

**APPLICATION OF HOMONUCLEAR OVERHAUSER EFFECT TO MOLECULES WITH INTERNAL MOTION\***J. DĚDINA<sup>a</sup> and J. SCHRAML<sup>b</sup><sup>a</sup> *Institute of Physiology,  
Czechoslovak Academy of Science, 142 20 Prague 4 and*<sup>b</sup> *Institute of Chemical Process Fundamentals,  
Czechoslovak Academy of Science, 165 02 Prague 6 - Suchbát 2*

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The formal theory of the nuclear Overhauser effect (NOE) is extended to molecules undergoing isotropic reorientation and consisting of rigid fragments which move one relative to the other at an arbitrary rate. Numerical methods are described by which relaxation rates due to dipole-dipole interaction can be calculated for: 1) protons from two different fragments the relative motion of which is slower than the molecular reorientation, 2) a proton interacting with a methyl group jumping 120° at an arbitrary rate, and 3) a proton interacting with a freely rotating methyl group. The analytical expressions for the last of the interactions is derived here. Numerical experiments show that, in any real molecule, the relaxation rates due to dipole-dipole interaction of a proton with protons of a rotating methyl group can be estimated with a sufficient accuracy on the basis of only populations of two extreme conformations and internal rotation rate.

Using the concept of relaxationally equivalent nuclei a simplified phenomenological description of relaxation in multiproton system is achieved. Two methods, direct and indirect, of solving the set of equations describing the stationary case of NOE experiments are discussed from the point of view of the applicability of NOE measurements to structure elucidation.

The theoretical results are applied to NOE enhancements in model compounds. The agreement between the theory and experiment is satisfactory in the case of mesitylene and  $\alpha$ -methylstyrene. In other molecules studied which are  $\alpha,\beta$  unsaturated carbonyl derivatives, a discrepancy is observed on an interaction between  $\alpha$ -methyl group protons and *trans* olefinic proton. The calculated dipole-dipole relaxation rate for this type of interaction is considerably smaller than the experimental value in methacrylic acid, methyl methacrylate, methacroleine, and *cis* crotonaldehyde. It is suggested that this discrepancy is caused by a selfassociation which brings the protons into a close vicinity intermolecularly.

More than ten years have passed since Anet's and Bourn's first application of nuclear Overhauser effect (NOE) to structure determination<sup>1</sup>. Many papers dealing with NOE have appeared since. The approach to the interpretation of NOE experiments has, however, remained essentially the same as in the first pioneering work<sup>1</sup>. The efforts<sup>2-8</sup> to use the NOE values in a more quantitative way have been left isolated; the general acceptance of the quantitative approach to NOE being hampered by the lack of an NOE theory applicable to molecules in which an internal rotation is as fast or faster than the molecular reorientation.

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A way how to build such a theory was shown in the work of Rowan, Mc Cammon and Sykes<sup>9</sup>. Using Woessner's theoretical results for spin relaxation<sup>10</sup> and 120° random jumping model for the motion of a methyl group Rowan and coworkers<sup>9</sup> derived expressions relating interprotonic distances to NOE's between the protons of the rigid part of the molecule and of the methyl group rotating at an arbitrary rate.

Since the model employed by Rowan and coworkers<sup>9</sup> does not necessarily describe every methyl group motion we have derived analogous expressions for some other models of internal motion. The derived expressions are valid in the cases of fast internal motion. Their applicability will be demonstrated on a few examples.

### THEORETICAL

In the present work we are concerned with molecules in which no other internal motion is allowed but the motion of rigid fragments one relative to the other. It is assumed that in an ansamble of such molecules the overall reorientation of one of the fragments taken for the core of the molecule can be characterized by a molecular correlation time  $\tau_c$  (or by the set of  $\tau_c$ 's if the molecular motion is anisotropic). Similarly, the internal motion of a fragment relative to the core is described by an internal correlation time  $\tau_r$ . Depending on the relation of the internal correlation time  $\tau_r$  to the spin-lattice relaxation time  $T_1$  of a typical proton in the fragment the internal motion can be classified as:

1) very slow, if  $\tau_r \gg T_1$ , 2) intermediate rate, if  $\tau_r \sim T_1$ , and 3) fast, if  $\tau_r \ll T_1$ .

Two boundary cases of the fast motion are particularly important under the conditions of extreme narrowing. Namely, they are a) moderate fast (called moderate rate in ref.<sup>11</sup>) motion in the slow limit of the fast motion region when  $\tau_r \gg \tau_c$  and b) superfast motion taking place if  $\tau_r \ll \tau_c$ .

#### *Relaxation Rates and Molecular Structure*

The NOE theory which was derived and described in detail by Noggle and Schirmer<sup>11</sup> is applicable to molecules in which the internal motion is not faster than moderate fast. Under the usual assumptions (absence or neglect of scalar relaxation, cross correlation and intermolecular effects, extreme narrowing), the behaviour of the spin of a proton  $k$  interacting with other protons  $j$ 's in a rigid molecule containing  $N$  protons is governed by the equation

$$d\langle I_{zk} \rangle / dt = -R_k \{ \langle I_{zk} \rangle - I_{0k} \} - \sum_{j \neq k}^N \sigma_{kj} \{ \langle I_{zj} \rangle - I_{0j} \} \quad (1)$$

with the total direct relaxation rate  $R_k$  of the proton  $k$  given as

$$R_k = \sum_{j \neq k}^N \rho_{kj} + \rho_k^* \quad (2)$$

In a rigid molecule, the direct ( $\varrho_{kj}$ ) and cross ( $\sigma_{kj}$ ) dipole-dipole relaxation rates between the protons  $k$  and  $j$  are related to the correlation time  $\tau_c$  of molecular reorientation and interprotonic distance  $r_{kj}$

$$\varrho_{kj} = \varrho_{jk} = K \tau_c (kj) G_{kj} , \quad (3)$$

$$K = \gamma^4 \hbar^2 , \quad (4)$$

$$G_{kj} = r_{kj}^{-6} , \quad (5)$$

$$\sigma_{kj} = \sigma_{jk} = \frac{1}{2} \varrho_{jk} . \quad (6)$$

(The symbols have their standard meanings explained in detail in ref.<sup>11</sup>).

In the case of very slow internal motion, the expectation values  $\langle I_{zk}(\beta) \rangle$  of individual conformers (or rotamers)  $\beta$  appear mutually independent and hence the set of Eqs (1)–(6) can be applied to each of them.

Under the conditions of intermediate rate internal motion, the  $\langle I_{zk}(\beta) \rangle$  of individual conformers are not independent but, also, they are not identical. The general equation (1) with additional terms for the spin transfer holds but a general solution which would be valid under the experimental NOE conditions is not available; each particular case of motion has to be treated separately, an example of a solution for a two-proton system was described by Combrisson and coworkers<sup>12</sup>.

In the fast motion region, however, the expectation value  $\langle I_{zk}(\beta) \rangle$  of a conformer  $\beta$  is related to the average observable value  $\langle I_{zk} \rangle$

$$\langle I_{zk}(\beta) \rangle = P_\beta \langle I_{zk} \rangle ,$$

where  $P_\beta$  is the population of the  $\beta$  conformer. This expression, though derived<sup>13</sup> for the moderate fast motion, obviously applies equally to all faster internal motions. It follows from this relation that unless some outside knowledge of the conformer populations is available, one can consider the average value  $\langle I_{zk} \rangle$  only. The motion of the average value  $\langle I_{zk} \rangle$  of the proton  $k$  is described by Eqs (1)–(6); the parameters occurring in these equations being also the motional averages. In deriving the average  $G_{kj}$  value we can follow Rowan and coworkers<sup>9</sup> and use the expressions derived by Woessner<sup>10</sup> for the dipole-dipole relaxation of two 1/2 spins in a molecule with an internal motion (independent of the overall reorientation of the molecule). In the case of isotropic reorientation, which is assumed here similarly as in ref.<sup>9</sup>, the extreme narrowing condition considerably simplifies the general expressions<sup>9,10</sup> into the form

$$G_{kj} = (1/2\tau_c) \int_0^\infty \langle A_{\beta\gamma} \rangle_\tau e^{-\tau/\tau_c} . d\tau , \quad (7)$$

$$A_{\beta\gamma} = [3(l_\beta l_\gamma + m_\beta m_\gamma + n_\beta n_\gamma)^2 - 1] \cdot r_{kj}^{-3}(\beta) r_{kj}^{-3}(\gamma). \quad (8)$$

In these expressions indices  $k$  and  $j$  denote any two protons in the molecule; their interprotonic separation vector  $\mathbf{r}_{kj}$  is given by its magnitude,  $r_{kj}$ , and direction cosines  $l$ ,  $m$ , and  $n$  in the frame of reference firmly connected with the molecular core. The quantities marked by index  $\gamma$  refer to those values which exist after time  $\tau$  from the time when the values marked by  $\beta$  persisted.

In order to take the internal motion into the account, the quantity  $A_{\beta\gamma}$  has to be averaged (as denoted in Eq. (7) by the angular brackets) over all the initial and final values of the internuclear vector that are allowed by the internal motion. The average depends on the type and rate of the internal motion. We shall consider several cases of the internal motion.

The trivial case of  $\mathbf{r}_{kj} = \text{const.}$  in which the two protons are from the molecular core, serves as a check of the theory. Equation (7) simplifies in such cases into Eq. (5) which was derived for rigid molecules.

The averaging of  $A_{\beta\gamma}$  term at time  $\tau$  can be expressed as

$$\langle A_{\beta\gamma} \rangle_\tau = \sum_{\beta} P_{\beta} \sum_{\gamma} A_{\beta\gamma} p(\beta - \gamma, \tau), \quad (9)$$

where  $p(\beta - \gamma, \tau)$  is the relative probability that conformer  $\beta$  changes into conformer  $\gamma$  in time  $\tau$ . Only such values of  $\langle A_{\beta\gamma} \rangle_{\tau'}$  contribute to the integral in Eq. (7) that are associated with time  $\tau'$  not much longer than  $\tau_c$ .

For moderate fast motion the relative probability is  $p(\beta - \gamma, \tau') = \delta_{\beta\gamma}$  ( $\delta$  is Kronecker delta). Therefore  $\langle A_{\beta\gamma} \rangle_{\tau'} = \sum_{\beta} P_{\beta} A_{\beta\beta}$  and Eqs (7) and (8) give

$$G_{kj} = \sum_{\beta} P_{\beta} r_{kj}^{-6}(\beta), \quad (10)$$

which is equivalent to that derived by Noggle and Schirmer<sup>13</sup> for this type of motion. It should be noted that Eq. (10) holds both for protons  $k$  and  $j$  from the same or from two different fragments. In the former case the expression (10) reduces into simple relation (5) in accord with the physical picture.

When the motion is faster than moderate fast it is no longer sufficient to consider populations of the conformers only but the actual form of motion must be taken into the account in averaging  $A_{\beta\gamma}$ . We shall restrict our discussion to two limiting models of internal motion: the rotation by  $360^\circ/L$  random jumps and the free rotation. For the brevity the two models will be called J- and F-models, resp., here.

J-model assumes that the rotating fragment takes with the same probability  $L$ -different positions separated by  $360^\circ/L$  and that the probabilities of the jumps from any of the positions into any other position are the same. Generalizing the expressions

of ref.<sup>10,14</sup> the model can be mathematically described by the following equations

$$p(\tau) = 1/L + ((L-1)/L) e^{-\tau/\tau_r},$$

$$p_1(\tau) = 1/L - (1/L) e^{-\tau/\tau_r}$$

in which  $p(\tau)$  is the probability that the rotating fragment is at the time  $\tau$  at the same position as in time  $\tau = 0$  and  $p_1(\tau)$  is the probability that the fragment is in some other position in time  $\tau$  than at  $\tau = 0$ .

F-model in which the rotating fragment passes through a very large number of equilibrium positions is simulated here by a stochastic diffusion rotation. In such a rotation the probability of the rotational position characterized by the torsional angle  $\alpha$  is independent of the angle  $\alpha$ . The probability that the torsional angle would change by angle  $\Delta$  is given by the Gaussian distribution<sup>14</sup>

$$p(\Delta, \tau) = (\tau_r/4\pi\tau)^{1/2} e^{-\Delta^2\tau_r/4\tau}.$$

Using these two models, interactions between protons from different molecular fragments can be treated. We shall consider here the following combinations: a) one proton from the molecular fragment rotating fast relatively to the core to which the other proton is attached, b) one proton from a fast rotating fragment and the other proton from another fragment rotating moderate fast (both relatively to the core), and c) both protons in fast rotating fragments.

a) *Interaction between the proton from the fast rotating fragment and the proton from the core.* In J-model the average takes the form

$$\begin{aligned} \langle A_{\beta\gamma} \rangle_\tau &= (1/L)^2 \{S_0 + S_1 + [(L-1)S_0 - S_1] e^{-(\tau/\tau_r)}\}, \\ S_0 &= \sum_{\beta} A_{\beta\beta} = 2 \sum_{\beta} r_{kj}^{-6}(\beta), \\ S_1 &= \sum_{\beta} \sum_{\gamma \neq \beta} S_{\beta\gamma}, \end{aligned} \quad (11)$$

where indices denote individual positions of the fragment rotating by  $360^\circ/L$  jumps. After substitution into Eq. (7) we receive

$$G_{kj} = (1/2L^2) \{S_0 + S_1 + [(L-1)S_0 - S_1] \tau_p/(\tau_p + 1)\}, \quad (12)$$

where  $\tau_p = \tau_r/\tau_c$ . In the low frequency limit of this motion (*i.e.* moderate fast) the expression (12) reduces to a special form of expression (10). Equation (12) can be

rewritten as the following combination of the two limiting cases

$$G_{kj} = G_{kj}(\text{super}) + (\tau_p/(\tau_p + 1))(G_{kj}(\text{moderate}) - G_{kj}(\text{super})). \quad (13)$$

According to F-model the average is given as

$$\langle A_{\alpha\alpha'} \rangle_\tau = (1/2\pi) \int_{\alpha=0}^{2\pi} \int_{\Delta=-\infty}^{+\infty} A_{\alpha\alpha'} p(\Delta, \tau) \cdot d\Delta \cdot d\alpha \quad (14)$$

for  $\alpha' = \alpha + \Delta$ .

As the integrals of the type  $\int_0^\infty [\exp\{ax + bx^{-1}\}] x^{-1/2} dx$  cannot be expressed in analytical form<sup>15</sup> the  $G_{kj}$  values must be obtained by numerical integration of the formula

$$G_{kj} = (\tau_p/8\pi) \int_{x=0}^{\infty} \exp\{-x\tau_p\} \int_{\alpha=0}^{2\pi} \int_{\Delta=-\infty}^{+\infty} A_{\alpha\alpha'} \exp\{-\Delta^2/4x\} (\pi x)^{-1/2} \cdot d\Delta \cdot d\alpha \cdot dx \quad (15)$$

$$(x = \tau/\tau_p).$$

Also in this model the formula (15) reduces in the limiting case of the medium fast rotation into a special example of relation (10).

b) *Interaction between the proton of a fast rotating fragment and the proton of a moderate fast rotating fragment.* Since we assume that the motions of the two fragments are uncorrelated, the averaging over the two motions can be done independently, *i.e.*

$$\langle A_{\beta\gamma} \rangle_\tau = \langle \langle A_{\beta\gamma} \rangle^I \rangle^{II}$$

The angular brackets  $\langle \rangle^I$  and  $\langle \rangle^{II}$  indicate averaging over the motion of the fast rotating fragment *I* and moderate fast rotating fragment *II*, resp. If the conformers of the fragment *II* are labeled by the index  $\delta$  and have relative populations  $P_\delta$  the above averaging can be expressed as

$$\langle A_{\beta\gamma} \rangle_\tau = \sum_{\delta} P_\delta \langle A_{\beta\gamma}^\delta \rangle_\tau^I,$$

where  $A_{\beta\gamma}^\delta$  is the value of  $A_{\beta\gamma}$  in rotamer  $\delta$  of the fragment *II*. Substitution into

Eq. (7) gives

$$G_{kj} = (1/2\tau_c) \sum_{\delta} P_{\delta} \int_0^{\infty} \langle A_{kj}^{\delta} \rangle^2 e^{-\tau/\tau_c} \cdot d\tau,$$

which can be written in the form

$$G_{kj} = \sum_{\delta} P_{\delta} G_{kj}^{\delta}. \quad (16)$$

According to these equations the  $G_{kj}$  value is the weighted average of  $G_{kj}^{\delta}$  values of rotamers of the moderate fast rotating fragment. The  $G_{kj}^{\delta}$  values can be calculated according to J- or F-models of fast motion taking the conformation of the rotamer  $\delta$  for the core.

c) *Interaction between protons of fast rotating fragments.* If the two protons are from two different fast rotating fragments their relative motion is so complicated that a general treatment of their relaxation is beyond of our present discussion. If, however, they are both from the same fragment the treatment is simple especially if we limit to the most usual case of the plane of vectors  $r$  perpendicular to the internal rotation axis. Then the angle  $\Delta_{\beta\gamma}$  is equal to the angle rotated by the whole fragment in the same time and Eq. (8) takes the form

$$A_{\beta\gamma} = (3 \cos^2 \Delta_{\beta\gamma} - 1) r_{kj}^{-6}.$$

Averaging according Eqs (12) and (15) leads to expressions for J-model

$$G_{kj} = (r_{kj}^{-6}/4) (1 + 3\tau_p(1 + \tau_p)) \quad (17)$$

and for F-model

$$G_{kj} = (r_{kj}^{-6}/4) (1 + 3\tau_p/(4 + \tau_p)). \quad (18)$$

Such relations can be also directly derived from the equations of Woessner<sup>14</sup>.

### *Relaxation Rates and NOE Experiments*

The relations derived above give the dependence of the  $G$  value (and hence of the relaxation rate) on the interprotonic distance and on the rate of its change. Through these relations interprotonic distances can be evaluated from relaxation rates in the molecule. The relaxation rates are related to the directly observable quantities through Eq. (1).

In principle, for a molecule with  $N$  protons a set of  $N$  Eq. (1) has to be solved. Since for a real molecule the set of equations becomes rather large some simplification would be useful.

The number of the equations can be reduced if the nuclei (protons) in the molecule can be divided into classes of relaxationally equivalent nuclei. From the point of view of NOE theory, two nuclei are relaxationally equivalent if their average values of  $\langle I_z \rangle$  are the same through the whole NOE experiment. In practice the nuclei are relaxationally equivalent if they are equivalent because of symmetry or because of effective symmetry brought about by fast internal rotation. If there are  $m_K$  relaxationally equivalent nuclei in the class  $K$  each with the expected value  $\langle I_{zKk} \rangle$  then the total observable value of the class  $K$  is

$$\langle I_{zK} \rangle = m_K \langle I_{zKk} \rangle.$$

This leaves the number of equations to be solved equal to the number  $n$  of classes of equivalent protons in the molecule. The equations can be further simplified if the quantity

$$f_k = f_K = \frac{\langle I_{zK} \rangle - I_{0K}}{I_{0K}}$$

is introduced into Eq. (1). Then, after some manipulation, we have a set of  $n$  equations

$$df_k/dt = -R_K f_k - 0.5 \sum_{j \neq K}^n m_j R_{Kj} f_j, \quad (19)$$

where

$$R_K = \sum_{j \neq K}^n m_j R_{Kj} + (3/2) R_{KK} + \varrho_K^*, \quad (20)$$

$$R_{Kj} = (1/m_j) \sum_{j=1}^{m_j} \varrho_{KkKj} = R_{jK}, \quad (21)$$

$$R_{KK} = \sum_{k \neq j}^{m_K} \varrho_{KkKj}. \quad (22)$$

(For a proton  $k$  from the class  $K$ ,  $R_k = R_K + 0.5R_{KK}$ ). The indices  $k$  and  $j$  run through the protons of the class denoted by the preceding class index in capital letter. The class relaxation rates  $R_K$ ,  $R_{Kj}$ ,  $R_{KK}$ , and  $\varrho_K^*$  do not depend on the choice of the proton  $k$  within the class  $K$  (in contrast,  $\varrho_{jKk}$  is not a class property).



The concept of relaxationally equivalent nuclei as defined here is broader than the concept of a group of equivalent nuclei as used by Noggle and Schirmer<sup>16</sup> since the relaxational equivalence does not require *e.g.* all  $\rho_{jk}$  rates within a class (*i.e.*  $\rho_{jk}$  for all relaxationally equivalent nuclei  $j$  and  $k$ ) to be the same.

One of the methods by which the relaxation rates can be determined experimentally is the measurement of NOE enhancements. Under the conditions of NOE experiment in which the spins of the classes  $S$  are saturated, the steady-state solution of the set of Eq. (19) gives the Overhauser effect observed on the protons of other classes. If we denote  $f_k(S)$  the steady-state Overhauser effect (*i.e.* the steady-state value of  $f_k$  when all protons of classes  $S$  are being saturated), then the steady-state solution of Eq. (19) can be written

$$2R'_K f_k(S) + \sum_{J \neq K}^n m_J R'_{KJ} f_J(S) = 0, \quad (23)$$

where the relative relaxation rates  $R'$  are proportional to the class relaxation rates  $R$ . For isotropic motion it is particularly convenient to take the proportionality constant equal to  $K\tau_c$  (Eq. (4)), then, the  $R'$  values are given as

$$R'_K = R_K/K\tau_c = \sum_{J \neq K}^n m_J R'_{KJ} + (3/2) R'_{KK} + G_K^*, \quad (24)$$

$$R'_{KJ} = R_{KJ}/K\tau_c = (1/m_J) \sum_j^{m_J} G_{Kk_j} = R'_{JK}, \quad (25)$$

and

$$R'_{KK} = R_{KK}/K\tau_c = \sum_{k \neq j}^{m_K} G_{Kk_j}. \quad (26)$$

The relaxation of a molecule containing  $n$  classes is described by  $n(n+1)/2$  different  $R'$  values. If all of them are unknown, at least  $n(n+1)/2(n-1)$  simple NOE experiments (each giving the enhancements for  $(n-1)$  classes) must be performed in order to determine the set (23) fully. Clearly, NOE experiments alone cannot yield sufficient number of equations in the case of a molecule with two classes of protons; for three classes, all possible simple NOE experiments must be carried out but for a higher number of classes it is not necessary to measure all NOE's.

Solutions for particular spin systems have been given in literature<sup>9,11</sup>. In general, due to the nature of Eq. (23) it is necessary to combine the NOE data with some other molecular data (which are available either from an experiment or can be reasonably estimated) in order to obtain the values of all the pertinent relative relaxation rates even when the sufficient number of NOE experiments has been

performed. If  $G$  values are needed for structure determination and if their number exceeds that of  $R'$  values additional assumptions must be made. There are two possible methods, direct and indirect, by which Eq. (23) can be solved.

In the direct method, the NOE data ( $f_k(S)$  values) are utilized in Eq. (23) to solve this set of equations for the unknown  $R'$  values. After trivial rearrangements the set of linear homogeneous equations (23) can be written in the form

$$\mathbf{Ax} = \mathbf{0}. \quad (27)$$

Since the physical meaning of the unknowns (the relaxation rates) does not allow for the trivial solution ( $\mathbf{x} = \mathbf{0}$ ) it must hold  $\det \mathbf{A} = 0$  (if the matrix  $\mathbf{A}$  is a square matrix or  $\det \mathbf{A}^T \mathbf{A} = 0$  if the solution is sought in the least-square sense for a larger number of NOE experiments than necessary). This condition puts the precision and correctness of the NOE experiments as well as the underlying application of the general theory at a test. Though the condition can be satisfied for the experiments involving saturation of some classes it might not be so for others and hence erroneous experiments might be detected. As the general solution of the Eq. (27) has the form

$$\mathbf{x} = \sum_{i=1}^q a_i \mathbf{x}^{(i)},$$

(where  $q$  is the nullity of the matrix  $\mathbf{A}$  (or  $\mathbf{A}^T \mathbf{A}$ ) and  $\mathbf{x}^{(i)}$  is  $i$ -th linearly independent solution) the other data are needed in order to find a meaningful set of  $a_i$ 's. The data should yield  $q$  different relaxation rates so that all  $a_i$  coefficients could be determined.

For example if the structure of the molecule is partially known, a relaxation rate  $R'_{kj}$  can be evaluated or, if some relaxation time  $T_1(j)$  and correlation time  $\tau_c$  are known, the relaxation rate  $R'_j$  can be evaluated and the obtained values used in the substitution. Such direct method was utilized in the works of Schirmer and coworkers<sup>2</sup> and Rowan and coworkers<sup>9</sup>. Other approaches are also possible. Thus, some known  $R'_{kj}$  values can be combined with  $R_k$  data (obtained from relaxation times) and  $1/K\tau_c$  and the remaining  $R'_{kj}$  values are obtained by solving Eqs (23)–(26).

In the indirect method, the  $R'$  values are calculated first for possible structures of the molecule and then the true structure is found by comparing the experimental NOE enhancements with those calculated from the  $R'$  values according to Eq. (23). Since in this method the Eq. (23) is treated for unknown  $f_k(S)$ 's and since by definition all  $f_S(S) = -1$ , the Eq. (23) takes then the form of a set of non-homogeneous linear equations

$$\mathbf{Bx} = \mathbf{y},$$

where the vector of unknowns is an arranged set of  $f_k(S)$  and the right hand side

vector  $\mathbf{y}$  has  $\sum_{s=1}^l m_s R'_{KS}$  values as its components (where  $l$  is the number of saturated classes). The indirect method was employed in solving the problem of conformation of some nucleosides<sup>3-6</sup>.

Each of the methods has its advantages and disadvantages which determine the choice of the method most convenient for a specific application. Undoubtful disadvantage of the indirect method is that knowledge of the value of the relative independent relaxation rate  $G_K^*$  is required for all the involved classes in addition to the structure of the molecule and to the types of internal motions. Usually, this difficulty is overcome by additional assumption that either the independent relaxation can be neglected<sup>4,7</sup> or that it is the same<sup>3</sup> for all the classes considered. No additional assumptions concerning  $G_K^*$  are required if the direct method is applied but it is necessary to measure NOE values for a relatively large number of proton classes. The indirect method is, however, capable of providing some structural information even if the system of Eq. (23) is not fully determined for solving it by the direct method.

## RESULTS AND DISCUSSION

In order to test the applicability of the theory treated above and to illustrate its use we have searched the literature for NOE studies of the molecules the structure of which was known to some extent and in which, at the same time, several NOE values were determined. Only investigations of methyl methacrylate<sup>17</sup>, methacroleine<sup>18</sup>, and *cis* and *trans*-crotonaldehyde<sup>9</sup> have met these requirements and therefore the data had to be supplemented by our measurements of some additional model compounds (mesitylene, methacrylic and  $\beta,\beta$ -dimethylacrylic acids, and  $\alpha$ -methylstyrene).

### *Derivation of Relaxation Rates from NOE Experiments*

*The direct method.* Mesitylene is a good model for NOE studies; its geometry can be reasonably estimated, the methyl group is practically free rotating, the internal rotation being much faster than the overall molecular tumbling<sup>19</sup>, it can be approximated by the superfast free rotation. With the two classes of protons present in the molecule (class A — aromatic protons (3) and class M — methyl group protons (9)) two different Overhauser enhancements could be measured; their experimental values

$$f_A(M) = 0.385 \pm 0.013$$

and

$$f_M(A) = 0.050 \pm 0.007$$

should fit two equations (23). According to Eqs (24)–(26) the following  $R'$  values have to be evaluated:

$$R'_{AM} = (1/3)(2G_{ortho} + G_{para})$$

$$R'_A = 3(2G_{ortho} + G_{para}) + 3G_{aa} + G_A^*$$

$$R'_M = (2G_{ortho} + G_{para}) + 3G_{mm} + 9G_{mm'} + G_M^*$$

where  $G_{ortho}$  and  $G_{para}$  are the  $G$  values due to the interactions of a methyl group proton with the aromatic proton in the *ortho* and *para* position, resp. The relative relaxation rate  $G_{mm}$  is caused by the interaction of two protons within the same methyl group while  $G_{mm'}$  is that between protons of different methyl groups. Obviously, so many  $G$  values cannot be calculated from two Eqs (23). The  $G_{aa}$  and  $G_{mm}$  values can be calculated from the interprotonic distances (which are  $r_{aa} = 4.28 \text{ \AA}$  and  $r_{mm} = 1.78 \text{ \AA}$ ) and formulas (5) and (18), resp. The  $G_{mm'}$  value can be neglected. (According to an approximate calculation,  $G_{mm'}$  is by at least two orders of magnitude smaller than the  $G_{mm}$  value.) This leaves two equations with three unknowns ( $2G_{ortho} + G_{para}$ ),  $G_A^*$  and  $G_M^*$ :

$$3(2G_{ortho} + G_{para})(2f_A(M) - 1) + 2G_A^* f_A(M) = -6(4.28)^{-6} f_A(M),$$

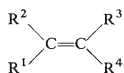
$$(2G_{ortho} + G_{para})(2f_M(A) - 1) + 2G_M^* f_M(A) = -6(1.78)^{-6} f_M(A)/4.$$

These equations can be solved if some additional relation among the unknowns is assumed. The solutions for three usual assumptions are given below:

Assumption	Unknown $10^{-57}, \text{ m}^{-6}$		
	$(2G_{ortho} + G_{para})$	$G_M^*$	$G_A^*$
$G_M^* = G_A^*$	$2.8 \pm 0.4$	$2.1 \pm 0.5$	$2.1 \pm 0.5$
$G_M^* = 0$	$2.6 \pm 0.4$	0	$1.9 \pm 0.4$
$G_A^* = 0$	$0.5 \pm 0.7$	$-19 \pm 5$	0

Clearly the direct method yields correct results with  $G_M^* = 0$  or with  $G_M^* = G_A^*$ ; the actual value of  $(2G_{ortho} + G_{para})$  being only little affected by the value of  $G_M^*$ . Since the theoretical calculation for superfast rotation of the methyl groups gave  $(2G_{ortho} + G_{para}) = 2.60 \cdot 10^{57} \text{ m}^{-6}$  the agreement between the theory and experiment is very satisfactory in this case in which the structure of the molecule is known with a sufficient accuracy. This justifies application of the theory to molecules with less known structure.

The NOE enhancements observed in some ethene derivatives (Scheme 1) are listed in Table I, the geometry of some of these compounds is known from crystallographic



	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>
<i>I</i> (methacrylic acid)	H	H	CH <sub>3</sub>	COOH
<i>II</i> (methacroleine)	H	H	CH <sub>3</sub>	CHO
<i>III</i> (methyl methacrylate)	H	H	CH <sub>3</sub>	COOCH <sub>3</sub>
<i>IV</i> ( <i>cis</i> -crotonaldehyde)	CH <sub>3</sub>	H	H	CHO
<i>V</i> ( <i>trans</i> -crotonaldehyde)	H	CH <sub>3</sub>	H	CHO
<i>VI</i> ( $\beta,\beta$ -dimethylacrylic acid)	CH <sub>3</sub>	CH <sub>3</sub>	H	COOH
<i>VII</i> ( $\alpha$ -methylstyrene)	H	H	CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub>

SCHEME 1

TABLE I  
Nuclear Overhauser Enhancements  $f_D(S)$  for Ethene Derivatives

Compound	<i>I</i> <sup>a</sup>	<i>II</i> <sup>b</sup>	<i>II</i> <sup>c</sup>	<i>III</i> <sup>c</sup>	<i>IV</i> <sup>f</sup>	<i>V</i> <sup>f</sup>	<i>V</i> <sup>f</sup>	<i>VI</i> <sup>a</sup>	<i>VII</i> <sup>a</sup>	
<i>D</i> <sup>g</sup>										
<i>S</i> <sup>h</sup>										
1	2	0.314 ± 0.014 <sup>i</sup>	0.42	0.28	0.48	0.29	0.015	0.116	— <sup>j</sup>	— <sup>j</sup>
	3	−0.003 ± 0.026	0.00	0.06	0.01	0.06	0.015	−0.020	0.020 ± 0.020	−0.021 ± 0.011
	4	— <sup>k</sup>	0.07	0.06	0.00	0.05	0.047	0.174	— <sup>j</sup>	0.079 ± 0.011 <sup>l</sup>
2	1	0.357 ± 0.020	0.39	0.30	0.42	0.28	0.186	0.006	— <sup>j</sup>	— <sup>j</sup>
	3	0.076 ± 0.017	0.09	0.09	0.09	0.09	0.233	0.038	0.048 ± 0.009	0.065 ± 0.010
	4	— <sup>k</sup>	0.00	0.00	0.00	0.05	0.008	−0.021	— <sup>j</sup>	−0.026 ± 0.009 <sup>l</sup>
3	1	0.000 ± 0.005	— <sup>k</sup>	— <sup>k</sup>	0.00	0.01	0.038	0.040	— <sup>j</sup>	0.004 ± 0.010
	2	0.053 ± 0.010	— <sup>k</sup>	— <sup>k</sup>	0.01	0.02	0.328	0.242	0.365 ± 0.012	0.055 ± 0.010
	4	— <sup>k</sup>	— <sup>k</sup>	— <sup>k</sup>	0.00	0.04	0.044	0.024	— <sup>j</sup>	0.027 ± 0.011 <sup>l</sup>
4	1	— <sup>j</sup>	— <sup>k</sup>	— <sup>k</sup>	0.00	0.02	0.326	0.257	— <sup>j</sup>	0.021 ± 0.005 <sup>l</sup>
	2	— <sup>j</sup>	— <sup>k</sup>	— <sup>k</sup>	0.00	0.02	0.013	0.001	— <sup>j</sup>	−0.003 ± 0.019 <sup>l</sup>
	3	— <sup>j</sup>	— <sup>k</sup>	— <sup>k</sup>	0.00	0.06	0.015	0.023	— <sup>j</sup>	0.034 ± 0.010 <sup>l</sup>

<sup>a</sup> This work; <sup>b</sup> ref.<sup>18</sup>, measured in 5% solution in dimethyl sulphoxide-d<sub>6</sub>, error not given; <sup>c</sup> ref.<sup>18</sup>, measured in 5% solution in CS<sub>2</sub>, error not given; <sup>d</sup> ref.<sup>17</sup>, measured in 10% solution in CS<sub>2</sub>, error ±0.01; <sup>e</sup> ref.<sup>17</sup>, measured in neat compound, error ±0.01; <sup>f</sup> ref.<sup>9</sup>, measured in 0.6M acetone-d<sub>6</sub> solution of 24 : 76 *cis* : *trans* mixture, error ±0.02; <sup>g</sup> *D*-detected proton class, labelling according to Scheme 1; <sup>h</sup> *S*-saturated proton class, labeling according to Scheme 1; <sup>i</sup> for the details see Experimental; <sup>j</sup> could not be measured with sufficient accuracy; <sup>k</sup> not measured; <sup>l</sup> total NOE for all the classes of benzene ring protons.

studies<sup>20,21</sup> and, owing to the rigidity of the C=C double bond, it can be estimated in others. In contrast to the above case of mesitylene the rates of internal rotations are not known for compounds *I-III*, *VI* and *VII*.

Sufficient number of NOE enhancements was determined for each of these compounds except for  $\alpha$ -methylstyrene and  $\beta,\beta$ -dimethylacrylic acid. The treatment of the NOE data is exemplified here on the case of methacrylic acid (*I*) which is dealt with as a molecule with three classes of protons since the line of the carboxylic proton is too broad to allow a measurement of its NOE. Necessarily, the relaxation of the other protons *via* the carboxylic one had to be included into  $G^*$  values.

In solving Eq. (23) the matrix **A** (Eq. (27)) takes the following form for methacrylic acid:

$$\mathbf{A} = \begin{pmatrix} -1 & 3f_3(1) & 0 & 2f_2(1) & 0 & 0 \\ f_1(3) & -3 & 0 & 2f_2(3) & 0 & 0 \\ -1 & 0 & 3f_3(2) & 0 & 2f_1(2) & 0 \\ f_2(3) & 0 & -3 & 0 & 2f_1(3) & 0 \\ 0 & -1 & f_1(2) & 0 & 0 & 2f_3(2) \\ 0 & f_2(1) & -1 & 0 & 0 & 2f_3(1) \end{pmatrix}$$

if the vector  $\mathbf{x}$  (in the transposed form  $\mathbf{x}^T$ ) is

$$\mathbf{x}^T = (R'_{21}, R'_{23}, R'_{13}, R'_2, R'_1, R'_3),$$

where the indices 1, 2, and 3 refer to the protons as labeled in Scheme 1.

TABLE II

Relative Relaxation Rates Calculated from NOE in Ethene Derivatives

Compound	$R' \cdot 10^{-57}, \text{m}^{-6}$				
	$R'_{13}$	$R'_{23}$	$R'_1$	$R'_2$	$R'_3$
<i>I</i> <sup>a</sup>	0.52 ± 0.02	1.63 ± 0.02	37.0 ± 0.1	32.6 ± 0.1	13.8 ± 0.6
<i>II</i> <sup>b</sup>	0.71 ± 0.04	1.78 ± 0.05	27.5 ± 0.2	29.8 ± 0.2	—
<i>II</i> <sup>c</sup>	2.4 ± 0.1	3.2 ± 0.1	41.3 ± 0.6	38.5 ± 0.5	—
<i>III</i> <sup>d</sup>	0.85 ± 0.04	1.75 ± 0.04	24.2 ± 0.1	27.8 ± 0.1	67 ± 6
<i>III</i> <sup>d,e</sup>	0.85 ± 0.03	1.74 ± 0.03	—	—	—

<sup>a</sup> An incorrect value of  $f_1(2)$  (Table I) would affect only  $R'_1$  value, e.g.  $f_1(2) = 0.40$  gives  $R'_1 = 29.0 \pm 0.1$  and the other  $R'_k$  and  $R'_{kj}$  remain within the indicated errors; <sup>b</sup> based on the data obtained in dimethyl sulphoxide solution and  $f_3(1) = 0.01$  and  $f_3(2) = 0.1$ ; <sup>c</sup> based on the data obtained in carbon disulphide solution and  $f_3(1) = 0.01$  and  $f_3(2) = 0.014$ ; <sup>d</sup> based on the data obtained in carbon disulphide solution; <sup>e</sup> calculated from NOE and  $T_1$  data.

In the view of the mentioned rigidity of the ethylene fragment and since this fragment is common to all the considered compounds, it appeared that substitution of the value of  $R'_{12}$  calculated from the interprotonic distance between the geminal olefinic protons (1.87 Å) would serve the purpose of this study best. After substituting  $R'_{12} = (1.87 \cdot 10^{-10})^{-6} \text{ m}^{-6}$  the resulting set of equations was solved by least-squares method. The solutions for methacrylic acid (I) are given in Table II together with the solutions for methacroleine (II) and methyl methacrylate (III) which were obtained analogously. Specifically, the same above value of  $R'_{12}$  was employed and it was assumed that the interactions of proton classes 1, 2, and 3 with proton 4 was adequately treated if these interactions were included into  $G^*$  terms only. The latter assumption being justified by the small NOE enhancements involving proton 4. In the particular case of methacroleine (II) the lacking values of NOE enhancements for methyl proton lines forbid evaluation of  $R'_3$ ; model calculations with the missing NOE enhancements  $f_3(1)$  and  $f_3(2)$  varied between 0.00–0.02 and 0.0–0.1, resp., showed that these NOE values (if  $\det \mathbf{A} = 0$ ) have little effect (*i.e.* smaller than the indicated errors) on the resulting values of  $R'_{13}$ ,  $R'_{23}$ ,  $R'_1$ , and  $R'_2$ .

The measurements of spin-lattice relaxation times of methylmethacrylate (III) protons ( $T_1(1) = 24.3$ ,  $T_1(2) = 21.4$ , and  $T_1(3) = 9.3$  s) reported by Fukumi and coworkers<sup>17</sup> permit a test of the calculated values.

Since  $R_J = 1/T_1(J)$ , equation (24) gives an opportunity to determine the product  $K\tau_c$ :

$$K\tau_c = 1/(R'_J T_1(J)).$$

TABLE III

Relative Relaxation Rates Calculated for Models of  $\text{H}_2\text{C} = \text{C}(\text{CH}_3)$  Fragment

Rotation <sup>a</sup>	Conformation <sup>b</sup>	Rate <sup>c</sup>	$R' \cdot 10^{-57}, \text{ m}^{-6}$					
			$R'_{12}$	$R'_{23}$	$R'_{13}$	$R'_2 - G_2^*$	$R'_1 - G_1^*$	$R'_3 - G_3^*$
J	Ec	M	23.3	2.8	0.28	32	24	92
		S	23.3	1.6	0.22	28	24	24
	St	M	23.3	1.8	0.28	29	24	91
		S	23.3	1.1	0.22	27	24	24
F	—	M	23.3	2.3	0.28	30	24	92
		S	23.3	1.4	0.22	27	24	24

<sup>a</sup> Model of the methyl group rotation: J-random 120° jumps, F-free rotation; <sup>b</sup> conformation of the methyl group relative to the double bond: Ec-eclipsed, St-staggered.; <sup>c</sup> rate of internal rotation of the methyl group: M-moderate fast, S-superfast.

The average value calculated from the data for proton classes  $J = 1$  and  $2$  is  $K\tau_c = 1.69 \cdot 10^{-60} \text{ m}^6 \text{ s}^{-1}$ . This value and the values of  $R'_{13}$  and  $R'_{23}$  calculated by the above procedure (given in the last but one row of Table II) should be compared with the corresponding values obtained by a modified procedure in which the problem of homogenous set of Eqs (23) is solved by substituting  $R'_j = 1/(K\tau_c T_1(J))$  ( $J = 1, 2,$  and  $3$ ) in addition to the  $R'_{12}$ . The resulting value of  $K\tau_c$  is  $1.69 \pm 0.06 \cdot 10^{-60} \text{ m}^6 \text{ s}^{-1}$ , the values of  $R'_{13}$  and  $R'_{23}$  are given in the last row of Table II. In view of all the assumptions and approximations the values agree remarkably well.

The relative relaxation rates  $R'$  in compounds *I–III* can be compared with those calculated for two conformations of  $\text{H}_2\text{C} = \text{C}(\text{CH}_3)$  fragments. The calculated values for methyl group rotation models are given in Table III. Analogous comparison for *cis* and *trans* crotonaldehyde (*IV* and *V*) is given in Table IV. The experimental relative relaxation rates  $R'_{ij}$  were derived from the relaxation rates  $R_{ij}$  using the value of  $\tau_c$  given in the same source<sup>9</sup>.

TABLE IV  
Relative Relaxation Rates in Crotonaldehydes *IV* and *V*

Aldehyde	Model <sup>a</sup>	$R' \cdot 10^{-57}, \text{m}^{-6}$					
		$R'_{12}$	$R'_{13}$	$R'_{14}$	$R'_{23}$	$R'_{24}$	$R'_{34}$
<i>IV</i>	exptl.	$1.7 \pm 0.2$	$0.7 \pm 0.2$	$2.1 \pm 0.2$	$6.3 \pm 0.6$ $5.9 \pm 0.7$	$0.5 \pm 0.5$ $0.7 \pm 0.5$	$0.5 \pm 0.5$ $1.0 \pm 0.4$
	J-Ec-M	3.0	0.26	5.8	5.5	0.32	1.1
	J-Ec-S	1.8	0.21	3.1	5.5	0.32	1.1
	J-St-M	3.4	0.26	3.3	5.5	0.32	1.1
	J-St-S	2.0	0.21	1.4	5.5	0.32	1.1
	F–M	3.2	0.26	3.9	5.5	0.32	1.1
	F–S	1.9	0.21	2.1	5.5	0.32	1.1
	<i>V</i>	exptl.	$1.2 \pm 0.2$	$0.7 \pm 0.3$	$5.5 \pm 0.6$ $5.5 \pm 0.7$	$1.2 \pm 0.1$	$0.3 \pm 0.2$
J-Ec-M		2.8	1.2	6.6	1.7	0.11	1.1
J-Ec-S		1.7	1.2	6.6	1.1	0.09	1.1
J-St-M		3.1	1.2	6.6	1.2	0.11	1.1
J-St-S		1.8	1.2	6.6	0.82	0.09	1.1
F–M		3.0	1.2	6.6	1.5	0.11	1.1
F–S		1.8	1.2	6.6	0.94	0.09	1.1

<sup>a</sup> Model descriptions as in Table III, exptl. denotes the values determined from experiments<sup>9</sup> as described in the text.



There is not enough NOE data on  $\beta,\beta$ -dimethylacrylic acid (VI) to allow a complete calculation of experimental relaxation rates, but some of their values can be estimated. Thus, equation (23) gives for the NOE observed on the line of *cis* methyl protons if the olefinic proton is saturated

$$2R'_2 f(3) + 3R'_{12} f_1(3) - R'_{32} = 0.$$

Combining this with Eq. (24) and with the experimental values of  $f_2(3)$  and  $f_1(3)$  and considering that the relaxation rates cannot be negative then it follows that

$$R'_{23} < 0.26G_{mm}, \quad (28)$$

where  $G_{mm}$  is the relative relaxation rate due to the interaction between two protons of the methyl group.

*The indirect method.* Interpretation of the NOE enhancement in  $\alpha$ -methylstyrene (VII) is somewhat more difficult. The important NOE's between the olefinic protons and between the aromatic protons could not be measured. Protons of  $R^4$  substituent (Scheme 1) must be divided into three classes labelled here O (two protons in the *ortho* position), M (two protons in *meta* position), and P (one proton in *para* position) though only the total NOE could be measured. For these reasons the indirect method was advantageous. In the calculation of the relative relaxation rates all models listed in Table III of methyl group rotation had to be evaluated since no information about the rate of this rotation was available; in considering the rotation of the phenyl group we assumed moderate fast reorientation between two conformations characterized by  $+42^\circ$  and  $-42^\circ$  dihedral angles between the aromatic

TABLE V  
Relative Relaxation Rates  $R'_{KK}$  and  $R'_{KJ}$  Calculated for (J-Ec-S) Model of  $\alpha$ -Methylstyrene<sup>a</sup>

$J$ $K$	1	2	3	0	M	P
1	—	23.31	0.22	3.04	0.71	0.02
2		—	1.63	0.24	0.02	0.01
3			—	0.84	0.03	0.01
O				—	0.16	0.01
M					—	0.16
P						—

<sup>a</sup> All the values are in  $10^{57} \text{ m}^{-6}$  units.

and olefinic planes which were established in biphenyl<sup>22</sup>. Assuming the same value  $G^*$  of the independent relaxation for all proton classes the set of Eqns. (23) was solved for each model to obtain the unknown NOE enhancement. The values resulting from the relaxation rates (Table V) corresponding to the model of superfast rotation of the methyl group by 120° random jumping between the eclipsed conformations (J-Ec-S model) agreed best with the experimentally observed NOE enhancements. The calculated enhancements are listed in Table VI for two values of  $G^*$ . It is immediately apparent, that a neglect of the independent relaxation, though in many cases successfully employed<sup>4,7</sup>, is unsuitable here similarly as in the case of mesitylene.

The good agreement may be interpreted as a confirmation of the assumed angle between the plane of the benzene ring and the plane of the double bond as well as a confirmation of the eclipsed conformation<sup>23</sup> of the methyl group and the type and rate of its rotation.

### Interpretation of Relaxation Rates

*Internal rotation.* Application of NOE to molecules with an internal rotation possibly faster than moderate fast requires evaluation of relative relaxation rates  $R'_{KJ}$  between protons of different fragments. For a successful application the fol-

TABLE VI  
Nuclear Overhauser Enhancements  $f_D(S)$  Calculated for  $\alpha$ -Methylstyrene VII<sup>a</sup>

$D^b$	$S^c$	$G^* \cdot 10^{-60}, m^{-6}$	0.00	0.01
1	2		0.39	0.29
	3		-0.04	-0.02
	4		0.12	0.08
2	1		0.40	0.30
	3		0.10	0.07
	4		-0.04	-0.02
3	1		-0.01	-0.01
	2		0.03	0.02
	4		0.03	0.02
4	1		0.06	0.03
	2		-0.02	-0.01
	3		0.05	0.03

<sup>a</sup> All the  $f_D(4)$  values were calculated assuming saturation of all aromatic protons;  $f_4(S)$  were calculated as  $(2f_O(S) + 2f_M(S) + f_P(S))/5$ ; <sup>b</sup> proton class detected; <sup>c</sup> proton class saturated.

lowing questions must be answered first: 1) what is the rate of the internal rotation and 2) which model describes this rotation adequately.

The rotation rate and the suitability of the model of rotation are determined by the barrier of the rotation ( $V_0$ ). Since the correlation time is related to the diffusion constant of the internal rotation which depends on the rotational barrier<sup>24</sup>, the order of magnitude of the correlation time  $\tau_r$  (in s) can be estimated as:

$$\tau_r \approx 10^{-14} e^{+V_0/RT}$$

According to this relation, as the molecular reorientation correlation time ( $\tau_c$ ) varies usually between  $10^{-11}$  and  $10^{-14}$  s (ref.<sup>25-28</sup>), only barriers lower than 3 kcal/mol can correspond to a rotation faster than moderate fast. Such rotational barriers are typical for methyl groups<sup>29,30</sup> the rates of their rotations covering the whole region of fast rotation.

The model of methyl group rotation by  $120^\circ$  jumps is acceptable if the rotational barrier is higher than  $2RT$  (i.e. higher than 1.2 kcal/mol) and, in contrast, the model of free rotation is applicable for barriers lower than 0.2 kcal/mol. For the intermediate rotational barriers the two models are not very adequate. Since the exact description of a rotation of a such intermediate barrier would be extremely involved we have attempted a simplified approach that should allow an estimation of relative relaxation rates  $G_{kj}$ .

Numerical experiments have shown that in the considered cases, the  $G_{kj}$  value calculated for the free rotation model ( $G(F)$ ) is equal with a sufficient precision, to the average of the  $G_{kj}$  values calculated according to the model of random  $120^\circ$  jumps for staggered ( $G(J-St)$ ) and eclipsed ( $G(J-Ec)$ ) conformations of the methyl group.

TABLE VII

Indirect Relative Relaxation Rates  $G$  Calculated for the Protons of the Methyl Group in *trans*-Crotonaldehyde ( $V^a$ )

Internal rotation rate	Moderate fast		Superfast	
$G \cdot 10^{-57}, m^{-6}$	$G_{23}$	$G_{12}$	$G_{23}$	$G_{12}$
$0.5G (J-Ec) + 0.5G (J-St)$	1.48	2.97	0.95	1.76
$G (F)$	1.47	2.97	0.94	1.75

<sup>a</sup> Based on the data of Table IV.

As illustrated by the results of the calculations showed in Table VII the relation

$$G(F) = 0.5G(J-Ec) + 0.5G(J-St) \quad (29)$$

holds in both limiting cases, moderate and superfast, of the fast internal rotation. Though the calculations were carried out only for the interaction between methyl protons on one side and geminal or *cis* olefinic proton on the other, the results prove general applicability of the above relation. In almost all other cases the other interacting proton would be either more remote or it would be closer to the rotational axis of the methyl group and hence the effect of rotation would be smaller than in the considered cases.

Since  $G(J-Ec)$  and  $G(J-St)$  are the two extreme values of  $G$  and since the averaging described by Eq. (29) is so well observed, a generalization of this relation

$$G = \varepsilon G(J-Ec) + (1 - \varepsilon) G(J-St) \quad (30)$$

is likely to hold for the intermediate barriers of 0.2–1.2 kcal/mol. As the values of  $G(J-Ec)$  and  $G(J-St)$  are readily calculated (according to Eq. (13) from  $\tau_p$  value and geometry) and as the coefficient  $\varepsilon$ , which is a ratio of population of the eclipsed conformer to the sum of populations of eclipsed  $P_{Ec}$  and staggered conformers  $P_{St}$  (the coefficient  $\varepsilon$  has to be distinguished from a relative population since the populations of the other rotamers actually present do not enter the expression for  $\varepsilon$ )

$$\varepsilon = P_{Ec} / (P_{Ec} + P_{St}),$$

can be estimated from the rotational barrier the relation (30) offers a manageable way for estimating the  $G$  values in the cases of intermediate rotational barriers which do not correspond to the two discussed models (J and F).

For the two exactly defined models with known  $\tau_c$  the needed ratio of correlation times  $\tau_p$  can be derived from the relaxation time  $T_1$  of  $^{13}C$  nuclei of the rotating methyl group and  $^{13}C-^{1}H$  NOE enhancement<sup>14,31</sup>.

*Deviations from Theoretical Values.* The very small variations (Table II) in the values of  $R'_{23}$  and  $R'_{13}$  derived from the NOE experiments in the ethene derivatives I–III (except for III measured in  $CS_2$ ) are remarkable especially as each of the compounds was measured in different laboratory and in a different solvent. Though the raw experimental NOE values have already suggested such an agreement, it becomes more visible when the relative relaxation rates are compared. The agreement illustrates the fact that in the fragment common to these compounds, ( $H_2C = C(H)C(O)R$ ) the geometry is not, within the sensitivity of the NOE method, appreciably affected by changing the substituent R from H to OH or  $OCH_3$ .

The exceptional case of larger  $R'_{23}$  and  $R'_{13}$  values of methacroleine (II) in  $CS_2$  solution can be explained by a molecular association which is hindered in a strongly basic solvent and hence „good” values are obtained in a dimethyl sulfoxide solution. Such a conclusion could be made directly from the experimental NOE values which are in the carbon disulfide solution very similar to those in neat methylmethacrylate (III) as already noted by Bell and Saunders<sup>18</sup>.

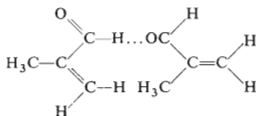
It should be ascertained to what extent are the “good” values in compounds I–III affected by a similar intermolecular association. This can be done by a comparison of the  $R'$  values derived from the experiments with those calculated theoretically (Table III). While the  $R'_{23}$  values (which are due to the interaction of the methyl proton with *cis* olefinic proton) are in agreement for some types of rotation of the methyl group, non of the considered types of motion brings the  $R'_{13}$  values (which are due to the interaction with the *trans* olefinic proton) into an agreement; the experimental values are considerably larger than the theoretical ones. Since these larger values cannot be explained by other than dipol–dipol relaxation mechanism only three possible sources of this discrepancy are left for consideration: a failure of the theory (as such or because of a violation of the assumptions it is based on), different geometry than assumed, and intermolecular association. In view of the success of the theory in the other cases it seems rather unlikely that the theory would fail in this particular case. Calculations showed that the theoretical value of  $R'_{13}$  becomes close to the experimental value if the interprotonic distance between the geminal olefinic protons is reduced to 1.67 Å (from the assumed 1.87 Å) or if the distance between the methyl proton and *trans* olefinic proton is shortened by 0.5 Å which is both very unlikely.

The isotropic intermolecular interaction, which contributes by the same amount  $G_{int}$  to the relaxation rate of each proton in the molecule, also cannot explain the discussed deviations. The magnitude of  $G_{int}$  which would bring  $R'_{13}$  to an agreement with the experiment leads to another disagreement:

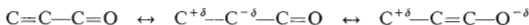
The  $R'_{23}$  values reduced by  $G_{int}$  would agree with the experiment only for J–St–S model of the methyl group rotation while the eclipsed conformation was found in ref.<sup>23</sup>.

A combination of the isotropic intermolecular interaction with distorted ethene geometry is more likely to account for this discrepancy without causing another one. For example, if the bond angles  $H_3C-C=C$  and  $H-C=C$  are assumed to be 126 and 123°, resp. (as in *trans*-crotonaldehyde<sup>21</sup>) the calculated  $R'_{23}$  values match the experimental ones for superfast rotation. But in *cis* crotonaldehyde, the geometry of which is known, any isotropic interaction which would bring an agreement for  $R'_{13}$  (Table IV) would cause unrealistic values of other relaxation rates. Obviously, the isotropic interaction alone or in a combination with an assumption of non-ideal geometry cannot account for larger values of experimental relaxation rate involving the interaction of the methyl proton and the olefinic *trans* proton.

A specific intermolecular "trans" interaction which would bring the methyl proton of one molecule into the vicinity of the trans olefinic proton of the other molecule would account for the observed deviations even if the assumption of isotropic molecular motion is retained in the theory. In compounds *I*, *II*, and *IV* such interactions occur if associates containing units of the type



are formed. (Such units should be present in planar cyclic hexamers.) In ester (*III*) owing to the following mesomeric forms<sup>32</sup>



formation of some other types of associates in which the trans interaction will be important is also possible.

Dilution NOE experiments would be needed to test this interpretation of the deviations. The results show that when a deviation from a theoretical value involves only one relaxation rate, it is likely to be due to a molecular association.

**Methyl Group Rotation Rates.** It would serve the purpose of this study to summarize the results regarding methyl group rotation rate as determined here from proton-proton NOE measurements though these rates can be also obtained by other techniques involving e.g. <sup>13</sup>C-NMR (as demonstrated<sup>9</sup> by  $\tau_p = 0.1$  for crotonaldehydes *IV* and *V*).

The rate of methyl group rotation in  $\beta,\beta$ -dimethylacrylic acid can be estimated from the relation (28) as follows: Assuming the same geometry of the methyl group as in compound *I* the theory (Eq. (17) or (18) gives the values  $29.8 \cdot 10^{57} \text{ m}^{-6}$  and  $7.45 \cdot 10^{57} \text{ m}^{-6}$  for  $G_{mm}$  if the rotation is moderate fast and superfast, resp. The theoretical results for the relative relaxation rates due to the interaction of a methyl proton with the olefinic proton in cis position ( $R'_{23}$  in Table III) as compared with the experimental values clearly rule out the possibility of moderate fast rotation under the assumed geometry of the molecule. In contrast, relation (28) gives  $R'_{23} > 1.9 \cdot 10^{57} \text{ m}^{-6}$  in the case of superfast rotation. This value is close to the value calculated for the methyl group with the eclipsed conformation in accord with the work<sup>23</sup>. Similarly, superfast rotation is found to be more likely in  $\alpha$ -methylstyrene (*VII*).

In the remaining ethene derivatives *I–III* comparison of experimental and theoretical relaxation rates  $R'_{23}$  and  $R'_3$  (Table II and III) would be most appropriate for the estimation of the rotation rate had not been these values possibly affected by the association. Nevertheless, the comparison of  $R'_{23}$  values points to a superfast rate of rotation in these compounds. After correcting for the effect of the association (which causes an increase of these values) the comparison is even more convincing. Comparison of  $R'_3$  values indicates approximately the same rate for methacrylic acid (*I*) but for methyl methacrylate (*III*) the results can be only interpreted as suggesting a rate faster than moderate fast.

## EXPERIMENTAL

**NOE measurements.** All the measurements reported here were carried out on a modified Tesla BS 477 spectrometer operating at 60 MHz in the CW mode at ambient temperature. An internal lock was employed for the stabilization of frequency/field ratio. Irradiating frequencies were obtained by the standard sideband field modulation technique using Tesla BM 524 and BM 269A audio generators. The reported NOE values are the averages of the measurements taken at two different amplitudes of the saturating field which were both larger than the minimum amplitude giving the full NOE (*i.e.* not reduced by an incomplete saturation). The sweep-rate and  $H_1$  field were optimized similarly. The NOE values were calculated from the integrated intensities according to the relation  $f_D(S) = (I_D^S - I_{D0})/I_{D0}$  where  $I_D^S$  is the integrated intensity of the signals of protons of the class *D* when the protons of the class *S* are being saturated and  $I_{D0}$  is this intensity if the irradiating frequency is shifted into the region of the spectrum with no lines. Each of the integrals was measured at least 20 times, the errors indicated for NOE values are 95% confidence limits based on *t*-distribution as calculated from the integrals. In the case of compound *I* the reported value of  $f_1(2)$  might be in error due to a small separation of signals and broadening of the line 1.

The measured samples were 0.4M solutions in deuteriochloroform (Merck, Uvasol) (except for mesitylene which was measured as 0.6M solution in perdeuterated dimethyl sulphoxide) containing 0.05–0.08M hexamethyldisilane (prepared in this laboratory) the signal of which served for the lock. The samples were degassed by the repeated freeze-pump-thaw cycles (using a vacuum better than  $10^{-3}$  Torr).

**Calculation of interprotonic distances.** In calculations of interprotonic distances according to the procedure by Thompson<sup>33</sup> the following bond lengths (*r*) and angles were used: in benzene derivatives:  $r(C_{ar}-C_{ar}) = 1.39 \text{ \AA}$ ,  $r(C_{ar}-H) = 1.08 \text{ \AA}$ , all C—C—C angles  $120^\circ$ ; in methyl groups on benzene ring:  $r(C-H) = 1.09 \text{ \AA}$ ,  $r(C_{alk}-C_{ar}) = 1.50$ , tetrahedral angles; in ethene derivatives (except in *IV* and *V* for which the values from ref.<sup>9</sup> and ref.<sup>21</sup>, resp., were used):  $r(C=C) = 1.39 \text{ \AA}$ ,  $r(=C-CH_3) = 1.52 \text{ \AA}$ ,  $r(=C-H) = 1.08 \text{ \AA}$ , all bond angles  $120^\circ$ ,  $r(C_{alk}-C_{ar}) = 1.51 \text{ \AA}$ ; in methyl groups on ethene  $r(C-H) = 1.10 \text{ \AA}$  except for *IV* and *V* where  $r(C-H) = 1.09 \text{ \AA}$ , tetrahedral.

**Calculation of the indirect relative relaxation rates  $G_{Hm}$  due to the interaction of a methyl group proton *m* with a proton *H* of the core.** In accord with the theoretical part, the rotating methyl group was viewed as a rigid fragment. The  $C_3$  symmetry axis of the methyl group was taken to coincide with the bond by which the group is connected to the molecular core. The coordinate system employed in the calculations is shown in Fig. 1. In this coordinate system expressions





and

$$G_{\text{Hm}}(J-\alpha-S) = \{A_{\alpha(\alpha+120^\circ)} + A_{\alpha(\alpha+240^\circ)} + A_{\alpha(\alpha+120^\circ)(\alpha+240^\circ)} + r_\alpha^{-6} + r_{(\alpha+120^\circ)}^{-6} + r_{(\alpha+240^\circ)}^{-6}\}/9$$

The  $G$  values for free rotation were calculated by numerical integration of the following expressions which were derived from Eq. (15):

$$G_{\text{Hm}}(F-M) = \frac{1}{2\pi} \int_0^{2\pi} r_\alpha^{-6} d\alpha$$

$$G_{\text{Hm}}(F-S) = \frac{1}{8\pi} \int_{\Delta=0}^{2\pi} \int_{\alpha=0}^{2\pi} A_{\alpha\alpha} d_\alpha d_\Delta$$

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