16	$ClCH_2OCH_2Cl$	210	114	1.84
17		168	308	0.54
18		320	110	2.90
19		880	320	2.75

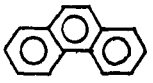
Table IV—Oral Toxicity in Rats: $C_{\infty v}$ Group Symmetry

Number	Compound	LD_{50}, mg/kg	Molecular Weight	LD_{50}, mmoles/kg
1	$Br{-}C{\equiv}C{-}Br$	117	184	0.64
2	$Cl{-}C{\equiv}C{-}Cl$	770	94	8.19
	C_{1h} Group Symmetry			
1	$CH_2{=}CHOH$	64	44	1.45
2	$4\text{-}Cl{-}1,3\text{-}(NO_2)_2{-}C_6H_3$	640	202	3.17
3	$Cl_2C{=}CCl{-}CCl{=}CCl_2$	300	258	1.16
4		500	106	4.71
5	$CH_2{=}CH{-}CH{=}CH{-}CH{=}CH_2$	1210	80	15.12
	D_{2h} Group Symmetry			
1	$1,2,4,5\text{-}(Cl)_4{-}C_6H_2$	1500	214	7.01
2		200	166	1.20
3	$ClCH_2CH_2Cl$	680	98	6.94
4	$BrCH_2CH_2Br$	117	188	0.62
5	$BrCH{=}CHBr$ (*trans*)	62	186	0.29

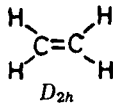
C_{nv} Symmetry Group—This group is derived from C_n and also has a vertical plane of symmetry σ_v through the axis. Examples of molecules of this group are:



C_{3v}

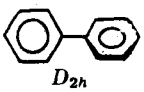

C_{2v}

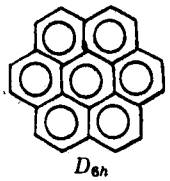
D_n, D_{nh}, and D_{nv} Symmetry Groups—Molecules that possess one or more twofold axes of rotation belong to the D_n group, while the addition of a σ_h (horizontal plane) produces the D_{nh} group and the addition of a σ_v (vertical plane) produces the D_{nv} group. Examples of these include:



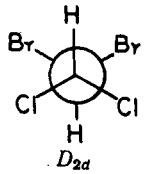
D_{2h}


D_{6h}


D_{2h}


D_{6h}

D_{nd} Symmetry Group—Another series of groups arises when a set of vertical planes, σ_v, is added to D_n through the axis C_n, bisecting the angles between the twofold axis. An example of D_{nd} is:



D_{2d}

$C_{v\infty}$ Symmetry Group—This group is obtained by adding a vertical plane, σ_v, to C_∞, where C_∞ implies an infinite-fold axis for which rotation by all multiples of an infinitesimal angle ($2\pi/n$, where $n \rightarrow \infty$) is a symmetry operation. A heteronuclear diatomic molecule has this symmetry, with the C_∞ axis along the bond axis, *e.g.*, carbon monoxide (CO).

EXPERIMENTAL

The molecular symmetry of the compounds under investigation was assigned based upon the observation of the spatial orientation of the "space-filling" molecular models[1]. These models depict the linearity and planarity of the molecules. For example, hydrocarbon side chains were found to be nonlinear due to sp^3 (tetrahedral) orbitals, whereas sp^2 bonded atoms such as in olefins were found to be trigonal and in the same plane. The possible alteration of spatial orientation by stretching, rotational, and translational energies was also considered. Although stretching energies of the molecules are the most significant of the three mentioned, there was no demonstrated alteration in the symmetry of the molecule. The rotational and translational energies were subjected to restrictions consistent with the most stable conformation. In all cases the most apparently stable conformation was selected. Compounds that were too large for model construction or too complex due to heterocyclic ring structures were assigned to symmetry groups after detailed inspection of their structural formulas or portions of the molecular models.

Compounds selected for study were assumed to be relatively stable in biological systems and do not readily undergo chemical or biological modification. For purposes of this discussion, only neutral and relatively inert compounds, which are presumed to act in an inhibitory or regulatory manner in biological systems, are included. Toxicity data for the compounds studied were obtained (7) and converted to LD_{50} values expressed in millimoles per kilogram.

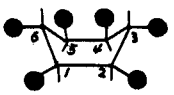
[1] CPK, Ealing Corp., Cambridge, Mass.

RESULTS AND DISCUSSION

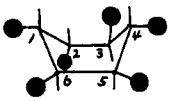
When using compounds from Ref. 7, an apparent relationship between the degree of molecular symmetry and acute lethal toxicity was found. For example, when the oral toxicity data in rats are arranged according to the different groups of symmetry of the compounds in Tables II–IV, the data can be summarized as in Table I.

Utilization of an unpaired *t* test indicated that the mean LD_{50} for the C_1 symmetry group is statistically different from the other three groups. This implies that the C_1 group, which is least symmetrical, has the lowest degree of toxicity. All constituents of living matter listed in "Geigy Documenta Scientific Tables" (8) also belong to the C_1 group. These include compounds from the following general classifications: monosaccharides, sugar phosphates, polyhydric alcohols, amino acids, purines and pyrimidines, porphyrins, fatty acids, steroids, *etc.* As expected, these endogenous compounds have low degrees of toxicity.

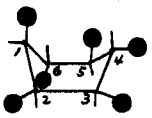
Although the sample sizes for the higher symmetry groups apparently account for the lack of statistical significance between the difference in mean LD_{50} values, the general trend indicates that the greater the degree of symmetry, the greater the degree of toxicity. Among the groups listed, D_{2h} has the greatest degree of symmetry and the highest acute lethal toxicity (lowest mean LD_{50}). The trend of this relationship and the usefulness of symmetry grouping are strengthened by the example of benzene hexachloride (lindane). Three different isomers (α, γ, and δ) are known, with the γ-isomer being the most potent (8) although their octanol-water partition coefficients (log *P*) differ only by 0.4 log unit[2]. Dreiding's models of these compounds show that the most stable conformation of the γ-isomer has all the chlorine atoms at the equatorial positions, which places it in the D_{6h} group while the other two isomers belong to the C_1 group:



benzene hexachloride γ-isomer
1,2,4,5/3,6
LD_{50} = 125 mg/kg (oral rat)
log *P* = 3.72
D_{6h} group symmetry



benzene hexachloride α-isomer
1,2,4/3,5,6
LD_{50} = 500 mg/kg
log *P* = 3.80
C_1 group symmetry



benzene hexachloride δ-isomer
1,2,3,5/4,6
LD_{50} = 1000 mg/kg
log *P* = 4.14
C_1 symmetry group

Another example of interest is the comparison of Compound 7 of the C_{2v} group in Table III with Compound 16 of the C_1 group in Table II. Compound 7, the more symmetrical of the two, is nearly four times as toxic yet the two molecules are isomers.

Considering the large number of chemical classes of compounds represented in Tables II–IV, the apparent relationship becomes even more interesting. Although a number of fundamental questions obviously remain to be answered, the symmetry parameter is apparently useful in predicting something about activity and toxicity of various chemical compounds. Why should all endogenous compounds used as natural building blocks belong to the C_1 symmetry group? Why do compounds of the same symmetry group have the same magnitude of toxicity regardless of their chemical nature and the mechanism of toxicity? Empirically, the answer to the first question may be governed by the building blocks of DNA which are all the C_1 purines and pyrimidines. This should not be confused with the gross symmetry of the two double-helical DNA molecules after replication occurs, which is probably responsible for the gross symmetry of almost all living organisms.

[2] T. Fujita, unpublished data through the courtesy of Dr. C. Hansch.

The answer to the second question may be that the acute lethal toxicity (LD_{50}) is a very nonspecific type of biological response which depends upon nonspecific interactions with biopolymers or macromolecules. It is well known that compounds of higher degrees of symmetry usually have higher melting points and/or boiling points, indicating a greater degree of binding force with the neighboring molecules.

CONCLUSIONS

An apparent relationship was found between the degree of molecular symmetry and the acute oral lethal toxicity in animals as represented by the LD_{50}. This approach has some obvious limitations including the uncertainty in conformation assignments for large molecules and the lack of comparable data on large numbers of compounds of interest of higher order symmetry groups. Furthermore, the approach may not be directly applicable to drugs or substances that exert very specific biological activity by interaction with enzymes, such as antimetabolites, or to drugs that are rapidly metabolized in the body to other species with altered biological activity.

In general, however, this approach may have uncovered a new parameter which should be considered in the design of potentially specific, nontoxic drugs. In a given chemical classification, for example, this parameter may be included along with other physical-chemical parameters such as lipophilicity and steric and electronic factors utilized in current approaches to structure-activity correlations. Group theory can also be taken one step further by using quantum mechanical calculations of molecular interaction in the case of a specific process where a significant amount of information is known about the interaction between the drug and the receptor. Additional studies on specific pharmacological groups or agents will further demonstrate the validity and usefulness of this working hypothesis.

REFERENCES

(1) "Drug Design," vol. I, E. J. Ariens, Ed., Academic, New York, N.Y., 1971, pp. 271–337.

(2) C. Hansch, "Biological Correlations—The Hansch Approach," Advances in Chemistry Series 114, American Chemical Society, Washington, D.C., 1972, pp. 20–40.

(3) J. G. Topliss and R. J. Costello, *J. Med. Chem.*, **15**, 1066(1972).

(4) P. N. Craig, *ibid.*, **14**, 680(1971).

(5) F. A. Cotton, "Chemical Application of Group Theory," 2nd ed., Wiley-Interscience, New York, N.Y., 1971.

(6) R. M. Hochstrasser, "Molecular Aspects of Symmetry," Benjamin, New York, N.Y., 1966.

(7) "The Toxic Substances List," H. E. Christiansen, Ed., U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health, Rockville, Md., 1971.

(8) "Documenta Geigy Scientific Tables," 6th ed., Geigy Pharmaceuticals, Ardsley, N.Y., 1968.

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GLC Determination of Plasma Levels of Enantiomers of α-[4-(1-Oxo-2-isoindolinyl)phenyl]propionic Acid

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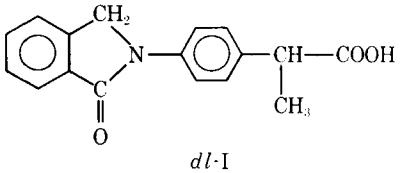
Abstract □ A quantitative GLC determination of each α-[4-(1-oxo-2-isoindolinyl)phenyl]propionic acid enantiomer in human plasma after oral administration of the racemate is described. After extraction and purification of the extract through partition steps, the substances were converted to the diastereoisomeric amides *via* the acid chlorides. These derivatives were separated and quantitated by GLC. The sensitivity limit is 0.3 μg of each enantiomer/ml plasma. In the concentration range of 0.62–5.00 μg/ml plasma, the percent recovery (± standard deviation) of the *d*- and *l*-enantiomer was 67.51 ± 3.11 and 67.44 ± 3.14, respectively, whereas the coefficients of variation of the ratios between these recoveries and those of the internal standard were 1.03 and 1.05%, respectively.

Keyphrases □ α-[4-(1-Oxo-2-isoindolinyl)phenyl]propionic acid—GLC determination of plasma levels of enantiomers □ α-Phenylpropionic acid derivatives—GLC determination of plasma levels of enantiomers □ GLC—determination, enantiomers of α-[4-(1-oxo-2-isoindolinyl)phenyl]propionic acid, plasma levels, humans

dl-α-[4-(1-Oxo-2-isoindolinyl)phenyl]propionic acid (*dl*-I) is a promising analgesic and anti-inflammatory agent. The chemistry (1) of this substance and its biological activities (2) were reported. Compound *dl*-I was approximately 20 times as active as phenylbutazone in inhibiting carrageenin-induced edema of the rat paw, 15 times as active as phenylbutazone in the granuloma pouch test, and about 20 times as potent as phenylbutazone in preventing adjuvant-induced arthritis in the rat. When using the parameter of phenylquinone-induced writhing in the mouse, the compound proved equipotent with indomethacin, 95 times more potent than phenylbutazone, and 50 times more potent than aspirin.

Pharmacokinetics studies (3) showed that the substance in its racemic modification was completely and rapidly absorbed from the GI tract when administered in capsules to healthy volunteers (peak plas-



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