Investigation of Atropisomerism in ortho-Substituted Tetraphenylporphyrins: An Experimental Module Involving Synthesis, Chromatography, and NMR Spectroscopy

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A new experimental module involving the synthesis, chromatography, and NMR spectroscopy of tetra(*o*-tolyl)porphyrin (T(*o*-Tol)P) has recently been incorporated into the advanced undergraduate laboratory curriculum at Davidson College. This experiment introduces the concepts of porphyrin structure and synthesis, atropisomerism, statistical distributions, and chromatographic separations. In addition, the NMR spectrum of the product provides an excellent example of anisotropic effects as well as evidence for the presence of atropisomers.

Atropisomerism arises because of hindered rotation about a single bond. ortho-Substituted biphenyl derivatives represent the most familiar example of atropisomerism; most organic chemistry textbooks include a brief discussion of the existence of resolvable optical isomers of unsymmetrically substituted biphenyls, such as 2,2′-dibromo-6,6'-diiodobiphenyl (*1*). Such isomers exist because the planar conformation through which the molecule must pass in order for the isomers to interconvert is very high in energy owing to steric interactions between the ortho substituents. A similar phenomenon is observed for ortho-substituted derivatives of *meso*-tetraphenylporphyrin, such as T(*o*-Tol)P, owing to hindered rotation of the phenyl rings.

Porphyrins are a class of naturally occurring and synthetic macrocyclic compounds consisting of a planar porphine core and its substituents. The fully conjugated core is constructed from four pyrrole rings joined at the α positions by four methine bridges. In T(*o*-Tol)P, a phenyl ring with an ortho methyl group is attached at each of the methine carbons. Steric interactions between the ortho substituents on the phenyl rings and the β-pyrrole hydrogens result in a high energy barrier to rotation about the porphine-phenyl bonds through a coplanar conformation. As a result, each ortho substituent is essentially fixed on one side of the porphyrin plane at room temperature. This leads to the existence of four atropisomers, which correspond to the four ways of distributing the substituents above and below the porphyrin plane. The isomers, designated 4,0 (all substituents on the same side); 3,1 (three substituents on one side); *cis*-2,2 (two adjacent substituents on one side); and *trans*-2,2 (two opposite substituents on the same side), are pictured in Figure 1. A statistical mixture of isomers contains the 4,0; 3,1; *cis*-2,2; and *trans*-2,2 isomers in a 1:4:2:1 ratio. The relative abundance of each isomer is proportional to the number of ways that isomer can be represented, as shown in Figure 2. The four methyl groups on each of the atropisomers are equivalent, with the exception of the 3,1 isomer, which contains three types of methyl groups in a 1:2:1 ratio (labeled *a*, *b*, and *c* in Figure 1). Thus there are six types of methyl groups present in a mixture of all four atropisomers.

Porphyrin atropisomerism was first reported in 1969 by Gottwald and Ullman, who separated the four atropisomers of tetra(*o*-hydroxyphenyl)porphyrin (*2*). In 1971, NMR evidence for the existence of atropisomers of the metalloporphyrin (tetra(*o*-tolyl)porphyrin)nickel(II) was presented by Walker and Avery (*3*). Since that time a number of studies involving the separation, interconversion, and characterization of atropisomers have appeared (*4*–*9*). In addition, the concept of atropisomerism has been exploited in the design of synthetic analogs of oxygen-binding heme proteins (*10*–*12*).

In this experiment, students synthesize a statistical mixture of atropisomers of T(*o*-Tol)P. The NMR spectrum of this sample, as well as of several porphyrin fractions obtained chromatographically, is recorded from −3 to 10 ppm, and the methyl signal is expanded to reveal multiple separate peaks. Changes in the relative integrals of these methyl resonances can be correlated to changes in the composition of the atropisomer mixture, and the six methyl peaks can be identified. The NMR spectrum of T(*o*-Tol)P, in addition to demonstrating the existence and distribution of atropisomers, also illustrates the concept of aromatic ring currents and the correlation of chemical shift with proton

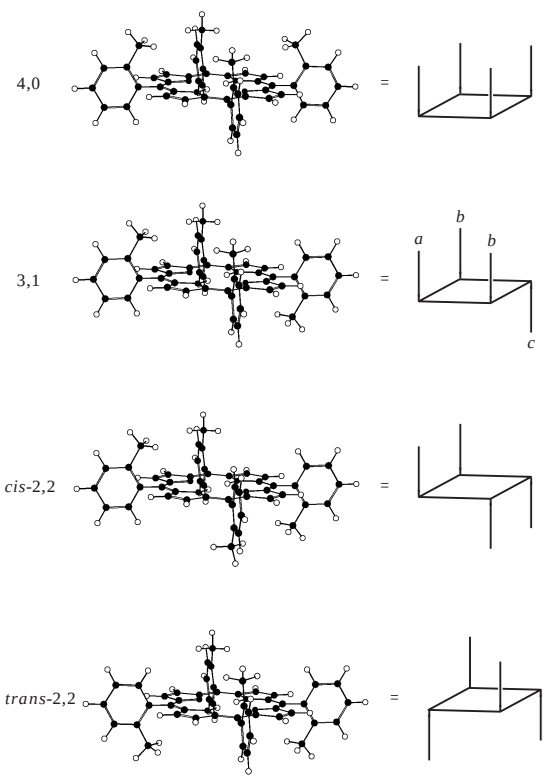

Figure 1. Structures and simplified representations of the four atropisomers of T(*o*-Tol)P.

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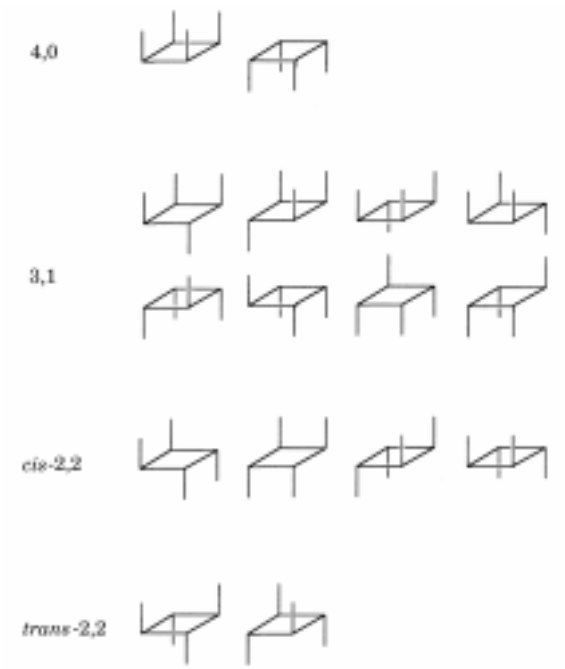

Figure 2. Representation of the statistical distribution of atropisomers for an ortho-substituted tetraphenylporphyrin.

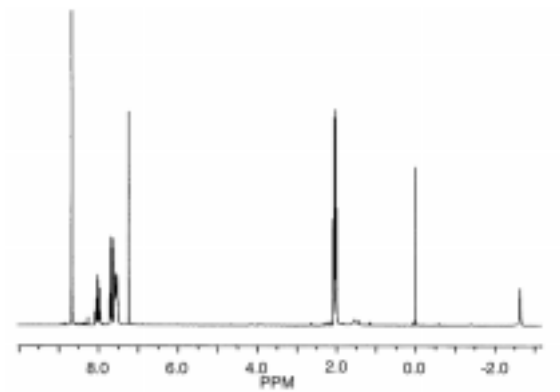

Figure 3. ^{1}H NMR spectrum of T(*o*-Tol)P in $CDCl_3$.

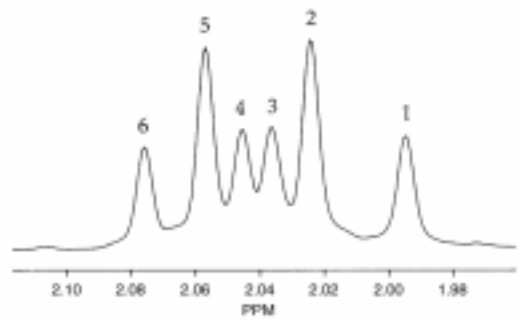

Figure 4. Expansion of ^{1}H NMR methyl signal for a statistical mixture of atropisomers of T(*o*-Tol)P.

position relative to the porphyrin π system. A related experiment illustrating magnetic anisotropy in porphyrins was published in this *Journal* in 1988 (*13*).

Results

The proton NMR spectrum of T(*o*-Tol)P is shown in Figure 3. One notable feature of the spectrum is the signal at –2.6 ppm, which arises from the highly shielded N–H protons located in the porphine core. The N–H proton of pyrrole gives a signal more than 10 ppm downfield from this (8.0 ppm). The porphyrin pyrrole C–H protons, which are deshielded by virtue of their location on the outskirts of the porphyrin π system, appear as a singlet at 8.7 ppm, compared to 6.2 ppm for the analogous protons in pyrrole. Students can make these comparisons utilizing an NMR spectrum of pyrrole provided by the instructor or found in the literature (*14*). The aromatic protons on the phenyl rings also show the deshielding effect of the porphyrin macrocycle. These protons appear as a series of multiplets from 7.5 to 8.1 ppm. The signal for the methyl protons consists of a series of closely spaced peaks at approximately 2.0 ppm. Models show that the methyl groups extend just inside the perimeter of the porphyrin π system and therefore show a slight shielding effect relative to, for example, the methyl protons on toluene (2.4 ppm in $CDCl_3$).

An expansion of the methyl region of the spectrum is shown in Figure 4. Six peaks are evident in this signal (labeled 1–6 on Figure 4), corresponding to the six different methyl environments in the four atropisomers. The integral ratio of 1 : 2 : 1 : 1 : 2 : 1 is consistent with a statistical distribution of atropisomers (where three peaks of relative intensity 1 : 2 : 1 are due to the three types of methyls in the 3,1 isomer). While it is certain that peaks 2 and 5 are due to the more abundant types of methyls (those in the *cis*-2,2 isomer and the two equivalent methyl groups in the 3,1 isomer, labeled *b* in Figure 1), it is not possible to make an exact peak assignment or to assign the other bands to a particular atropisomer based on this NMR spectrum alone. One can make an initial assumption that the methyl groups in the 4,0 isomer, which have three cofacial methyl neighbors, and the *c* methyl in the 3,1 isomer, which has no cofacial methyl neighbors, represent the two extremes and likely correspond to peaks 1 and 6.

Although the atropisomers are not completely separable via chromatography, fractions enriched in certain isomers and depleted in others can be obtained using a silica gel column with a nonpolar mobile phase. An examination of changes in the relative integrals of the six methyl peaks in the NMR spectra of these samples leads to positive peak identification. NMR spectra of early, middle, and late fractions of T(*o*-Tol)P obtained chromatographically are shown in Figure 5. The early fraction (Fig. 5a) shows a greatly enhanced intensity for peak 1, whereas peak 4 is nearly absent and peak 5 is diminished. In the spectrum of the middle fraction (Fig. 5b), peaks 4 and 5 increase at the expense of peak 1; this trend is continued in the later fraction (Fig. 5c). In all of the spectra, a 2 : 1 : 1 ratio is maintained for peaks 2, 3, and 6, and therefore it can be concluded that these peaks correspond to the 3,1 isomer. For reasons discussed above, peak 2 can be assigned to the two equivalent *b* methyl groups, and peak 6 most likely corresponds to the *c* methyl group. Therefore, peak 3 can be assigned to the *a* methyl group.

The 4,0 isomer can now be assigned to peak 1, the peak that is enhanced in the early fraction. This is consistent with the expected elution order for the atropisomers. With a nonpolar ortho substituent (for example, hexadecylamido),

the 4,0 isomer has the shortest retention time on silica gel (*6*). The remaining peaks, 4 and 5, correspond to the methyl groups on *trans*-2,2 and *cis*-2,2, respectively. This assignment can be made based on the fact that the integral of peak 5 is twice as large as that of peak 4 in the spectrum of the statistical mixture of atropisomers, in which the *cis*-2,2 and *trans*-2,2 isomers are expected to exist in a 2:1 ratio. From the absence of peak 4 in the spectrum of the early fraction, it can be concluded that the *trans*-2,2 isomer has the longest retention time on silica gel.

The peak assignments are summarized in Table 1 along with the chemical shift data and information about the number of cofacial methyl neighbors encountered for each type of methyl group. It is evident that the chemical shift of a particular methyl group decreases as the number of cofacial methyl groups increases.[1] Surprisingly, the shielding effect due to methyl groups on the same face of the porphyrin ring appears to be slightly greater for methyls on opposite phenyl rings than for those on adjacent phenyl rings.

Experimental Procedure

Synthesis of T(o-Tol)P (15)

In a 50-mL round-bottom flask, *o*-tolualdehyde (0.666 mL, 5.76 mmol) is combined with 20 mL of propionic acid. The solution is heated to reflux with stirring. When a steady rate of reflux is achieved, pyrrole (0.400 mL, 5.76 mmol) is quickly added via a digital pipet through the reflux condenser. The resulting black solution is allowed to reflux for an additional 40 min, and is then cooled to room temperature by immersing the flask in a cold water bath. After 15 min of additional cooling in an ice bath, the porphyrin is collected by vacuum filtration on a Hirsch funnel. The fine purple solid is washed with 3×5 mL of methanol and dried in a vacuum oven (80 °C) for 30 min. A yield of 8–10% is typical.

Column Chromatography

A silica gel (200–425 mesh) column (1 cm i.d.) is packed as a slurry in mobile phase (2:1 cyclohexane:chloroform) to a height of 18 cm. A 2–3-mm layer of sea sand is placed on the top of the silica gel layer. A 20–25-mg sample of T(*o*-Tol)P dissolved in 2 mL of the mobile phase is filtered through a cotton-plugged disposable pipet (to remove a dark, insoluble impurity) and carefully loaded onto the column. Additional small portions of mobile phase are added and loaded onto the column until the liquid above the column is clear. Elution is then carried out with the mobile phase at a flow rate of about 1 drop/3 s until the dark porphyrin band emerges. A green impurity band may be observed in front of the porphyrin band, and this should be collected separately and discarded. Three or four fractions of the dark purple/brown porphyrin are collected (approximately 3–4 mL each), evaporated, and dried in a vacuum oven. The chromatography requires approximately 100 mL of the mobile phase and about 2 hours.

Nuclear Magnetic Resonance Spectroscopy

Samples are prepared by dissolving 5 mg of T(*o*-Tol)P, or an evaporated residue from the chromatographic separation, in 0.5 mL of $CDCl_3$ and filtering through a cotton-plugged disposable pipet into a 5-mm NMR tube. The proton NMR spectrum is recorded from –3 to 10 ppm, and the methyl region is then expanded (1.96–2.12 ppm) and plotted. A Bruker AC-300 FT-NMR (16 scans) was used to obtain all spectra.

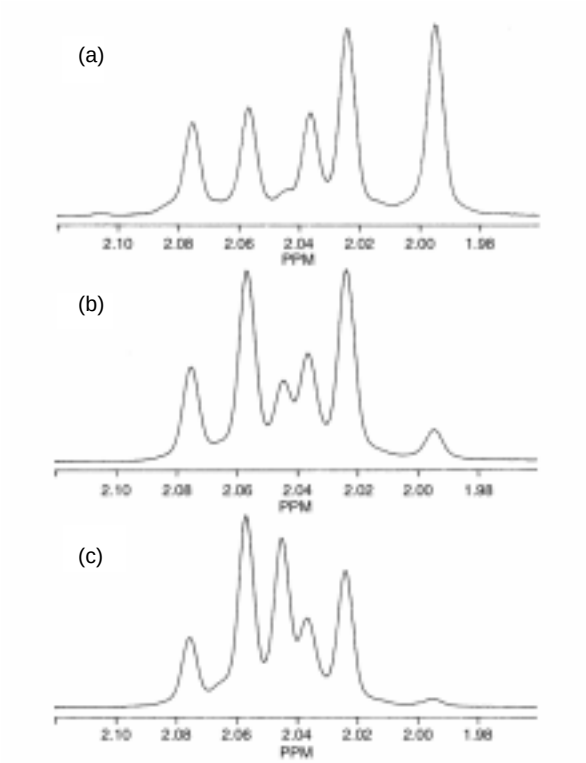

Figure 5. Expansion of ^{1}H NMR methyl signal for (a) early fraction; (b) middle fraction; and (c) late fraction following column chromatography of T(*o*-Tol)P.

Table 1. Methyl Signal Assignments in ^{1}H NMR Spectrum of T(*o*-Tol)P

Peak No.	δ (ppm)	Assignment	No. of Cofacial Methyl Neighbors
1	1.995	4,0	1 opposite, 2 adjacent
2	2.024	3,1 *b*	1 opposite, 1 adjacent
3	2.037	3,1 *a*	2 adjacent
4	2.045	*trans*-2,2	1 opposite
5	2.057	*cis*-2,2	1 adjacent
6	2.076	3,1 *c*	0

Implementation

At least two laboratory sessions are required to complete this experimental module. Students work in groups of two or more. While the synthesis is in progress, students are provided with structure diagrams of the four atropisomers of T(*o*-Tol)P (Fig. 1) and are asked to determine the relative amounts of the four atropisomers expected for a statistical distribution. They are instructed to use simplified drawings representing the structures, and to count the number of ways each isomer can be represented. Students must then predict the number of unique methyl groups as well as their relative abundances. The peaks in the expanded methyl region of the original synthetic atropisomer mixture are later examined and compared to the students' prediction. ^{1}H NMR spectra of pyrrole and toluene are provided for comparison to the spectrum of T(*o*-Tol)P.

Students are informed that because the 4,0 isomer has the greatest mobility on the silica gel chromatographic column, it tends to be enriched in the early fraction. A series of questions in the lab manual leads students through a stepwise inference of peak assignments based on careful examination of the NMR spectra obtained for the successive fractions.

This has become a popular experiment for our advanced undergraduate students. The synthesis of T(*o*-Tol)P is unusual and interesting, in that eight molecules must come together to form the highly colored, symmetric porphyrin macrocycle. The observation of individual methyl resonances, the correspondence of relative peak integrals to the expected statistical distribution, and the exercise of assigning peaks to the individual atropisomers provides a stimulating and satisfying laboratory experience. The module can be expanded to include a porphyrin metallation reaction and subsequent comparison of the absorption and emission spectra of the free base and metalloporphyrin products.

Note

1. The decrease in chemical shift with increasing number of cofacial methyl neighbors appears to be predominantly a solvation effect. The signal due to the methyl protons on toluene is shifted upfield by 0.3 ppm when the solvent is changed from $CDCl_3$ to toluene-d_8. Likewise, the signals due to the methyl groups in T(*o*-Tol)P are all below 2.0 in toluene-d_8, consistent with the idea that an upfield shift accompanies a change to a more hydrocarbon-like environment and that cofacial methyl groups provide such an environment.

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