Molecular Connectivity V:
Connectivity Series Concept Applied to Density

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Abstract ☐ The concept of the expanded series of the connectivity index, χ, is introduced and applied to a consideration of the density of three classes of molecules. Correlations are found using two terms in the expanded series. A preliminary reflection on the extended series terms is made. It is noted that the regression equation constant in the three studies is close to the phase volume, 0.7402, and the possible significance of this fact is discussed.

Keyphrases ☐ Molecular connectivity—expanded series, correlated with densities of alkanes, aliphatic alcohols, and carboxylic acids ☐ Densities—alkanes, aliphatic alcohols, and carboxylic acids correlated with molecular connectivity

Density is a property of a molecule in the bulk phase, defined as the mass in a unit volume. Density can be viewed as both an additive and constitutive property. The additive nature relates to the volume of the molecule, while the constitutive nature relates to the intermolecular alignments governed by surface area and other superficial features.

Recent studies examined various surface area and volume-related physicochemical and biological properties (1–4). Fundamental to both the volume and surface area of a molecule is a structural property defined as molecular connectivity (1). This property was expressed in numerical form with an index first devised by Randić (5) for hydrocarbons.

The index, χ, has been shown to relate to molecular cavity surface area, polarizability, nonspecific local anesthetic activity (1), water solubility, boiling point (2), oil–water partition coefficient, barnacle larvae narcosis, tadpole inhibition (3), and enzyme inhibition (4). The fundamental nature of χ can be seen from these studies in that it closely relates to properties with molecular volume or surface area, as well as to properties of the bulk phase or of individual molecules.

To explore further the fundamental nature and general applicability of χ, this study begins with the hypothesis that the density of a molecule should be related in some way to a physical property or properties governed by molecular connectivity. In view of experience with χ relating to properties associated with molecular volume (1), the relationship $\rho = a(1/\chi) + b$, where ρ is the density and a and b are constants, should obtain. A test of this relationship with 82 aliphatic hydrocarbons (Table I) proved to be encouraging; the correlation amounted to $r = 0.9029$. The density probably is more complex, involving more than just a simple volume relationship.

In the initial paper in this series, it was stated that any function of a molecule can be represented by a combination of terms representing various bond contributions (1). The previous studies in this series dealt with only the first-order term, i.e., the value of χ for each bond. In the case of density, it is apparently necessary to include additional terms in a molecular connectivity function.

CONNECTIVITY SERIES

The first term in the connectivity series is the index χ, derived from a summation of values describing each bond in the molecule. The second term is a summation of values describing each adjacent pair of bonds. This term can be symbolized as $^2\chi$. The calculation follows the presciption previously described (1, 2, 5). The δ values are assigned to each first row atom according to the connectivity or branching. The product of the three adjacent δ values (i, j, and k) for each pair of adjacent bonds is obtained. The square root of the reciprocal of the product of the δ values give a bond pair term, C_{ijk}. The value of $^2\chi = \Sigma C_{ijk}$.

The calculation of $^2\chi$ for isopentane illustrates the procedure.

$$\frac{1}{\sqrt{1\cdot3\cdot1}} + \frac{1}{\sqrt{1\cdot3\cdot2}} + \frac{1}{\sqrt{1\cdot3\cdot2}} + \frac{1}{\sqrt{3\cdot2\cdot1}} = 1.558$$
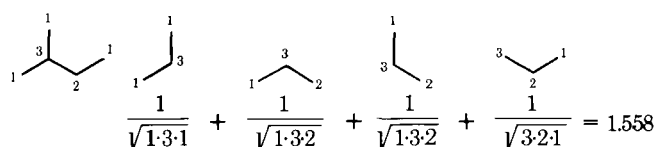
The third term in the χ series is an index derived from three linear adjacent bonds, designated as $^3\chi$. The C_{ijkl} terms are calculated as square root reciprocals of products of four first-row atoms i, j, k, and l comprising three linearly adjacent bonds. For isopentane, the value of $^3\chi$ is obtained as follows:

$$^3\chi = 0.816$$
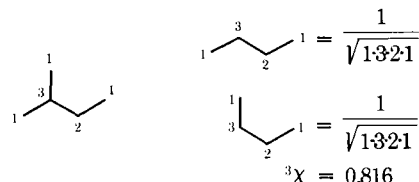
Subsequent terms in the series are calculated similarly.

Where double bonds occur, including carbonyl groups, the χ terms are computed with valence δ as described previously (3).

Analysis of Hydrocarbon Densities with χ Series—The question is, which terms in the expansion series of χ adequately describe structural features that are manifest in influencing the experimentally observed value of the density. The answer is not obvious, since one has no developed intuition as to the significance of each term in the expanded series of χ. As an approach to the answer, 82 aliphatic liquid hydrocarbons were analyzed. Each term in the χ expansion series, as well as combinations of terms, was tested for correlation with density. The results clearly show that $1/\chi$ and $^3\chi$ give excellent correlation with the density. The statistical analysis and regression equation are:

$$D_4^{20}\text{(aliphatic liquid hydrocarbons)} = 0.0030{}^3\chi - \frac{0.2927}{\chi} + 0.7348$$

$$r = 0.9889 \qquad s = 0.0046 \qquad n = 82 \qquad \text{(Eq. 1)}$$

Analysis of Aliphatic Alcohol Densities with χ Series—An analysis of 42 alcohols (Table II) also demonstrated the contribution of $^3\chi$ to the χ term in correlations with density. The statistical analysis and regression equation are:

$$D_4^{20}\text{(aliphatic monoalcohols)} = 0.0183{}^3\chi - \frac{0.0043}{\chi} + 0.7933$$

$$r = 0.9305 \qquad s = 0.0045 \qquad n = 42 \qquad \text{(Eq. 2)}$$

Based upon the regression equation, the density of two alcohols was predicted. For heptanol, $d_{\text{obs}} = 0.8224$; the predicted value was 0.8189. For decanol, $d_{\text{obs}} = 0.8292$; the predicted value was 0.8364. The residual for both was less than the standard error.

Analysis of Aliphatic Acid Densities with χ Series—The acids present a third test of the role of χ and $^3\chi$ in the density of a molecule. Twenty acids (Table III) were analyzed. The best relationship was

Table I—Densities (Specific Gravities) of Alkanes Predicted from Functions of χ

Alkane	$^3\chi$	$\dfrac{1}{\chi}$	D_4^{20} (obs)[a]	D_4^{20} (calc)[a]
Pentane	0.70700	0.41425	0.62620	0.63481
2-Methylbutane	0.81600	0.44053	0.61970	0.63040
2,2-Dimethylpropane	0.00000	0.50000	0.59100	0.58846
Hexane	0.50000	0.34317	0.65940	0.64940
2-Methylpentane	0.86600	0.36101	0.65320	0.65518
3-Methylpentane	1.39400	0.35613	0.66430	0.67248
2,2-Dimethylbutane	1.06100	0.39047	0.64920	0.65241
2,3-Dimethylbutane	1.33300	0.37836	0.66160	0.66414
2,4-Dimethylpentane	0.94300	0.31990	0.67270	0.66953
2,2-Dimethylpentane	1.00000	0.32669	0.67380	0.66925
2-Methylhexane	1.13300	0.30581	0.67860	0.67936
Heptane	1.20700	0.29291	0.68380	0.68536
3-Methylhexane	1.47800	0.30230	0.68710	0.69076
2,2,3-Trimethylbutane	1.73200	0.33979	0.69010	0.68742
3,3-Dimethylpentane	1.66400	0.32041	0.69330	0.69105
2,3-Dimethylpentane	1.78200	0.31447	0.69510	0.69634
3-Ethylpentane	1.73200	0.29886	0.69820	0.69940
Octane	1.45700	0.25549	0.70250	0.70383
2-Methylheptane	1.38500	0.26525	0.69790	0.69881
3-Methylheptane	1.45800	0.26261	0.70580	0.70178
4-Methylheptane	1.56300	0.26261	0.70460	0.70493
2,2-Dimethylhexane	1.28000	0.28082	0.69530	0.69110
3,3-Dimethylhexane	1.88400	0.27617	0.71000	0.71061
2,3-Dimethylhexane	2.00200	0.27167	0.71710	0.71548
2,4-Dimethylhexane	1.57100	0.27293	0.70040	0.70215
2,2,3-Trimethylpentane	2.09100	0.28727	0.71600	0.71358
2,2,4-Trimethylpentane	1.02100	0.29265	0.69190	0.67985
2,3,4-Trimethylpentane	2.10300	0.28145	0.71910	0.71565
2,3,3-Trimethylpentane	2.34500	0.28539	0.72620	0.72177
2-Methyl-3-ethylpentane	1.99200	0.26889	0.71930	0.71599
3,4-Dimethylhexane	2.18400	0.26889	0.71920	0.72176
3-Methyl-3-ethylpentane	2.56100	0.27159	0.72740	0.73230
3-Ethylhexane	1.85200	0.26001	0.71360	0.71438
Nonane	1.70700	0.22655	0.71760	0.71982
2-Methyloctane	1.63500	0.23419	0.71340	0.71542
3-Methyloctane	1.70900	0.23213	0.72070	0.71825
2,2-Dimethylheptane	1.53000	0.24624	0.71050	0.70873
2,3-Dimethylheptane	2.09800	0.23918	0.72600	0.72787
2,4-Dimethylheptane	1.65500	0.24015	0.71600	0.71427
2,2,3-Trimethylhexane	2.20000	0.25119	0.72920	0.72742
3-Ethylheptane	2.12100	0.23010	0.72700	0.73122
3,3-Dimethylheptane	2.16400	0.24266	0.72500	0.72884
Decane	1.95700	0.20350	0.73010	0.73408
2,2,6-Trimethylheptane	1.83800	0.22640	0.71950	0.72380
2,2,5-Trimethylhexane	1.47200	0.25530	0.70720	0.70434
2,2,4-Trimethylhexane	1.65800	0.25290	0.71560	0.71063
2,3,4-Trimethylhexane	2.32600	0.25140	0.72380	0.73115
2,3,5-Trimethylhexane	2.01200	0.24770	0.72190	0.72279
2,2,3,4-Tetramethylpentane	2.33600	0.25950	0.73900	0.72908
2-Methyl-4-ethylhexane	1.95900	0.23800	0.72300	0.72404
2,2-Dimethyl-3-ethylpentane	2.21000	0.24880	0.73480	0.72842
4,4-Dimethylheptane	1.85400	0.24260	0.72500	0.71954
2,6-Dimethylheptane	1.56300	0.24240	0.70890	0.71085
2,5-Dimethylheptane	1.93400	0.24020	0.71500	0.72265
3,4-Dimethylheptane	2.19900	0.23800	0.72300	0.73126
2,4-Dimethyl-3-ethylpentane	2.53500	0.24440	0.73790	0.73948
2,2,5-Trimethylhexane	1.62500	0.23770	0.71880	0.71409
2,3,3-Trimethylhexane	2.45700	0.24970	0.73800	0.73559
2-Methyl-3-ethylhexane	2.12700	0.23700	0.73100	0.72938
2,3,4-Trimethylhexane	2.35700	0.24440	0.73920	0.73413
2,2,3,3-Tetramethylpentane	2.91400	0.25980	0.75670	0.74637
3-Methyl-4-ethylhexane	2.49700	0.23490	0.74200	0.74112
3,3,4-Trimethylhexane	2.65400	0.24740	0.74540	0.74218
4-Ethylheptane	1.97100	0.23010	0.73000	0.72671
2,3,3,4-Tetramethylpentane	2.97600	0.25730	0.75470	0.74896
2,3-Dimethyl-3-ethylpentane	3.00900	0.24600	0.75400	0.75326
4-Methyloctane	1.83200	0.23210	0.71990	0.72195
2,4,6-Trimethylheptane	1.74800	0.22130	0.72250	0.72259
3,3-Diethylpentane	3.00000	0.23570	0.75360	0.75601
2,2-Dimethyl-4-ethylhexane	2.05600	0.22260	0.73300	0.73146
3,4-Dimethylheptane	2.12400	0.23700	0.73140	0.72929
3-Methyl-3-ethylhexane	2.56100	0.23910	0.74100	0.74182
2,2,4-Trimethylheptane	1.74200	0.22450	0.72750	0.72147
2,2,5-Trimethylheptane	2.08400	0.22450	0.72600	0.73175
2,2-Dimethyloctane	1.78000	0.21930	0.72450	0.72413
2,4-Dimethyloctane	1.92400	0.22440	0.72590	0.72697
2,7-Dimethyloctane	1.81300	0.21620	0.72420	0.72603
4-Isopropylheptane	2.26200	0.21190	0.74100	0.74079
4-Propylheptane	2.19100	0.20640	0.73640	0.74027
5-Methylnonane	2.07700	0.20800	0.73260	0.73637
Undecane	2.18300	0.18470	0.74020	0.74638
Dodecane	2.45700	0.16910	0.74870	0.75918

[a] Values from "Handbook of Tables for Organic Compound Identification," 3rd ed., CRC Press, Cleveland, Ohio, 1974.

Table II—Densities (Specific Gravities) of Aliphatic Alcohols Predicted from Functions of χ

Alcohol	$^3\chi$	$\frac{1}{\chi}$	$D_4{}^{20}$ (obs)[a]	$D_4{}^{20}$ (calc)[a]
Methanol	0.00000	1.00000	0.79150	0.78902
Ethanol	0.00000	0.70721	0.78140	0.79027
2-Propanol	0.00000	0.57737	0.78510	0.79083
1-Propanol	0.50000	0.52247	0.80360	0.80022
2-Butanol	0.81600	0.44053	0.80690	0.80636
2-Methyl-2-butanol	1.22500	0.39047	0.80890	0.81407
2-Methylpropanol	0.81600	0.44053	0.80200	0.80636
3-Methyl-2-butanol	1.33300	0.37850	0.81800	0.81610
3-Pentanol	1.47800	0.35625	0.82040	0.81885
1-Butanol	0.70700	0.41425	0.80960	0.80448
2-Pentanol	0.86600	0.36101	0.80920	0.80762
3,3-Dimethyl-2-butanol	1.73200	0.33979	0.81850	0.82357
2,3-Dimethyl-2-butanol	1.73200	0.33979	0.82080	0.82357
3-Methyl-3-pentanol	1.41400	0.32041	0.82330	0.81783
2-Methyl-2-pentanol	1.00000	0.32669	0.81340	0.81022
2-Methyl-3-pentanol	1.70700	0.31447	0.82490	0.82322
2-Methylbutanol	1.39400	0.35625	0.81930	0.81731
4-Methyl-2-pentanol	0.94300	0.31990	0.80710	0.80920
3-Methylbutanol	0.86600	0.36101	0.80920	0.80762
3-Hexanol	1.47800	0.30239	0.81850	0.81908
2,2-Dimethylbutanol	1.91400	0.32041	0.82830	0.82699
1-Pentanol	0.93300	0.34317	0.81480	0.80892
2-Hexanol	1.13500	0.30581	0.80980	0.81278
2,4-Dimethyl-3-pentanol	2.10300	0.28145	0.82880	0.83062
3-Ethyl-3-pentanol	2.56100	0.27624	0.83890	0.83903
2,3-Dimethylbutanol	1.70700	0.31437	0.82970	0.82322
2-Methylpentanol	1.47800	0.30230	0.82080	0.81908
2-Ethylbutanol	1.73200	0.29886	0.83350	0.82375
3-Methylpentanol	1.47800	0.30230	0.82420	0.81908
4-Methylpentanol	1.13500	0.30581	0.81310	0.81278
4-Heptanol	1.53600	0.26261	0.81830	0.82031
1-Hexanol	1.20700	0.29291	0.81890	0.81416
2-Heptanol	1.38500	0.26525	0.81670	0.81753
2-Methylhexanol	1.74700	0.26261	0.82700	0.82418
4-Methylhexanol	1.74700	0.26261	0.82390	0.82418
2,6-Dimethyl-4-heptanol	1.74800	0.22124	0.81290	0.82437
2-Octanol	1.63500	0.23419	0.82050	0.82225
2-Ethylhexanol	2.12100	0.23010	0.83280	0.83117
1-Octanol	1.70700	0.22655	0.82490	0.82360
2-Nonanol	1.88500	0.20964	0.81910	0.82693
2-Decanol	2.13500	0.18975	0.82500	0.83160
1-Nonanol	1.95700	0.20350	0.82710	0.82828

[a] Values from "Handbook of Tables for Organic Compound Identification," 3rd ed., CRC Press, Cleveland, Ohio, 1974.

again revealed with χ and $^3\chi$. The statistical analysis and regression equation are:

$$D_4{}^{20}\text{(aliphatic acids)} = 0.0252\ ^3\chi + \frac{0.4358}{\chi} + 0.7546$$

$$r = 0.9831 \qquad s = 0.0137 \qquad n = 20 \qquad \text{(Eq. 3)}$$

Predictions of densities for two acids, nonanoic (d_{obs} = 0.9055) and oleic (d_{obs} = 0.8935), yielded the values 0.8913 and 0.8966, respectively.

DISCUSSION

Relationship of χ Terms to Density—The analyses of three series of compound classes using χ and $^3\chi$ reveal good correlations with the densities of these molecules. With the hydrocarbons and acids, the correlation is excellent. In these two cases, the value of the densities increases in an opposite direction with increasing molecular weight. The same terms of the connectivity expansion, χ and $^3\chi$, still predict the densities rather well.

The correlation with the alcohol densities is good with χ and $^3\chi$ but not of the quality of the other two. One explanation lies in the failure of χ and $^3\chi$ to distinguish between isomers such as 2-pentanol and 3-methylbutanol and 3-methyl-3-pentanol and 2,2-dimethylbutanol. Each pair has the same χ and $^3\chi$ value. The first pair of isomers has exactly the same density. The second pair has a density difference of 0.005. This duplication exists in seven cases in the list of alcohols studied. A more definitive index must be developed to characterize uniquely these molecules.

Nature of $^3\chi$—At this time, one can only make a preliminary analysis of the nature of the structural features defined by χ and $^3\chi$ that influence density. The authors have already observed a rela-

tionship between χ and the volume of a molecule (1). The reciprocal of χ would, therefore, be expected to contribute to the density definition.

The nature of $^3\chi$ becomes more apparent on closer inspection. The value of $^3\chi$ is really a summation of C_k values for pairs of alternate bonds. For example, in the case of hexane, the $^3\chi$ value is a summation of the molecular fragments shown here:
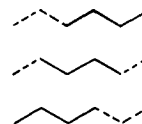
Each bond type is counted a different number of times in the total summation: terminal bonds once, the central bond three times, and others twice.

In contrast, the $^3\chi$ value for the hexane isomer 2,3-dimethylbutane is a summation of the fragments shown here:
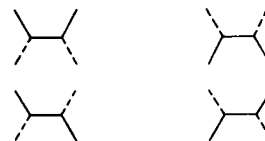
The four terminal bonds are each counted twice, while the central bond is not implicitly counted at all. This pattern of weighting of bonds in different molecules emphasizes certain structural features that bear a significance to density along with the χ term. In-depth studies of this kind are currently underway in this laboratory.

Regression Equation Constant—An interesting observation from the regression equation deserves comment. The three equation con-

Table III—Densities (Specific Gravities) of Aliphatic Acids Predicted from Functions of χ

Acid	$^3\chi$	$\dfrac{1}{\chi}$	$D_4{}^{20}$ (obs)[a]	$D_4{}^{20}$ (calc)[a]
Formic	0.00000	1.01420	1.22030	1.19654
Acetic	0.00000	0.73746	1.04930	1.07595
Propionic	0.60400	0.52247	0.99390	0.99751
2-Methylpropionic	0.98600	0.43535	0.94790	0.96919
Butanoic	0.67700	0.41425	0.95790	0.95219
2-Methylbutanoic	1.53800	0.35273	0.93800	0.94712
3-Methylbutanoic	0.75700	0.36101	0.92620	0.93101
3,3-Dimethylbutanoic	0.83200	0.32669	0.91240	0.91795
Pentanoic	0.95700	0.34317	0.93920	0.92829
2,2-Dimethylbutanoic	2.03900	0.31596	0.92760	0.94374
2,3-Dimethylbutanoic	1.79600	0.31172	0.92750	0.93576
2-Ethylbutanoic	1.70500	0.29647	0.92390	0.92682
2-Methylpentanoic	1.58500	0.29985	0.92300	0.92526
3-Methylpentanoic	1.39400	0.30221	0.92620	0.92147
4-Methylpentanoic	1.14800	0.30572	0.92250	0.91679
Hexanoic	1.20700	0.29291	0.93570	0.91270
4-Methylhexanoic	1.76100	0.26518	0.91940	0.91460
4-Ethyl-4-methylbutanoic	1.76100	0.26254	0.91490	0.91345
Heptanoic	1.63400	0.25549	0.91980	0.90717
Octanoic	1.88400	0.22655	0.90880	0.90087

[a] Values from "Handbook of Tables for Organic Compound Identification," 3rd ed., CRC Press, Cleveland, Ohio, 1974.

stants for the hydrocarbons, alcohols, and acids are 0.7348, 0.7933, and 0.7546, respectively. These values are close enough to cause one to suspect that this term may have some fundamental significance, particularly if one considers the different values of the densities in the three series and the fact that one set of densities decreases with increasing molecular weight.

The density (expressed as specific gravity) of a hypothetical hydrocarbon is predicted to be 0.7348 if $^3\chi$ is zero and χ is infinite in spite of the fact that the density values of the hydrocarbons in Table I range above and below this value. This number cannot be a limiting value of the density. For the acids, the constant is 0.7546, a value well below the range of values of the densities.

If only the hydrocarbons and acids are considered, in view of the quality of these two equations, the two constants are seen to be very close to the phase volume of 0.7402. This is the fraction of space occupied by spheres of identical size. Therefore, the derived equations may be of such a fundamental quality that the geometrical constant of phase volume is the limiting value when $^3\chi$ is zero and χ is infinite.

If this interpretation of the constant is true, it is possible to describe the influences of the χ terms on the experimental value of the density. In a hypothetical primary state, a molecule with $\chi = \infty$ and $^3\chi = 0$ would be a perfect sphere. This would describe an infinite number of bonds radiating from a single central atom; this is the connectivity definition of a perfect sphere. It would pack into a unit space, filling 0.7402 part of that space. In an actual molecule, the χ terms may be viewed as modifiers of the perfect sphericity that gave a density (specific gravity) of 0.7402. The χ terms reflect modifications in the sphericity that may increase or decrease the packing ability of the molecules in the bulk phase. Thus, an experimental density may be higher or lower than the phase volume of 0.7402.

CONCLUSIONS

Once again, the fundamental molecular property of connectivity is seen to have a close relationship to a physical property. In this case, the density is seen to be a function of two terms in a series expansion of χ.

The equations relating χ and $^3\chi$ to the densities of three groups of molecules results in a constant term quite close to the phase volume. This finding suggests a fundamental quality of the equations and, in turn, the roles of the χ terms. It also permits an interpretation of the contributions of the χ terms to the experimental value of the density, at least in a general sense.

Finally, the quality of the correlations of χ with density has a very practical consequence to the pharmaceutical scientist. Acids and alcohols are classes of molecules frequently used in formulation as solvents and buffers for their physical properties, such as osmotic influence, and in their own right as active agents. With this simple approach, other classes of molecules of equal interest in pharmacy

may be treated and physical properties such as density may be predicted.

APPENDIX

An alternate formulation of the extended series arises from a consideration of powers of the connectivity matrix of the graph. When using isopentane as an example and numbering the skeleton in any manner, the matrix is:

$$\begin{pmatrix} 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 1 \\ 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \end{pmatrix}$$

In the upper triangle, there are four unit entries corresponding to the four C_{ij} bond terms in the calculation of χ.

The square of the matrix is:

$$\begin{pmatrix} 1 & 0 & 1 & 0 & 1 \\ 0 & 3 & 0 & 1 & 0 \\ 1 & 0 & 2 & 0 & 1 \\ 0 & 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 & 1 \end{pmatrix}$$

In the upper triangle, there are four unit entries corresponding to each C_{ijk} term in the calculation of $^2\chi$. The diagonal of the matrix contains the δ_i values of the graph.

The cube of the matrix is:

$$\begin{pmatrix} 0 & 3 & 0 & 1 & 0 \\ 3 & 0 & 4 & 0 & 3 \\ 0 & 4 & 0 & 2 & 0 \\ 1 & 0 & 2 & 0 & 1 \\ 0 & 3 & 0 & 1 & 0 \end{pmatrix}$$

Again, the number of unit entries in the upper triangle corresponds to the C_{ijkl} terms used in computing $^3\chi$. The fourth power of the matrix has no unit terms and there are no C_{ijklm} terms; hence, $^4\chi$ is zero.

Studies on the matrix formulation of the extended series are in progress in this laboratory.

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Spectrofluorometric Determination of Bufuralol in Blood and Urine

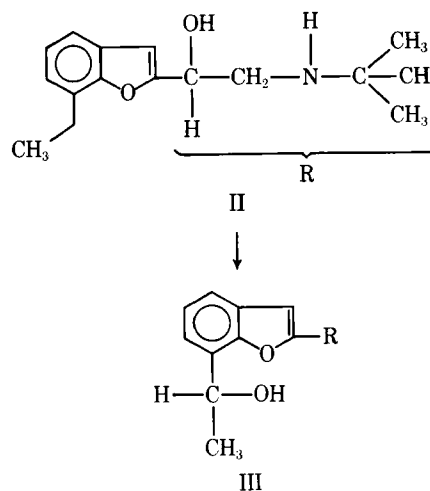## J. ARTHUR F. de SILVA ˣ, JOHN C. MEYER, and CARL V. PUGLISI

Abstract □ The benzofuran analog bufuralol, a β-adrenergic blocker, was determined in blood and urine by a specific and sensitive spectrofluorometric assay. The compound was extracted into ether from blood or urine adjusted to pH 10. The ether extract was separated by TLC to resolve the parent drug from any basic metabolites present, and the spots were eluted off the silica gel and quantitated fluorometrically in 0.1 N HCl. The overall recovery of the assay was 85 ± 3.0%; the sensitivity limit was 2–4 ng/ml of blood or urine, using a 2.5-ml specimen/analysis. The method was applied to the determination of blood levels in a dog following a single 10-mg/kg oral dose and in two human subjects administered a single 20-mg oral dose.

Keyphrases □ Bufuralol—spectrofluorometric analysis in blood and urine □ Spectrofluorometry—analysis, bufuralol in blood and urine □ β-Adrenergic blocking agents—bufuralol, spectrofluorometric analysis in blood and urine

Scheme I

Several phenethanolamines, polycyclic aromatic alkanolamines, and naphthyloxyethanolamines, possessing β-adrenergic blocking activity in humans, have been investigated as therapeutic agents in cardiovascular disorders such as angina pectoris, tachyarrhythmias, and essential hypertension (1–3). All of these compounds contain an N-isopropyl side chain similar to that in isoproterenol and propranolol. A spectrofluorometric method for the determination of plasma propranolol levels (4) and a clinical evaluation of plasma propranolol levels and β-adrenergic blockade in humans (5) were reported.

β-Adrenergic activity was noted in a series of benzofuran derivatives, and the pharmacology of 6,7-dimethyl-α-[(isopropylamino)methyl]-2-benzofuranmethanol hydrochloride (I) was reported (6). The clinical evaluation of this compound against propranolol was also reported (7), and a spectrofluorometric assay for I was developed and used in pharmacokinetic studies in normal volunteers (8).

Another member of the benzofuran series, bufuralol [7-ethyl-α-[(*tert*-butylamino)methyl]-2-benzofuranmethanol hydrochloride] (II) (9), is under clinical investigation as a β-adrenergic blocking agent in the treatment of cardiac arrhythmias (10). A spectrofluorometric assay was developed for the determination of II in blood and urine by a modification of a procedure published for I (8). The modified procedure includes a TLC separation step to resolve the parent drug (II) from its major metabolite (III) in blood and urine (Scheme I), thereby imparting specificity to the assay. Compounds II and III fluoresce in 0.1 N HCl with excitation at 250 nm and emission at 300–305 nm (Fig. 1). The sensitivity limit of the assay is 2 ng of compound/ml of blood, using a 2.5-ml specimen/analysis and specially selected instrumental conditions.

The method was used to determine blood levels of II in a dog given a 10-mg/kg oral dose and in two human subjects following administration of a single 20-mg oral dose.

EXPERIMENTAL

Standard Solutions—Compound II, $C_{16}H_{23}NO_2\cdot HCl$ (mol. wt. 297.82, mp 145–146°), of pharmaceutical grade purity (>99%) was used. Weigh out 11.40 mg of the hydrochloride salt equivalent to 10.0 mg of the free base. Transfer it into a 10-ml volumetric flask, dissolve in 5 ml of acetone, and dilute to volume with n-hexane. This stock solution (A) contains 1.0 mg of free base/ml. Prepare working solutions containing 0.25, 0.50, 0.75, or 1.0 μg/ml by making serial dilutions of Solution A with acetone–n-hexane (50:50).

Similarly, prepare standard solutions of Metabolite III, $C_{16}H_{23}O_3N(COOH)_2$ (mol. wt. 367.39, mp 115°), by weighing out 13.26 mg of the oxalate salt equivalent to 10 mg of the free base and proceeding as described for II. These solutions are used as external