tions. On the other hand, molecules whose substituents or heteroatoms interact quite differently from atoms in benzene would not be expected to adhere to the DEE method.

The principal conclusion to be derived here is that among the several levels of approximation in the theory of chemical shifts, there is an *optimum* level of agreement between theory and experiment at the DEE stage, when BJ-CNDO wave functions are used. Fewer approximations, that is, use of the Ramsey tensor, give bad results because the wave function is inadequate to the theory. More approximations than DEE result in the neglect of the lower energy excitations and inadequate $\sigma$ polarity; that is, the theory is insufficient.

At the DEE approximation with the BJ-CNDO MO's, which is suitable for calculating shifts, there is a delicate match of theory to wave functions with respect to their level within the theoretical framework.

### Proton Chemical Shifts

The results calculated by using eq 14-16 in different cases are given in Table V. The *jj* component of the paramagnetic susceptibility, $(\chi_p{}^B)_{jj}$, in eq 16 is calculated as follows. When the B nucleus is a carbon, in cases 1-6, equations corresponding to eq 10-13 were used and the summation runs over all carbon atoms except nitrogen and the atom bonded to the hydrogen of interest. As mentioned above, the large anisotropy of nitrogen is mostly due to the *xx* component of the paramagnetic susceptibility because of the low transition energies. Accordingly, $(\chi_p{}^N)_{xx}$ was calculated by use of eq 6 and the experimental first and second n → $\pi^*$ excitation energies, listed in Table VI. Other transitions are assumed to be 10 eV. In spite of the rough nature of this calculation, the results account well for the proton shifts from one molecule to another, or among positions in the same molecule. However, as noted before, the results should be regarded as qualitative even if they give rather good agreement.[12] These shifts are roughly proportional to the atom charges since $\sigma_d$ makes the larger contribution.

Table VI. n → $\pi^*$ Transition Energies in Azines

| | n → $\pi^*$ transition energy, eV | |
|---|---|---|
| | First | Second |
| Pyrazine | 3.78 (obsd)[a] | 7.19 (estd) (cases 1 and 2) |
| | | 7.35 (estd) (cases 3 and 4) |
| | | 7.72 (estd) (cases 5 and 6) |
| Pyridazine | 3.65 (obsd)[a] | 4.13 (estd) (cases 3 and 4) |
| | | 7.20 (estd) (cases 3 and 4) (third) |
| Pyrimidine | 4.15 (obsd)[a] | 4.40 (estd) (cases 3 and 4) |
| | | 7.59 (estd) (cases 3 and 4) (third) |
| *s*-Triazine | 4.46 (obsd)[a] | 7.62 (estd) (cases 1 and 2) |
| | | 7.85 (estd) (cases 3 and 4) |
| | | 7.51 (estd) (cases 5 and 6) |
| *s*-Tetrazine | 2.30 (obsd) | 3.87 (obsd) (cases 1-6) |

[a] L. Goodman, *J. Mol. Spectry.*, **6**, 109 (1961).

**Acknowledgment.** This research was supported in part by the Air Force Office of Scientific Research, Grant No. 68-1441, the National Institutes of Health, Grant No. GM-08686, and the National Science Foundation, Grant No. CB-8507. We are very grateful to Professor H. H. Jaffé and to Dr. M. Okuda and Dr. H. Masuhara, who supplied wave functions used in our calculations. We also acknowledge stimulating discussion with Professor C. W. Kern of this department.

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# Carbon-13 Magnetic Resonance. XIII.[1a] Assignment of Carbon-13 Resonances Using the Nuclear Overhauser Effect

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*Contribution from the Department of Chemistry, University of Utah,*
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*College, Hanover, New Hampshire 03755. Received February 17, 1969*

**Abstract:** The observed carbon-13 spectral intensities for the chemically nonequivalent quarternary carbon atoms in pyrene, acenaphthene, acenaphthylene, and fluoranthene enable assignment of these lines provided that the contributing nuclear Overhauser enhancement arises from the distance-dependent ($r^{-6}$) dipolar interaction between the carbon and nearest neighbor protons. A semiquantitative model which emphasizes the geometrical dependence of these phenomena is provided.

The assignment of carbon-13 resonance peaks in complex natural abundance carbon-13 spectra has to date been dependent upon one or more of the following factors: (a) observation of proton-induced splitting patterns,[2] (b) the absence of proton-induced splittings on lines associated with quarternary carbon atoms, (c) the relative intensities of isolated spectral multiplets and/or observed decoupled resonance lines, and (d) selective proton decoupling.[3] In complex molecular systems, however, major ambiguities in assignments occur for those quarternary carbon atoms which are chemically nonequivalent but would be expected to exhibit carbon-13 reasonance lines of equal intensity. We present herein a general method for assigning some

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(1) (a) Previous paper in this series, R. J. Pugmire and D. M. Grant, *J. Am. Chem. Soc.*, **90**, 4232 (1968); (b) University of Utah; (c) Dartmouth College.

(2) P. C. Lauterbur, *J. Am. Chem. Soc.*, **83**, 1838 (1961).

(3) E. G. Paul and D. M. Grant, *ibid.*, **86**, 2977 (1964).

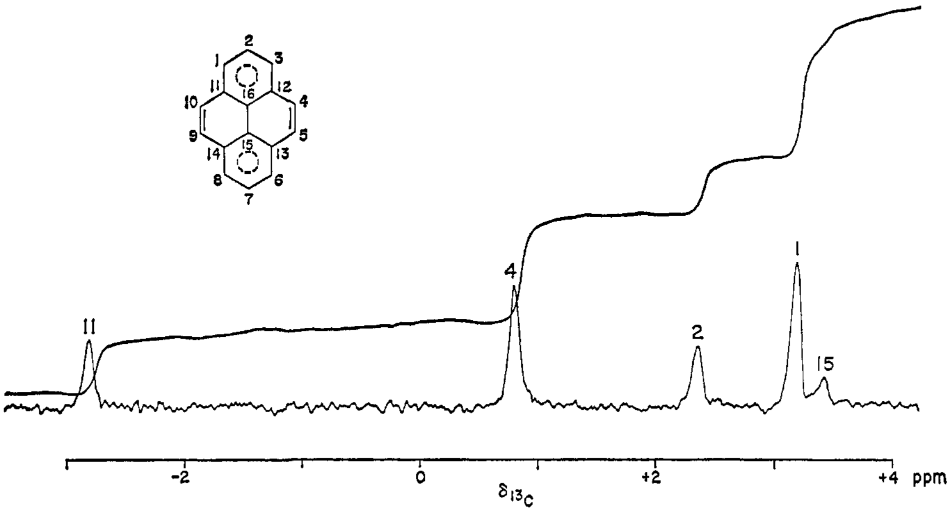

Figure 1. The proton-decoupled carbon-13 magnetic resonance spectrum of pyrene. Upper trace shows integral.

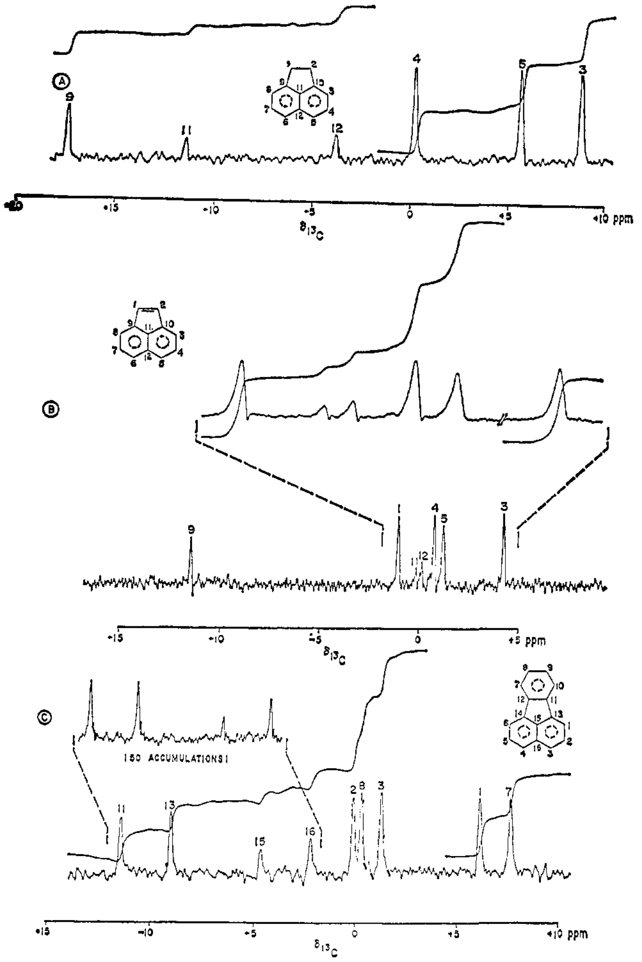

Figure 2. The proton-decoupled and integrated carbon-13 magnetic resonance spectra of (a) acenaphthene (10 accumulations); (b) acenaphthylene; inset shows integral after 10 accumulations for expanded high-field region; the integral for C-9,10 was determined in the single scan (lower spectrum) but is not shown; and (c) fluoranthene; inset shows quarternary carbon atom spectrum after 50 accumulations.

quarternary carbon atoms in polycyclic systems utilizing nuclear Overhauser phenomena.[3,4]

## Experimental Section

Carbon-13 spectra were obtained using a Varian AFS-60 analog frequency-sweep high-resolution spectrometer operating at 15.1 MHz. The 10-mm sample tubes were spun in a Varian V-4331 A probe, double tuned to 15 and 60 MHz. The field was locked by conventional techniques to an external water sample in the probe. Proton decoupling was accomplished using a Hewlett-Packard 5105 A frequency synthesizer in conjunction with a white noise generator.[5] The band width of the decoupling field was in excess of 500 Hz under these conditions. All samples were studied as saturated solutions in carbon disulfide, degassed using the nitrogen bubbling technique. Spectra are the average of not less than ten accumulations using a time-averaging device (Varian C-1024). Integrals were determined using a Varian V-3521 A integrator.

## Results

The decoupled and integrated carbon-13 spectral transitions in pyrene,[6] acenaphthene, acenaphthylene, and fluoranthene[7] are shown in Figures 1 and 2. The integrated and normalized ratios of these transitions are given in Table I. It should be emphasized that white noise decoupling techniques were employed in order to ensure that all the protons in the system under consideration were simultaneously decoupled. It is significant that in the spectrum of pyrene (Figure 1) the integral shows a ratio of 4:1 for C-11:C-15 compared with the expected 2:1. Similarly, the unit intensity lines attributable to C-11 or C-12 in acenaphthene and acenaphthylene and C-15 and C-16 in fluoranthene are not of equal intensity. From the pyrene result it is suggested that the lower intensity lines be attributed to the central quarternary carbon atoms C-11 and C-15 in these compounds. We provide a semiquantitative basis for these observations.

## Discussion

Applying the theory of Solomon[8] to a decoupled two-spin carbon–hydrogen system Kuhlmann and Grant[9] have shown that a maximum nuclear Overhauser en-

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(4) A. W. Overhauser, *Phys. Rev.*, **92**, 411 (1953). The nuclear Overhauser effect has also been employed to aid assignment of proton spectra: F. A. L. Anet and A. J. R. Bourn, *J. Am. Chem. Soc.*, **87**, 5250 (1965).

(5) R. R. Ernst, *J. Chem. Phys.*, **45**, 3845 (1966); see also F. J. Weigert, M. Jautelat, and J. D. Roberts, *Proc. Natl. Acad. Sci. U. S.*, **60**, 1152 (1968).

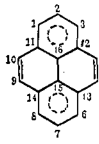
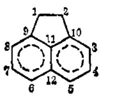
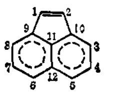
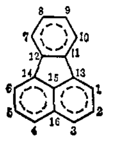
(6) T. D. Alger, D. M. Grant, and E. G. Paul, *J. Am. Chem. Soc.*, **88**, 5397 (1966).

(7) A detailed discussion of these spectra and the spectral parameters obtained therefrom will be presented shortly.

(8) I. Solomon, *Phys. Rev.*, **99**, 559 (1955).

(9) K. F. Kuhlmann and D. M. Grant, *J. Am. Chem. Soc.*, **90**, 7355 (1968).

**Table I.** Integrated and Normalized Ratios for Observed Carbon-13 Resonances in Selected Hydrocarbons

| | | Carbon atom | Integrated ratios[a] | Normalized ratios | Calculated normalized[b] ratios for best fit[c] | |
|---|---|---|---|---|---|---|
| Pyrene |  | C-1,3,6,8 | ~6.0 | 3.0 | 2.53 | $\chi = 0.06$ |
| | | C-2,7 | ~3.0 | 3.0 | | |
| | | C-4,5,9,10 | ~6.0 | 3.0 | | |
| | | C-11,12,13,14 | ~4.0 | 2.0 | 1.34 | |
| | | C-15,16 | ~1.0 | 1.0 | 1.00 | |
| Acenaphthene |  | C-1,2[d] | | | | |
| | | C-3,8 | 4.7 | 2.4 | 2.40 | $\chi = 0.10$ |
| | | C-4,7 | 4.7 | 2.4 | | |
| | | C-5,6 | 4.7 | 2.4 | | |
| | | C-9,10 | 2.4 | 1.2 | 1.20 | |
| | | C-11 | 1.0 | 1.0 | 1.00 | |
| | | C-12 | 1.3 | 1.3 | 1.27 | |
| Acenaphthylene |  | C-1,2 | 4.5 | 2.3 | | $\chi = 0.04$ |
| | | C-3,8 | 4.5 | 2.3 | 2.31 | |
| | | C-4,7 | 4.4 | 2.2 | | |
| | | C-5,6 | 4.4 | 2.2 | | |
| | | C-9,10 | 2.7 | 1.4 | 1.35 | |
| | | C-11 | 1.0 | 1.0 | 1.00 | |
| | | C-12 | 1.6 | 1.6 | 1.46 | |
| Fluoranthene |  | C-1,6 | 6.0 | 3.0 | 2.52 | $\chi = 0.06$ |
| | | C-2,5 | 6.0 | 3.0 | | |
| | | C-3,4 | 6.0 | 3.0 | | |
| | | C-7,10 | 6.0 | 3.0 | | |
| | | C-8,9 | 6.0 | 3.0 | | |
| | | C-11,12 | 2.4 | 1.2 | 1.20 | |
| | | C-13,14 | 2.4 | 1.2 | | |
| | | C-15 | 1.0 | 1.0 | 1.00 | |
| | | C-16 | 1.7 | 1.7 | 1.36 | |

[a] Average of not less than three independent spectral determinations. [b] Relative to lowest intensity peak in the spectrum. [c] The values given for $\chi$ yield best agreement between calculated and experimental enhancement factors. [d] Integral not determined.

hancement factor of 2.988 is theoretically possible wherever the carbon-13 relaxation mechanism is dominated by proton–carbon-13 dipole–dipole coupling. An enhancement factor of 2.98 ± 0.15 was measured for formic acid.[9] It was also suggested that since this factor is distance dependent ($1/r_{CH}^6$) other mechanisms (*e.g.*, interaction with residual $O_2$ or spin-rotational relaxation) will begin to compete whenever the carbon–hydrogen separation greatly exceeds that of a normal carbon–hydrogen bond. Consequently, a carbon atom not directly bonded to a proton is expected to give rise to a spectral line of lower intensity than the maximum possible from dipole–dipole relaxation.

When more than one proton contributes to the dipolar relaxation of the carbon atom, it can be shown from similar considerations to those of Solomon[8] that the intensity of a decoupled line obeys eq 1 provided all protons are completely decoupled;[10] in (1) the coefficient

$$\gamma_H^2\gamma_C^2\hbar^2\tau_c\alpha\left[(I - I_0) - \frac{1}{2}\frac{\gamma_H}{\gamma_C}I_0\right] = 0 \qquad (1)$$

$\alpha = \Sigma_i r_i^{-6}$, $I$ is the magnetization with decoupling and $I_0$ the magnetization in the absence thereof, and the ratio $\gamma_H/\gamma_C$ is 3.976. Equation 1 is identical with that derived in the two-spin case except for the summation over all carbon–hydrogen distances contained in $\alpha$. Relaxation mechanisms competing with the dipolar term contribute an additional term proportional to $(I - I_0)$. It is convenient, therefore, to express the competitive mechanisms in the same form as the above dipolar term as follows: $\gamma_H^2\gamma_C^2\hbar^2\tau_c\chi(I - I_0)$. The quantity $\chi$ (in the same units as $\alpha$) then measures the relative contribution of all relaxation mechanisms other than the dipolar one in units of the dipolar relaxation rate for two magnetic nuclei separated by a distance of 1 Å. When this empirical term is added to eq 1, the ratio of integrated intensities for a given carbon atom becomes

$$\frac{I}{I_0} = 1 + \frac{\gamma_H}{\gamma_C}\left(\frac{\alpha}{2(\alpha + \chi)}\right) \qquad (2)$$

Equation 2 predicts that the nuclear Overhauser enhancement of chemically nonequivalent carbon atoms in the same molecule will be dependent upon the carbon–hydrogen distances, as expressed in the coefficients $\alpha$, providing the following conditions obtain: (a) the correlation time, $\tau_c$, is the same for every carbon atom under consideration, (b) the value of $\chi$ does not vary significantly from carbon to carbon within a given molecule, and finally (c) changes in the magnitude of $\alpha$ are comparable to the value of $\chi$. Condition a is reasonable for rigid molecules of the type considered in this paper as internal molecular motion is minimized in these systems. The second assumption is less easily substantiated as $\chi$ may represent many different types of relaxation processes. As the chemical-shift range in the systems studied is relatively small it may be inferred that the electronic environments of the various carbon atoms are very similar. It would therefore be expected that $\chi$ would not vary greatly due to differing anisotropies in the electronic shielding terms. Similarly, random oscillating fields resulting from molecular diffusion should be reasonably constant over fairly large domains and this would yield similar $\chi$ values over the

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(10) K. F. Kuhlmann and D. M. Grant, manuscript in preparation.

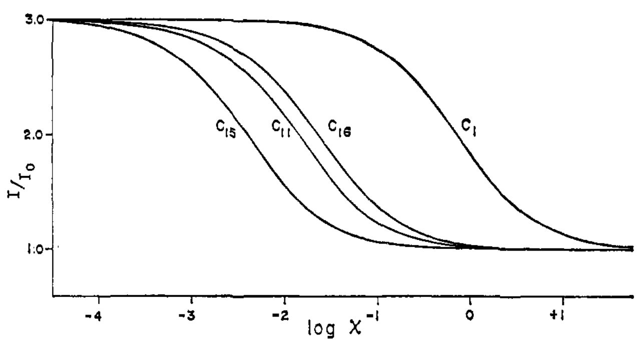

Figure 3. Plot of the Overhauser enhancement for the carbon atoms in fluoranthene as a function of log $\chi$.

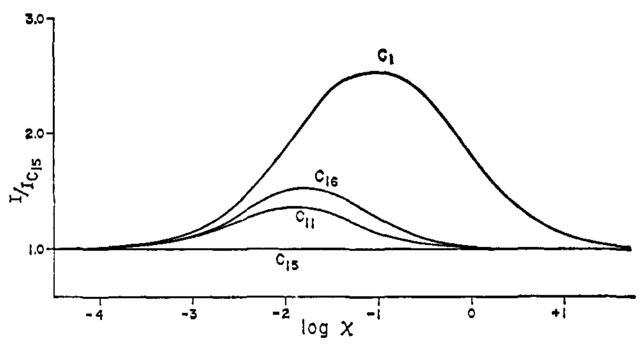

Figure 4. Plot of the calculated intensities of the spectral transitions relative to C-15 in fluoranthene.

whole molecule.[11] That condition c holds in any given instance can only be determined by comparing experimental and predicted values of $I/I_0$ for various values of $\chi$ and $\alpha$. This is done in detail in the following section for fluoranthene.

Values for $\alpha$ are obtained for the various carbon positions in fluoranthene by summing over all protons in the molecule. These quantities are summarized in Table II. The calculated $\alpha$ terms in fluoranthene are almost identical (~0.70) for all carbon atoms directly bonded to protons. The carbon atom C-1 is therefore taken as representative of this group. The quarternary carbon atoms clearly separate into three groups C-11–14, C-15, and C-16, as determined by their $\alpha$ values. A plot of the Overhauser enhancement for these nonequivalent carbon atoms as a function of $\chi$ over the range $\chi = 10^{-4}$ to $10^2$ is shown in Figure 3. It is apparent from this plot that the predicted Overhauser enhancement for the carbon atoms in fluoranthene follows the order C-1 > C-16 >C-11 >C-15. A plot of the normalized relative intensities using C-15 in fluoranthene as reference is given in Figure 4. Over the range $\chi$ = 0.05 to 0.10 the predicted relative intensities are in good agreement with the experimental values given in Table I assuming the correct peak assignments have been made. For $\chi$ = 0.06 the predicted intensities, taking account of populational weighting, are $I_1:I_{11}:I_{16}:I_{15}$ = 5.0:2.4:1.4:1.0, which compare favorably with the experimental values 6.0:2.4:1.7:1.0 taken from Table I.

Table II. Calculated $\alpha$ Values, Enhancement Factors, and Normalized Relative Intensities for Fluoranthene

| Carbon atom | $\Sigma\alpha_i^a$ | $I/I_0^b$ | $(I/I_0)_i/(I/I_0)_{\text{C-15}}$ |
|---|---|---|---|
| C-1,6 | 0.6933 | 2.82 | 2.52 |
| C-2,5 | 0.7019 | 2.82 | 2.52 |
| C-3,4 | 0.6951 | 2.82 | 2.52 |
| C-7,10 | 0.6936 | 2.82 | 2.52 |
| C-8,9 | 0.7023 | 2.82 | 2.52 |
| C-11,12 | 0.0132 | 1.35 | 1.20 |
| C-13,14 | 0.0135 | 1.35 | 1.20 |
| C-15 | 0.0040 | 1.12 | 1.00 |
| C-16 | 0.0221 | 1.52 | 1.36 |

[a] Ignoring contributions from protons greater than 5 Å from the carbon atom. [b] Calculated for a value of $\chi$ = 0.06.

An estimate of the variation in $\chi$ can be obtained by considering the sigmoid curves of Figure 3. The shape of these curves is independent of $\alpha$, but the inflection point occurs at $\chi = \alpha$. To represent a situation in which a different value of $\chi$ must be assigned to each carbon, one could consider that the origin of log $\chi$ is different for each curve. To effect an interchange between two curves characterized by $\alpha_i$ and $\alpha_j$ we must have $\log \chi_i/\chi_j = 2\Delta \log \chi = -2(\log \alpha_i - \log \alpha_j)$ where $\chi_i < \chi_j$ and $\chi_i/\chi_j = (\alpha_i/\alpha_j)^2$. Applying this result to fluoranthene, we find that to interchange the curves for C-11 and C-16 requires $\chi_{16}/\chi_{11}$ = 2.76. At the same time the predicted relative intensities would become 5.0:2.8:1.2:1.0, which would be in poor agreement with the experimentally determined intensities. Reversal of the assignments for C-15 and C-16, where a more reasonable intensity profile might be obtained, would require $\chi_{16}/\chi_{15}$ = 30. It is suggested that variations of this magnitude in the relative relaxation of chemically similar carbons by mechanisms such as spin-rotation interaction, chemical-shift anisotropy, or interaction with residual oxygen are too large to be reasonable. More reasonable variations of the order of several per cent on the other hand would not invalidate the assignment and in fact may account in part for the minor variations noted between experimental and predicted intensities.

Calculations of the relative enhancement factors in the other molecules are also given in Table I. These values were obtained from graphs similar to Figures 3 and 4 and as can be observed are in reasonable agreement with the experimental quantity. The limited scatter of $\chi$ values (av 0.07 ± 0.02) lends validity to the treatment. These values would be expected to have similar magnitudes for the molecules considered. The approximate calculations given in this treatment enable an estimate of the relative contribution to carbon-13 relaxation due to processes other than the dipolar mechanism. For $\chi$ = 0.07 these processes appear to relax a carbon atom about 10% as effectively as the dipolar mechanism arising from a directly bonded hydrogen atom.

We conclude that the normalized relative intensities of carbon-13 resonance signals can be predicted using geometrical arguments and consequently that the nuclear Overhauser effect is applicable in assigning carbon-13 resonances of the quarternary carbon atoms in complex polycyclic systems. Similar considerations may permit its use for assignment in other systems where

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(11) If paramagnetic substances are selectively adsorbed in one part of the molecule over another, it is expected that this assumption would no longer be valid.

carbons with no directly bonded protons are found. However, in each case one must be alert to variations in $\chi$. Further, an estimate of the contribution of the competing relaxation mechanisms can be obtained using this method provided enhancement data are available for a sufficient number of different carbon atoms in the molecule.

**Acknowledgment.** This work was supported primarily by Grant No. GM08521-08 from the National Institutes of Health.

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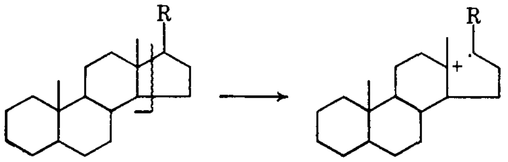
# Mass Spectrometry in Structural and Stereochemical Problems. CLXXVI.[1] The Course of the Electron Impact Induced Fragmentation of Androstane[2]

**L. Tökés[3] and Carl Djerassi**

*Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305.* *Received March 24, 1969*

**Abstract:** The fragmentation of the androstane skeleton differs drastically from that of C-17-substituted steroidal hydrocarbons such as cholestane or pregnane. The characteristic ring D fragmentation of the latter leading to peaks at *m/e* 217 and 218 is relatively unimportant in the absence of a C-17 substituent. Instead, androstane suffers appreciable fragmentation in ring A and through extensive deuterium labeling, the nature of this fragmentation—notably the course of a reciprocal hydrogen transfer—has been elucidated. Another striking difference between the mass spectrum of androstane and 17β-ethylandrostane (pregnane) is the origin of the expelled methyl radical associated with the characteristic M − 15 peak. Mechanistic rationalizations for the various fragmentation processes are presented as are synthetic details for the preparation of the various deuterated analogs.

Steroids possessing a saturated alkyl substituent at C-17, such as cholestane (I) or pregnane (II), undergo a very characteristic electron impact induced fragmentation of ring D, which is schematically illustrated by the wavy line in the structural formula. The precise course of this long-known fragmentation process has recently been elucidated[4] through extensive deuterium labeling and could best be rationalized through the intervention of the molecular ion a.



I, R = $C_8H_{17}$
II, R = $C_2H_5$
III, R = H

a

The presence of a double bond in various locations in the side chain (R in I) drastically affects[5] this ring D fragmentation, which is not surprising, since preferred charge localization at the double bond may be anticipated at the expense of contributions from species a. Work on the elucidation of the mass spectral fragmentation of these hydrocarbons was required to provide the basic information upon which the interpretation of the mass spectra of more highly substituted steroids rests. Consequently, we felt that a similar detailed study of the simplest steroidal hydrocarbon, androstane (III), was definitely warranted, especially since even a cursory examination[6] of its mass spectrum without any isotopic labeling indicated major differences between its fragmentation and that of cholestane (I). The presently described investigation involving extensive deuterium labeling proved well worth the effort, because in addition to defining in some detail the course of the major mass spectral fragmentation paths of the androstane skeleton, it uncovered two processes which are undetectable except through isotopic labeling. The first refers to the origin of the loss of a methyl radical from the molecular ion, which in the case of pregnane (II) had been found[4] to involve the C-19 angular methyl group[7] to the extent of 80%, but which in androstane (III) has now been found (see Table I) to proceed to a more nearly equal extent by elimination of either angular substituent. The second and even more interesting observation concerns the discovery of a reciprocal hydrogen transfer associated with fission of ring A, which is discussed in detail in the sequel.

## Synthesis of Deuterium-Labeled Analogs of 5α-Androstane

During the course of this study all hydrogen-bearing carbon atoms in 5α-androstane (III) except for positions 1, 11, and 15 were labeled with deuterium atoms

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[1] For paper CLXXV, see C. A. Brown and C. Djerassi, *J. Chem. Soc.*, in press.

[2] Financial assistance from the National Institutes of Health (Grant No. AM-12758) is gratefully acknowledged.

[3] Taken in part from the Ph.D. thesis of L. T.

[4] L. Tökés, G. Jones, and C. Djerassi, *J. Am. Chem. Soc.*, **90**, 5465 (1968).

[5] S. G. Wyllie and C. Djerassi, *J. Org. Chem.*, **33**, 305 (1968).

[6] H. Budzikiewicz and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1430 (1962).

[7] In this connection, attention should be called to a misprint in Table I of ref 4 in that the M − $CH_3$ peak in 19,19-$d_2$-5α-pregnane is distributed to the extent of 80/20% between *m/e* 273 and 275, rather than *m/e* 273 and 276. Another misprint in Table I of ref 4 refers to the *m/e* 219 peak in the 14α-$d_1$ analog, which by mistake is listed as *m/e* 218.