most active fraction (33) is ≥ 50 % of that of Cu in erythrocuprein.[5] This, together with its resistance towards EDTA, seems to exclude that the activity is of a non-specific metal-complex type.

Some people have genetic variant forms of erythrocuprein.[15] Variants are not found in the electrophoretically slow component from placentas of such persons, which indicates different genetic control.[16] Preliminary investigations indicate that an electrophoretically slow component staining for superoxide dismutase activity from Kb cells is associated with a particulate fraction, probably mitochondria, whereas a fast component is cytoplasmatic.[16] The slow component may be related to the manganese containing superoxide dismutase recently mentioned as present in chicken liver mitochondria.[17]

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Ring Inversion in Substituted Spiro[2.5]octanes

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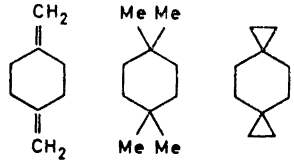
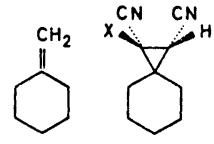
Some of the interest in the field of inversion of cyclohexane rings has been focused on compounds having hybridization different from sp^3 at one or more of the ring atoms. NMR spectroscopy has proven useful to evaluate rate constants for such processes provided the free energy of activation is larger than about 6 kcal/mol. By this technique 1,4-dimethylenecyclohexane (I) has been studied and a $\Delta G^{\ddagger}$ value of 7.5 kcal/mol was calculated for the inversion for which arguments are presented showing that this most likely is a chair-chair interconversion.[1] A change in the hybridization from sp^2 to sp^3 as in 1,1,4,4-tetramethylcyclohexane (II) raises the barrier to inversion to 11.6 kcal/mol.[2] An intermediate value of 10.7 kcal/mol has recently been found in dispiro[2.2.2.2]decane (III) and fits qualitatively in the picture of the ring atoms involved in substitution having a slightly higher *s*-character than in II.[3] Methylenecyclohexane (IV) has also been found to exist predominantly in a chair



I II III



a: x = CN
b: x = COOMe

IV V

conformation with a $\Delta G^{\ddagger}$ value of 8.4 kcal/mol for the inversion process.[4]

By analogy one would expect spiro-[2.5]octane to exhibit a barrier to inversion of approximately 10.4 – 10.5 kcal/mol *i.e.* intermediate between III and cyclohexane itself ($\Delta G^{\ddagger} \approx 10.2$ kcal/mol).

The ring inversion in unsubstituted spiro[2.5]octane has hitherto not been investigated, but the easy preparation of 1,1,2-trisubstituted spiro[2.5]octanes (V) gave us in hand compounds well suited for barrier determination.[5]

The NMR spectra at room temperature of the compounds Va or Vb indicated rapid interconversion with singlets for the cyclopropyl proton at 2.37 and 2.64 ppm, respectively (downfield from Me_4Si). The cyclohexyl resonance consisted of broad non-resolvable multiplets at 1.4 – 2.0 and 1.2 – 2.0 ppm, respectively. Lowering the temperature to about −40°C some changes were observed in the cyclohexyl region. On further cooling a splitting of the cyclopropyl resonance ($\Delta\nu = 6.7$ Hz) occurred which obviously must be due to the two possible conformers of the spiro compounds. A population difference was indicated by the unequal intensities of the two peaks. The rate of interconversion at seven different temperatures ranging from about −70°C to −100°C was then calculated using a complete line-shape analysis, (Gutowsky *et al.*[6]) and the free energy of activation was calculated using the Eyring equation $\Delta G^{\ddagger} = RT \ln (kT\tau/h)$ where τ is the lifetime at temperature T (Table 1).

Table 1.[a] Interconversion barrier and free energy difference between the two conformers of 1,1,2-trisubstituted spiro[2.5]octanes V.

	$\Delta G^{\ddagger}_{A\rightarrow B}$	$\Delta G^{\ddagger}_{B\rightarrow A}$	ΔG_0	t(°C)
Va	10.5	10.3	0.2	−78
Vb	10.5	10.4	0.1	−72.5

[a] A is the conformer of lowest energy; free energy difference (ΔG) in kcal/mol.

The calculated difference in free energy of the two conformers, ΔG_0, indicates only slight difference in population while the observed intensities in the spectra (by integration) point to a preference for the lower energy state of about 0.25 kcal/mol for both Va and Vb. However, the limit of error in the calculation, 0.1 kcal/mol, may account for this discrepancy.

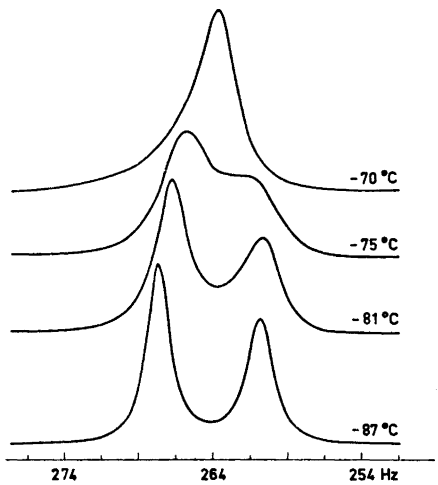


Fig. 1. The cyclopropyl region of the NMR spectrum of Vb as a function of sample temperature.

The thus observed values for the barrier to inversion, 10.3 – 10.5 kcal/mol, agree very well with those expected. It remains to be seen whether the substituents exhibit any effect on the barrier. If so, it will probably be more of electronic nature, *i.e.* an effect in the hybridization at the spiro atom. The substituents are probably too far away for the observation of a sizeable van der Waals repulsion.

Experimental. The spectra were obtained using a Varian HA 100-15D spectrometer (operating at 98 MHz) with Model V4333 variable temperature probe and Model V6040 Temperature Controller. The temperatures were calibrated using a copper-constantan thermocouple which was introduced in the sample tube before and after each run. Temperature accuracy was estimated to ±1°C.

The compounds were dissolved in dichlorofluoromethane to a concentration of about 0.2 M.

Spectra were recorded in the field sweep mode and at a sweep rate of 0.1 Hz/sec, thus approaching "slow passage" conditions. Internal lock with dichlorofluoromethane as

lock signal was used. Field homogeneity was checked for each run using the resonance signal from tetramethylsilane run at the same rate (0.1 Hz/sec).

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