equally satisfactory results in the absence of trifluoroacetic acid, in fact, but we feel that trifluoroacetic acid may, in some cases, be advantageous as a solvent for high molecular weight permethylated polysaccharides. Analysis of all reactions after workup by GC/MS demonstrated in each case that reductive cleavage was virtually quantitative. In each reaction the product of reductive cleavage was identical with the authentic anhydroalditol derivative as judged by electron impact mass spectrometry, chemical ionization mass spectrometry, ^{1}H NMR spectroscopy, gas-liquid chromatography, and optical rotation.

We conclude from these results that reductive cleavage of glycosides is potentially an attractive method for polysaccharide structure determination. Moreover, the reaction conditions described herein are synthetically useful as a means to prepare anhydroalditols, which have proven to be very useful analogues for the study of the mechanisms of carbohydrate-requiring enzymes.

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On the Concept of Graph-Theoretical Individual Ring Resonance Energies

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Hückel molecular orbital theory has been reexamined and redefined in terms of graph-theoretical concepts,[1,2] Graph-theoretical definitions of resonance energies (GTRE) have arisen out of this work,[3-6] and the applications of these definitions comprise a sizable part of the chemical graph-theoretical literature. Recent papers have stressed the point that GTRE's can be divided among the individual rings of polycyclic π molecular graphs. Both individual ring aromaticities[5,9,10] and the theory of London diamagnetism[11,12] have been interpreted on this basis.

The purpose of this communication is to point out that the principal definition[7-10,12] for individual ring aromaticies is based on polynomial equations that have *imginary roots* in several key, nontrivial cases. The roots of these polynomials must be taken to correspond to energy levels, and the existence of imaginary roots therefore obviates the use of these GTRE's in discussing ring aromaticities or susceptibilities due to individual ring currents. This failing of the GTRE definition, when added to other, less formal types of difficulties,[13-19] should lead to caution in the use of the GTRE concept.

The details of a GTRE calculation are as follows. The coefficients of the HMO secular polynomial $P^{HMO}(G)$ can be written by using graph theory since each term is a prescribed function of the number of bonds (edges), rings (cycles), and atoms (vertices) in the molecular graph.[20-23] The polynomial for a hypothetical cyclic resonance-free reference system $P^{ref}(G)$ is obtained by deleting all cyclic component terms from the original polynomial.[3-6,23]

$$P^{ref}(G) = P^{HMO}(G) - \sum(\text{ring terms}) \quad (1)$$

The ordered set of the roots of $P^{HMO}(G)$ and $P^{ref}(G)$ allow one to define GTRE as

$$\text{GTRE} = E(P^{HMO}) - E(P^{ref}) = \sum_i g_i(x_i^{HMO} - x_i^{ref}) \quad (2)$$

where g_i is an occupation number and the sum is over all i occupied levels.[24]

In a polycyclic π molecular graph, it is presumed[7-10,12] that the GTRE can be divided among the various rings by defining individual ring reference polynomials

$$P^{ref}(G/R_n) = P^{HMO}(G) - (\text{ring terms})(R_n) \quad (3)$$

where $P^{ref}(G/R_n)$ refers to the polynomial with algebraic terms for the individual ring R_n deleted. The use of eq 2 with the roots of eq 3 gives individual ring resonance energies.

The interested reader can check all calculations by using standard computer programs that determine the eigenvalues of the adjacency matrix of a graph (HMO programs). This follows because the HMO polynomial can be written as the product function

$$P^{HMO}(G) = \prod_{\substack{G \\ (k \text{ levels})}} (x - x_k) \quad (4)$$

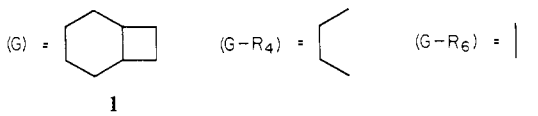
where the x_k are the k HMO eigenvalues. In addition, each set of ring terms is given by

$$(\text{ring terms})(R_n) = -2P^{HMO}(G - R_n) \quad (5)$$

where $G - R_n$ is the molecular graph with the individual ring deleted.[8,11,20] Therefore, all reference polynomials are obtainable in terms of HMO secular polynomials for the graph and subgraphs of the original system.[23]

$$\begin{aligned} P^{ref}(G/R_n) &= P^{HMO}(G) + 2P^{HMO}(G - R_n) \\ &= \prod_G (x - x_k) + 2 \prod_{\substack{G-R_n \\ l \text{ levels}}} (x - x_l) \end{aligned} \quad (6)$$

These definitions are illustrated in the following by using the π molecular graph of benzocyclobutadiene (**1**).



1

$$P^{HMO}(G) = x^8 - 9x^6 + 22x^4 - 16x^2 + 1 \quad (7)$$

R_4 terms $= -2(x^4 - 3x^2 + 1)$ R_6 terms $= -2(x^2 - 1)$

R_8 terms $= -2$

$$P^{ref}(G/R_4) = x^8 - 9x^6 + 24x^4 - 22x^2 + 3 \quad (8)$$

$$P^{ref}(G/R_6) = x^8 - 9x^6 + 22x^4 - 14x^2 - 1 \quad (9)$$

$$P^{ref}(G/R_8) = x^8 - 9x^6 + 22x^2 - 16x^2 + 3 \quad (10)$$

Equations 8-10 are fourth-degree equations in the variable x^2. According to Descartes' rule of signs,[25] $P^{ref}(G/R_6)$ (eq 9) has

(1) Gutman, I.; Trinajstić, N. *Top. Curr. Chem.* **1973**, *42*, 49-93.
(2) Graovac, A.; Gutman, I.; Trinajstić, N. *Lect. Notes Chem.* **1977**, *4*, 1-123.
(3) Gutman, I.; Milun, M.; Trinajstić, N. *MATCH* **1975**, *1*, 171-175.
(4) Aihara, J. *J. Am. Chem. Soc.* **1976**, *98*, 2750-2758.
(5) Aihara, J. *J. Am. Chem. Soc.* **1977**, *99*, 2048-2053.
(6) Gutman, I.; Milun, M.; Trinajstić, N. *J. Am. Chem. Soc.* **1977**, *99*, 1692-1704.
(7) Gutman, I.; Bosanac, S. *Tetrahedron* **1977**, *33*, 1809-1812.
(8) Bosanac, S.; Gutman, I. *Z. Naturforsch.* **1977**, *32a*, 10-12.
(9) Gutman, I. *J. Chem. Soc. Faraday Trans. 2*, **1979**, *75*, 799-805.
(10) Gutman, I. *Croat. Chem. Acta* **1980**, *53*, 581-586.
(11) Ihara, J. *J. Am. Chem. Soc.* **1979**, *101*, 5913-5917.
(12) Aihara, J. *J. Am. Chem. Soc.* **1981**, *103*, 5704-5706.
(13) Gutman, I. *Chem. Phys. Lett.* **1979**, *66*, 595-597.
(14) Gutman, I.; Mohar, B. *Chem. Phys. Lett.* **1980**, *69*, 375-377.
(15) Gutman, I. *Theor. Chim. Acta* **1980**, *56*, 89-92.
(16) Gutman, E.; Mohar, B. *Chem. Phys. Lett.* **1981**, *77*, 567-570.
(17) Aihara, J. *Chem. Phys. Lett.* **1980**, *73*, 404-406. This paper replies to criticisms in ref 13.
(18) Herndon, W. C. *J. Org. Chem.* **1981**, *46*, 2119-2125.
(19) E. Heilbronner, submitted for publication. A copy of this article was kindly supplied by Professor Heilbronner.
(20) Hosoya, H. *Theor. Chim. Acta* **1972**, *25*, 215-222.
(21) Graovac, A.; Gutman, I.; Trinajstić, N.; Zikovic, T. *Theor. Chim. Acta* **1972**, *26*, 67-78.
(22) Aihara, J. *J. Am. Chem. Soc.* **1976**, *98*, 6840-6844.
(23) Herndon, W. C.; Ellzey, M. L., Jr. *J. Chem. Inf. Comput. Sci.* **1979**, *19*, 260-264.
(24) Gutman[13] and Aihara[17] show that there may not be a continuous one-to-one mapping of the roots of the HMO polynomial with those of the reference polynomial. The occupation numbers g_i then refer to the occupied eigenlevels in the case of the original π molecular system but are taken to correspond to the $n/2$ (n = number of electrons) most negative roots in the case of the reference polynomial.

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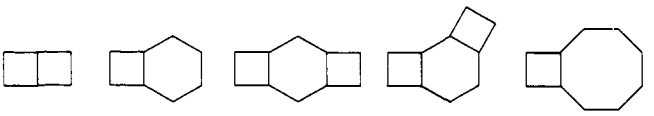
exactly one negative value of x^2, i.e., the values of x are imaginary numbers. $P^{ref}(G/R_4)$ (eq 8) also has imaginary roots, a result that can be demonstrated by direct numerical solution for the two real positive values of x^2 and factorization to give a quadratic equation in x^2. The discriminant of the quadratic equation is negative, which shows that there are four complex roots for the original eq 7.[26] In either case, the obtaining of imaginary values obscures the meaning and the chemical interpretation of the graph-theoretical resonance energy.

Published works[7,8,10] on graph-theoretical individual ring resonance energies contain real number entries for the π molecular graphs shown in **1–5**, each of which can be demonstrated to possess

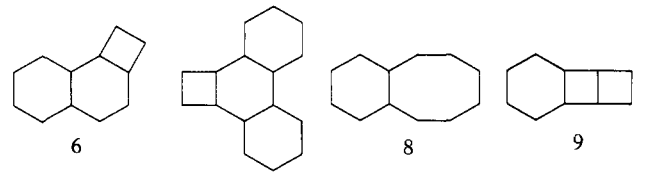


2 1 3 4 5

two or more complex or pure imaginary roots for particular G/R reference polynomials. The reason for these errors may be the use of an approximate formula and numerical integration to obtain the total ring resonance energy, which bypasses finding the actual exact solutions to the polynomials.

The recent discussion[12] of a "unified theory of aromaticity and London diamagnetism" uses bicyclo[6.2.0]decapentaene molecular graph **5** as one of its two examples, also reporting the total sum of the ring resonance energies as a real number.[27] Here the explanation is that roots are obtained by an expression based on Newton's method to approximate roots that is not capable of finding complex solutions.

In general, annelated alternant polycyclic π molecular graphs with corrected structure count (CSC)[28] of unity or zero will have complex roots for ring reference polynomials defined as in eq 3. This class includes many benzannelated derivatives of the compounds **1–5** and many other types of related systems, e.g., **6–9**,



6 7 8 9

which comprise a group of compounds that are of particular interest to theoreticians concerned with definitions of aromaticity and calculations of resonance energies. The use of the reference polynomial, eq 3, is interdicted for this group, and its successful use in other systems including benzenoid alternants and nonalternants *should be considered problematic.*[29]

A less explored way to define GTRE has been suggested in earlier work by Aihara.[5] The reference polynomial is constructed by subtracting all ring terms, eq 1, and reintroducing the algebraic terms only for the ring under consideration. The roots of such ring polynomials have all been found to be real numbers in several benzenoid systems[5] and in the π graphs considered in the present paper. Whether or not one wishes to employ this alternate definition where no proof exists that difficulties of the type outlined above are absent is a matter of individual predilection.

Acknowledgment. I thank Professor Edgar Heilbronner for a useful exchange of unpublished information. The financial support of the Robert A. Welch Foundation of Houston, TX, is also gratefully acknowledged.

(25) Uspensky, J. V. "Theory of Equations"; McGraw-Hill: New York, 1948; pp 121–124.

(26) The numerical values of the roots for eq 8 are $x^2 = 0.163901$, 5.143381, and $(1.846359 \pm 0.386849)(-1)^{1/2}$.

(27) The reference polynomial for the 10-membered ring component of **5** has one negative value of x^2, showing that the corresponding roots are $\pm x\text{-}(-1)^{1/2}$. Columns 3 and 4 of Table II, ref 12, should therefore contain some entries with imaginary numbers.

(28) Herndon, W. C. *Tetrahedron* **1973**, *29*, 3–12.

(29) The significance of the necessity for real roots of reference polynomials is pointed to by the number of papers where this question is discussed. For leading references see: Graovac, A. *Chem. Phys. Lett.* **1981**, *82*, 248–251. Godsil, C. D.; Gutman, I. *Croat. Chem. Acta* **1981**, *54*, 53–59. The real root requirement is considered to be inconsequential in the following: Aihara, J. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 2010–2012. Gutman, I. *Z. Naturforsch.* **1978**, *33a*, 840–841. The use of the so-called "sextet" polynomial is described here.

Enzyme-Catalyzed Transhydrogenation between Nicotinamide Cofactors and Its Application in Organic Synthesis[1]

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Several of the most convenient systems that have been proposed for the regeneration of the nicotinamide cofactors are specific for NAD(H).[2,3] Although NADP(H) is less common in synthetically interesting biochemical pathways than NAD(H), on those occassions when NADP(H) is required, one is restricted for its regeneration to two procedures.[4,5] Here we describe three practical systems that accomplish transhydrogenation between NAD(H) and NADP(H) cofactors (eq 1) and demonstrate their

$$\text{NADH} + \text{NADP}^+ \rightleftharpoons \text{NAD}^+ + \text{NADPH} \qquad (1)$$

application to problems in practical synthesis (Scheme I).[6] These systems permit regeneration procedures that are specific for one cofactor (NAD(H) *or* NADP(H)) to be coupled to the other.

The first of these schemes utilizes the flavoenzyme diaphorase (EC 1.6.4.3) from *Clostridium klyveri*; the second uses alcohol dehydrogenase from *Leuconostic mesenteroides* (ADH, EC 1.1.1.1) in a system containing both ethanol and acetaldehyde; the third involves glutamic dehydrogenase (GluDH, EC 1.4.1.3) in solutions containing glutamate (Glu), 2-ketoglutarate (2-KG), and ammonium ion. Added redox-active substrates are required for transhydrogenation activity with ADH and GluDH but not for diaphorase,[7] which contains FAD and which catalyzes hydride transfer between oxidized and reduced nicotinamide coenzymes directly. We used concentrations of the added substrates for ADH and GluDH that were $5\text{–}10K_m$. Relevant kinetic parameters for these enzymes are summarized in Table I.

Demonstration of the coupling of a $NADP^+$-specific enzymatic oxidation to a regeneration system specific for $NADH \rightarrow NAD^+$ was accomplished by conversion of pyruvate to L-lactate (**1**) and

(1) Supported by the National Institutes of Health (GM 26543 and GM 30367).

(2) Shaked, Z.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 7104.

(3) Jones, J. B.; Beck, J. F. In "Application of Biochemical Systems in Organic Chemistry"; Jones, J. B., Perlman, D., Sih, C. J., Eds.; Wiley-Interscience: New York, 1976; p 107–401.

(4) Wong, C.-H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 4890–4899.

(5) Wong, C.-H.; Whitesides, G. M. *J. Org. Chem.*, in press.

(6) The flavoenzyme pyridine nucleotide transhydrogenase (EC 1.6.1.1) from *pseudomonas* or spinach catalyzes direct transhydrogenation (Kaplan, N. O.; Colowick, S. P.; Neufeld, E. F. *J. Biol. Chem.* **1953**, *205*, 1–15). Since $NADP^+$ is a potent dead-end inhibitor for this enzyme, the forward reaction ($NADP^+ + NADH \rightarrow NAD^+ + NADPH$) is very slow (Cohen, P.T.; Kaplan, N. O. *J. Biol. Chem.* **1970**, *245*, 4666–4672). Moreover, the enzyme isolated from either source (ca. 20 units from 1 kg of spinach or *pseudomonas* cells) has low specific activity (0.1–0.3 unit mg^{-1}). We did not attempt to use this enzyme for large-scale synthesis.

(7) Pig heart lipoamide dehydrogenase is essentially specific for NAD^+ ($NADP^+$ is only 5% as active a substrate as NAD^+, in either the presence or the absence of added lipoic acid). Addition of lipoic acid to the transhydrogenation system containing diaphorase from *C. klyveri* did not improve its catalytic activity, because lipoic acid (or lipoamide) is a poor substrate for this enzyme.

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