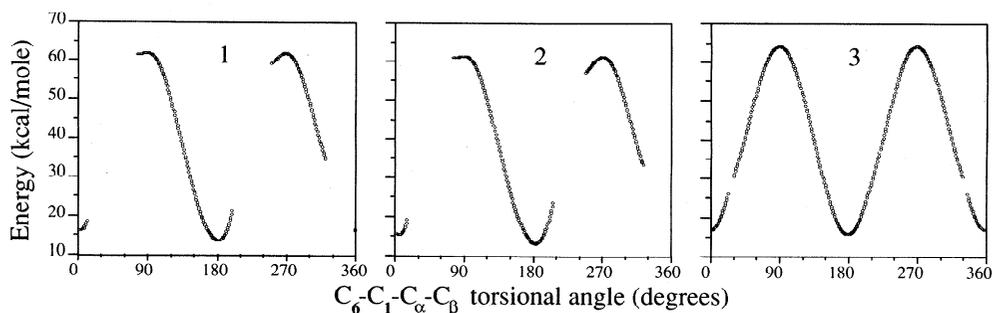


**Fig. 2.** Three-dimensional structure of minimized *syn*-1. Note that the orientation of the methoxyl was not fully examined – it is usually considered to be in the plane of the ring.

bond angles within SYBYL. The original structure for each compound began with the  $C_6-C_1-C_\alpha-C_\beta$  torsional angle (the numbering is as indicated in Fig. 1), set at  $180^\circ$ , corresponding to the *syn* (or *Z*-) isomer (Ralph and Adams 1983), Figure 2.

The energies of the initial structures were calculated using the Tripos 3.2 general purpose force-field (Clark *et al.* 1989), and conjugate gradient minimization. Minimizations were stopped when the energy of subsequent iterations differed by less than  $0.001 \text{ kcal.mole}^{-1}$ . Beginning with these energetically minimized structures a conformational search was performed by rotation about the  $C_6-C_1-C_\alpha-C_\beta$  dihedral angle, in  $1^\circ$  increments through  $360^\circ$ , with energy calculations performed at each step.



**Fig. 3.** Energy vs. torsional angle  $C_6-C_1-C_\alpha-C_\beta$  in 1, 2 and 3

NMR spectra of  $CDCl_3$  solutions were recorded on Bruker instruments at 270, 300, 500, and 600 MHz. Two-dimensional phase-sensitive NOESY spectra, Figure 4, used the standard Bruker pulse program NOESYPH.AU, and were acquired at 600 MHz for  $\alpha$ -D-1 and  $\alpha$ -D-2, and at 500 MHz for  $\alpha$ -D-3; 8K data points were used in  $t_2$  (ca  $0.5 \text{ Hz/point}$ ) and 128 increments of 16 scans in  $t_1$  (zero-filled to 256 points); the relaxation delay was 5 s and the mixing time was 1 s; processing was in the phase-sensitive mode with simple line-broadening (2 Hz) in  $t_2$ , and  $\pi/2$  shifted sine-bell in  $t_1$ .  $^1H$  NMR chemical shift data (For Table 1  $\Delta\nu$  values) were taken from the original data collected for Ralph and Adams (1983).  $^{13}C$  NMR chemical shift data (for Table 1  $\Delta\nu$  values) were taken from the authenticated data in Ralph and Ede (1988).

Calculations of rotational rates, and coalescence temperatures for Table 1 were made from the following equations (Kessler 1970; Binsch and Kessler 1980; Note: Both of these papers use a value of 10.32 for  $\log(2k/h)$ ; the correct value is 10.62 (Shannan-Atidi and Bar-Eli 1970).

$$k_r = k_B T / h \cdot e^{-\Delta G^\ddagger / RT}$$

$$\Delta G^\ddagger = RT_c [\ln(2k/h) + \ln(T_c/k_r)]$$

$$k_r (\text{coalescence}) = 2\pi\Delta\nu/\sqrt{2} \text{ (for equally populated rotamers)}$$

## Results and Discussion

The energies for the initially minimized structures were all around  $12 \text{ kcal.mole}^{-1}$ . Our modelling methods failed (as did Jakobsons' and Shevchenko's) to predict the slightly increased stability of the *syn* over the *anti* isomer that is seen experimentally (Ralph and Adams 1983; Ede *et al.* 1990). A MOPAC calculation also predicted *anti* to be more stable than *syn*. However the predicted differences are less than  $1 \text{ kcal.mole}^{-1}$ , and the 70:30 ratio determined experimentally translates to only  $0.5 \text{ kcal.mole}^{-1}$ . Energy differences of these magnitudes, far less than the energy required for ethane to rotate about its C-C bond, are extremely difficult to predict and it is unreasonable to expect computational methods to be accurate to this degree.

The results of the conformational analyses are indicated in Figure 3. The discontinuities in the graphs are due to the elimination of structures that would have bad steric interactions, and therefore much higher energies. It can be seen that the conformers with  $C_6-C_1-C_\alpha-C_\beta$  angles of  $90^\circ$  and  $270^\circ$  have energies that are in excess of  $48 \text{ kcal.mole}^{-1}$  greater than the

original conformations. These, of course, represent considerable barriers to rotation, and indicate that the rotamers are not readily interconverted.

It has been reported, however, that conformational analyses using rigid-residues can result in overestimated rotational barriers between minima (French and Brady 1990). To assess this possibility in the current study, a conformer for each structure was selected with a dihedral angle of  $270^\circ$  and allowed to completely optimize. The resultant structures had low energies of around  $12 \text{ kcal.mole}^{-1}$ . The problem arose, however, that the torsional angle had also changed to ca.  $0^\circ$  or  $180^\circ$ , corresponding to the *syn* or *anti* conformations. What was happening was that the optimization process relieved the tremendous strain associated with a rotated double bond by re-attaining the normal conformation. Since, at some point in the rotation, the quinone methide ring must be able to assume a perpendicular orientation, further calculations were performed with the  $C_6-C_1-C_\alpha-C_\beta$  torsional angle fixed at  $270^\circ$ , with all other factors allowed to optimize. This resulted in structures with energies of 60.95, 60.48, and 63.40  $\text{kcal.mole}^{-1}$  for quinone methides **1**, **2**, and **3**, only slightly below those reported by the rigid-residue conformational search.

Such molecular modelling does have a number of underlying assumptions (Burkert and Allinger 1982) but the predicted  $\Delta G^\ddagger$  values, Table 1, are unlikely to be wildly deviant. And, as with all theoretical data, they must agree with and/or predict experimental data to be of any value. The claim of free rotation and the comments regarding nuclear Overhauser enhancements (nOe) (Jakobsons and Shevchenko 1990) simply overlook the indisputable fact that these isomers are clearly not interconverting on the proton NMR timescale (which, at around 300 MHz, can resolve rotations in the 75  $\mu\text{s}$  to 200 ms range, corresponding to rates of 5 to 10,000  $\text{s}^{-1}$  and activation energies of 5 to 25  $\text{kcal.mole}^{-1}$  (Bryant 1983; Kessler 1970). This is clear because the quinone methide spectra (see the high-resolution projections in Fig. 4) are a superposition of two, non-interconverting isomers (Ralph and Adams 1983; Ralph and Young 1983; Ede *et al.* 1990). If they interconverted rapidly on the NMR timescale, only a single 'compound' or averaged conformer would be visible in the spectra. If the rotation was occurring on the NMR timescale, the peaks for each isomer would be broadened and, at some temperature close to ambient, would show coalescence behavior typical of hindered rotation (Kessler 1970). The fact that the peaks are sharp and well resolved simply refutes any claim of free rotation at reasonable rates.

The nOe data reported previously (Ralph and Adams 1983) also confirms the non-interconvertibility of isomers on the NMR timescale. The following argu-

**Table 1.** NMR frequency differences, calculated rotational activation energies, and derived correlation temperatures and rotational rates

Atom <sup>1)</sup>	$\Delta\nu$ <sup>2)</sup>	$\Delta G^\ddagger_{\text{syn}}$ <sup>3)</sup>	$T_c$ ( $^\circ\text{C}$ ) <sup>4)</sup>	$k(T_c)$ <sup>5)</sup>	$k(25^\circ\text{C})$ <sup>6)</sup>
<b>1</b>		48.49			
H <sub>2</sub>	78.70		686.33	350	$3.29 \times 10^{-23}$
H <sub>5</sub>	7.90		609.53	35	$3.29 \times 10^{-23}$
H <sub>6</sub>	113.25		699.70	503	$3.30 \times 10^{-23}$
C <sub>2</sub>	382.51		747.22	1699	$3.30 \times 10^{-23}$
C <sub>5</sub>	65.43		679.68	291	$3.29 \times 10^{-23}$
C <sub>6</sub>	437.87		752.78	1945	$3.30 \times 10^{-23}$
<b>2</b>		48.14			
H <sub>2</sub>	83.90		682.07	373	$5.90 \times 10^{-23}$
H <sub>5</sub>	11.40		614.82	51	$5.89 \times 10^{-23}$
H <sub>6</sub>	115.10		693.65	511	$5.90 \times 10^{-23}$
C <sub>2</sub>	392.57		741.31	1744	$5.91 \times 10^{-23}$
C <sub>5</sub>	60.40		670.31	268	$5.90 \times 10^{-23}$
C <sub>6</sub>	458.00		747.63	2035	$5.91 \times 10^{-23}$
<b>3</b>		50.74			
H <sub>2</sub>	94.70		736.12	421	$7.41 \times 10^{-25}$
H <sub>5</sub>	15.10		670.25	67	$7.39 \times 10^{-25}$
H <sub>6</sub>	120.60		745.47	536	$7.41 \times 10^{-25}$
C <sub>2</sub>	372.44		791.44	1655	$7.42 \times 10^{-25}$
C <sub>5</sub>	65.43		722.14	291	$7.41 \times 10^{-25}$
C <sub>6</sub>	442.90		798.86	1968	$7.42 \times 10^{-25}$

1) See Fig. 1 for numbering

2) Absolute frequency difference (Hz) between *syn* and *anti* resonances

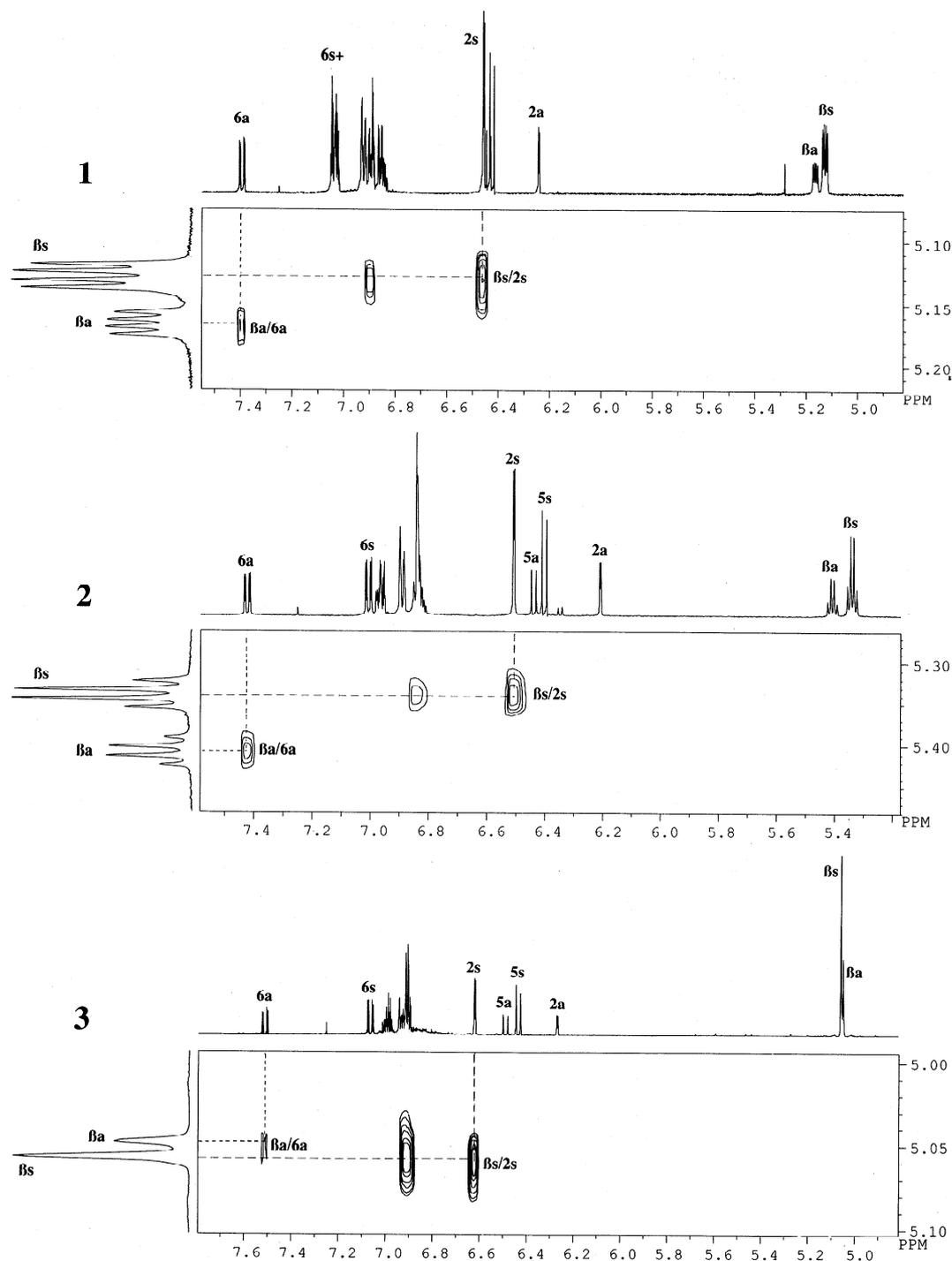
3) The rotational activation energy ( $\text{kcal.mole}^{-1}$ ) determined from molecular modelling,  $\Delta G^\ddagger = G^\ddagger - G^\circ_{\text{syn}}$

4) The NMR coalescence temperature ( $^\circ\text{C}$ ) calculated from the frequency differences and the  $\Delta G^\ddagger$ .

5) The rate of rotation ( $\text{s}^{-1}$ ) at the coalescence temperature

6) The calculated rotational rate ( $\text{s}^{-1}$ ) at room temperature

ment can be made. In a 1D experiment, if a single proton, say  $H_{\beta\text{syn}}$ , is irradiated, it can interact with other protons within ca. 0.5 nm by through-space interactions, giving rise to nOe enhancement. Thus  $H_{2\text{syn}}$  will experience nOe. If, during the course of the experiment,  $H_{\beta\text{syn}}$  becomes  $H_{\beta\text{anti}}$  (due to rotation) then, since it would retain its magnetization,  $H_{\delta\text{anti}}$  would also experience this nOe and  $H_{\beta\text{syn}}$  would correlate with both  $H_{2\text{syn}}$  and  $H_{\delta\text{anti}}$ . Here we present more convincing 2D phase-sensitive NOESY spectra, Figure 4. As previously, the quinone methides were  $\alpha$ -deutero analogues which had the advantage of simplifying the spectra (the  $\alpha$ -protons resonate within the  $H_2/H_5$  region) and of enhancing the NOE by elimination of the undesirable magnetization pathway (Tsang *et al.* 1990) from  $H_\beta$  through  $H_\alpha$  to the ring 2 and 6 protons. It should be stressed that the same correlations were observed in the normal (non-deuterated) models **1–3** however. From the high-resolution spectra plotted as projections in Figure 4, it is clear that two isomers exist for all three quinone methides. The predominance of the *syn* over the *anti* isomer is also clear in



**Fig. 4.** Partial 2D phase-sensitive NOESY spectra of quinone methide  $\alpha$ -D-1,  $\alpha$ -D-2 and  $\alpha$ -D-3, showing correlation of the  $\beta$ -protons. s = *syn* (Z), a = *anti* (E). Spectra of **1** and **2** were taken at 600 MHz, **3** at 500 MHz. Projections show the high-resolution spectra.

all three compounds. At 600 MHz (500 MHz for **3**) there is sufficient dispersion to fully separate the  $\beta$ -proton resonances, and this allows for particularly diagnostic correlations, Figure 4. In the case of quinone methide **1** however, even 600 MHz was not sufficient field strength to remove the overlap of  $H_{6syn}$  with other aromatic protons, nor to fully disperse the 5-protons. Nevertheless, it is abundantly clear that, for all three quinone methides,  $H_{\beta syn}$  correlates with

$H_{2syn}$  and not  $H_{6anti}$ , whereas  $H_{\beta anti}$  correlates with  $H_{6anti}$  and not  $H_{2syn}$ . Thus again, on the NMR timescale, there can be no interconversion of  $H_{\beta syn}$  and  $H_{\beta anti}$ , i.e. no rotation about the  $C_1-C_\alpha$  bond.

The molecular modelling data, coupled with the NMR chemical shift data, Table 1, allow prediction of the temperatures necessary to observe peak coalescence and, hence, rotation on the NMR timescale, and prediction of that rate. The calculations were

simplified by using the equations for coalescence of equally populated rotamers. It is worth keeping in mind the data for systems that are known to be freely rotating or experiencing hindered rotation. The energy associated with the rotation of ethane from the staggered to the eclipsed conformation is 2.9 kcal.mole<sup>-1</sup> (Lowry and Richardson 1981). Interconversion of chair forms of cyclohexanes requires 10.8 kcal.mole<sup>-1</sup> (Lowry and Richardson 1981). Clearly these processes can occur at significant rates at room temperature. Amides have received considerable attention since the classic application by Phillips (1955) of NMR methods to study kinetic processes of dimethylformamide hindered rotation. Amide rotations typically have  $\Delta G^\ddagger$  values of 15–22 kcal.mole<sup>-1</sup> (Kessler 1970) due to the ca. 40% double bond character of the amide N–C bond. 2,2'-Dimethylbiphenyl has a rotational activation energy of 15.1 kcal.mole<sup>-1</sup> (Theilacker and Bohm 1967). The  $t_{1/2}$  for racemization of this compound has been determined at 420 s at –32°C, and estimated at 1.1 s at 25°C. Thus 15 kcal.mole<sup>-1</sup> represents a rotational activation energy at which resolution of rotamers (as isomers) becomes possible at depressed temperatures. From our own work (Ralph *et al.* 1984), an acetylated adduct between anthraquinone and quinone methide **2** had rotational activation energies of 14.25 and 13.97 kcal.mole<sup>-1</sup> (the two conformers were not equally probable and had different energies) representing rates of 42 s<sup>-1</sup> and 70 s<sup>-1</sup>. In addition, a series of simple quinone methides derived from di-*t*-butylphenols was studied by Rieker and Kessler (1968). They were unable to observe any alteration in NMR spectra at 190°C, and concluded that  $\Delta G^\ddagger$  had to be > 25 kcal.mole<sup>-1</sup>. Finally, 2,2'-dimethyl-9,9'-bitriptycyl, with a calculated energy barrier of 54 kcal.mole<sup>-1</sup>, underwent no detectable rotational isomerism after 171 h at 300°C (Schwartz *et al.* 1977; Oki 1976). Kessler (1970) states that, when  $\Delta G^\ddagger$  is > 23 kcal.mole<sup>-1</sup>, the isomers are stable at room temperature (toward rotation).

From the rotational activation energies calculated above from molecular modelling, Table 1, and from the NMR frequency differences for H<sub>2</sub>, H<sub>5</sub> and H<sub>6</sub> between the *syn* and *anti* isomers of quinone methides **1–3** at 270 MHz (Ralph and Adams 1983), it is possible to calculate the expected temperature at which these peaks would coalesce, and the rates of the rotations at those temperatures from the equations in the methods section. Thus in quinone methide **1**, even for the most closely spaced peaks, H<sub>5</sub>, which would coalesce at the lowest temperature, coalescence would not occur until about 600°C and with a rotational rate of only 30 s<sup>-1</sup> (Table 1). Other protons would coalesce at higher temperatures and exhibit correspondingly higher rotation rates at those temperatures, Table 1. The calculated rotational rate at room

temperature is < 10<sup>-22</sup> s<sup>-1</sup> (Table 1). In order to see coalescence in the <sup>13</sup>C NMR spectra at 200 MHz (Ralph and Ede 1988), the temperatures would have to be greater than 670°C and the rotational rates would be less than ca. 2000 s<sup>-1</sup> (Table 1). Again, the expected rates at room temperature calculate out to < 10<sup>-22</sup> s<sup>-1</sup>.

It is clear from these data that, even if the molecular modelling rotational barriers determined in this study were tremendously in error, the quinone methides would be far from freely rotating even at pulping temperatures of 170°C. The calculated  $\Delta G^\ddagger$  required to see NMR coalescence at pulping temperatures is ca. 23.5 kcal.mole<sup>-1</sup> for quinone methide **1**. Since our molecular modelling data gives us a value of ca. 48.5 kcal.mole<sup>-1</sup>, we would have to be overestimating that energy by 25 kcal.mole<sup>-1</sup>, or ca. 100%. While mechanistic details, such as uncoupling of the pi electrons in the transition state (Kessler 1970) may lower the rotational barriers somewhat, we consider it unlikely that these data are flawed to that extent. Consequently the enhanced reactivity of the *anti* over the *syn* isomer noted recently (Ede *et al.* 1990) is still likely to hold. Additional confidence in the  $\Delta G^\ddagger$  values predicted by these computational methods was obtained by modelling systems with known rotational barriers. Those with low barriers where rotation is detectable at room temperature, such as 2,2'-dimethylbiphenyl (Theilacker and Bohm 1967), were the hardest to predict accurately but were within 20%.

## Conclusion

Both experimental data and our theoretical modelling data show conclusively that guaiacyl quinone methides exist as non-interconverting isomers at room temperature and predict that the same holds true at elevated temperatures typical of pulping reactions.

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