Table I. Chemical Shift Nonequivalence of Diastereotopic Protons in Some 2-Phenyl-2-methoxyethylphosphonium Bromides (3)

Starting phosphine (2)				Δν, Hz, at 60 MHz		
R_1	R_2	R_3	Solvent	OCH_3[a]	PCH_3[b]	Anisyl OCH_3
Methyl	Cyclohexyl	Benzyl[c]	D_2O	2.7	0.5	
Methyl	Phenyl	*n*-Propyl[d]	D_2O	4.2	9.2	
Methyl	Phenyl	*p*-Anisyl	D_2O	0.9	*e*	*e*
Methyl	Phenyl	*o*-Anisyl	D_2O	2.2	5.8	3.3
Methyl	Phenyl	*o*-Anisyl	$(CD_3)_2CO$	6.0	3.0	2.3
Phenyl	β-Naphthyl	*o*-Anisyl	$CDCl_3$	1.5		1.9
Phenyl	β-Naphthyl	*o*-Anisyl	C_6H_5CN	*e*		4.2

[a] Refers to the methoxyl group derived from **1**. [b] J_{PCH} = 13.5–14.5 Hz. [c] At 60 MHz, the benzyl methylene protons in both diastereomers appear as broadened A_2 (rather than AB) spin systems, split by phosphorus. Δν for the two diastereomers is 2.5 Hz; J_{PCH} is 15 Hz for each. [d] Using **4**, Δν for OCH_3 and PCH_3 protons is 5.1 and 7.4 Hz, respectively. Δν for the diastereotopic methine protons is 9 Hz; J_{PCCH} is 7.5 Hz for each. [e] Only a broad signal was observed at 60 MHz.

mides (**3**), and, by virtue of the chemical shift nonequivalence observed for the diastereotopic protons in the product mixture, it provides a convenient means for estimating the optical purity of aliphatic, aromatic, and mixed tertiary phosphines.[4]

$$\underset{\mathbf{1}}{C_6H_5CH(OCH_3)CH_2Br} + \underset{\mathbf{2}}{PR_1R_2R_3} \longrightarrow \underset{\mathbf{3}}{C_6H_5CH(OCH_3)CH_2P^+R_1R_2R_3Br^-}$$

Reduction of optically pure methyl (*S*)-O-methylmandelate[5] with lithium aluminum hydride gives 2-phenyl-2-methoxyethanol (bp 75° (1 mm)); mesylation under standard conditions, followed by treatment of the mesylate with sodium bromide in DMSO at 80° (12 hr), affords **1**, bp 59° (0.5 mm), $n^{22.5}D$ 1.5419, $[\alpha]D$ +73° (methanol), in 40–80% over-all yield from O-methylmandelic acid. Where signals from the methylene protons in **3** interfere with the pmr analysis, the reagent of choice is 2-phenyl-2-methoxyethyl-1-d_2 bromide (**4**), $n^{22.5}D$ 1.5399, $[\alpha]D$ +73° (methanol), prepared as above by reduction with lithium aluminum deuteride.

When various representative racemic phosphines (**2**) were heated under reflux for 7 hr with a twofold excess of **1** in oxygen-free dry benzene, **3** precipitated from solution. After addition of hexane (to complete precipitation), the mixture of diastereomers was isolated and dried, and the pmr spectrum was recorded. The chemical shift differences of diastereotopic protons are listed in Table I. There is a pronounced doubling of resonance signals and an appreciable solvent effect. Given that the reaction is essentially quantitative,[6] that quaternization proceeds stereospecifically with retention of configuration at phosphorus,[7] that thermal racemization of **2** is minimized by the mildness of the reaction conditions,[8] and that no racemization at the asymmetric carbon center can take place, the method thus lends itself ideally to the stated purpose.

To test the accuracy of the method, methylphenylpropylphosphine (**5**), $[\alpha]D$ −8.6° (toluene), obtained by hexachlorodisilane reduction[9] of methylphenylpropylphosphine oxide (**6**), $[\alpha]D$ +10.5° (methanol), was quaternized with **4**. From the relative intensities of the diastereotopic OCH_3 and PCH_3 pmr signals in the phosphonium salt (Table I), an optical purity of 44% was calculated[3] for the precursor **5**. A portion of the same sample of **5** was oxidized with hydrogen peroxide to the phosphine oxide, **7**, $[\alpha]D$ −8.8° (methanol),[10] and another portion quaternized with benzyl bromide to the P-benzylphosphonium salt, **8**, $[\alpha]D$ −16.0° (methanol). It follows that the absolute rotations of **5**, **7**, and **8** are 19.5, 20.0, and 36.4°, respectively, in good accord with the highest rotations reported[7,11] for these compounds, 18.4, 19.6, and 36.8°, respectively.

(4) Where applicable, doubling of nmr signals of tetrahedral bis(phosphine)nickel(II) bromide complexes in chloroform (W. D. Horrocks, Jr., private communication) or of phosphine oxides in chiral solvents (W. H. Pirkle, private communication) may also be employed.

(5) J. Jacobus, M. Raban, and K. Mislow, *J. Org. Chem.*, **33**, 1142 (1968).

(6) As judged by the ratio of intensities of the signals due to the diastereotopic protons, which is unity within the precision of the measurements. When racemic **2** and *racemic* **1** are allowed to react, however, this ratio deviates from unity by as much as 10%, reflecting[5] the difference in the rate constants of formation of the diastereomeric phosphonium salts.

(7) L. Horner, *Pure Appl. Chem.*, **9**, 225 (1964).

(8) For example, methylphenylpropylphosphine (**5**) racemizes in decalin at 130° with a half-life of 301 min (L. Horner and H. Winkler, *Tetrahedron Lett.*, 461 (1964)).

(9) K. Naumann, G. Zon, and K. Mislow, *J. Amer. Chem. Soc.*, **91**, 2788 (1969).

(10) From a comparison of the specific rotations of **6** and **7**, it follows that the hexachlorodisilane reduction[9] proceeded with only 84% net inversion. As detailed in a forthcoming paper, the incomplete stereospecificity can be attributed to chemical racemization of produced **5** under the conditions of the reduction.

(11) D. B. Denney and J. W. Hanifin, Jr., *Tetrahedron Lett.*, 2177 (1963).

(12) (a) Public Health Service Postdoctoral Fellow, 1968–1969; (b) Public Health Service Predoctoral Fellow, 1968–1969.

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Conformational Analysis of the 2,5-Di-*t*-butylcyclohexanol System. Evaluation of the Steric Effect of the *t*-Butyl Group in Cyclohexane Chair and Twist-Boat Conformations[1]

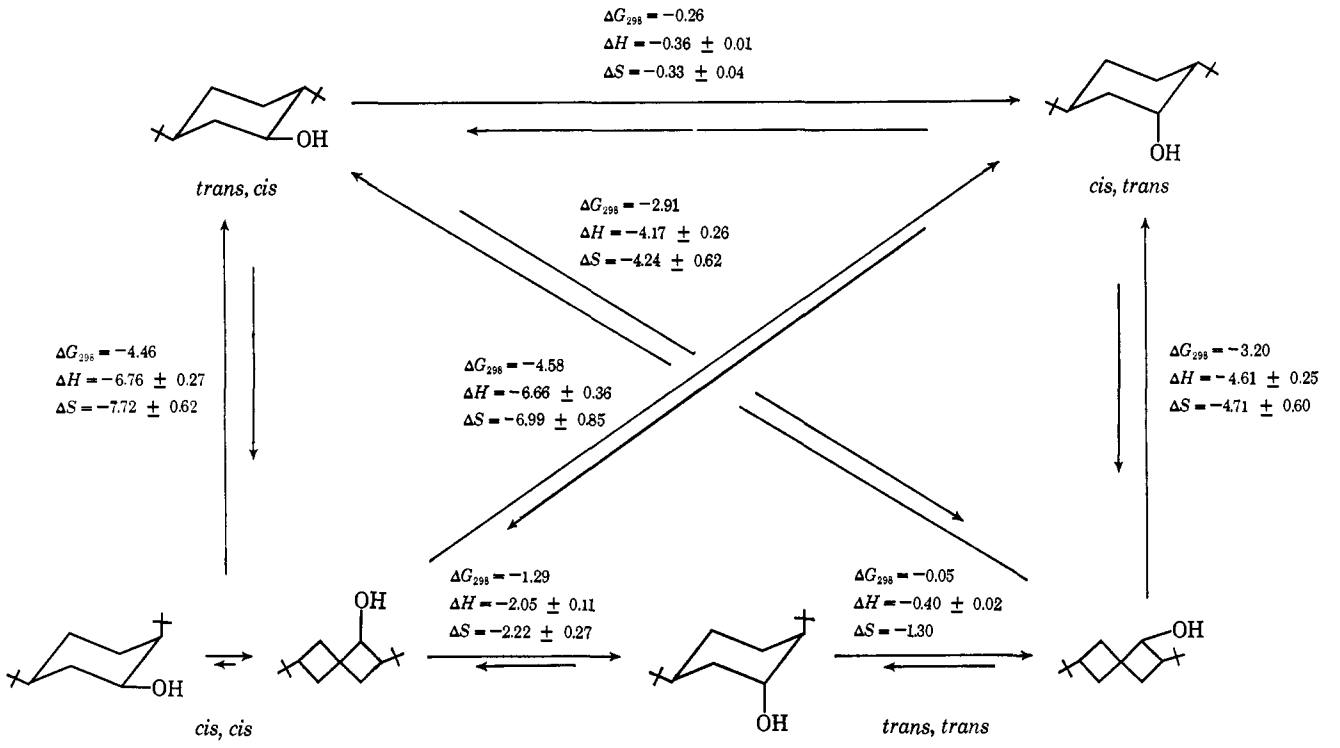
Sir:

Conformational analysis of the cyclohexane ring system has demonstrated that the chair form is appreciably more stable than the boat or twist-boat form. Allinger and Freiberg[2] have measured the enthalpy and entropy difference between *trans*- and *cis*-1,3-di-*t*-butylcyclohexane to be 5.9 ± 0.6 kcal/mol and +4.9

(1) Research supported by the Alred P. Sloan Foundation.

(2) N. L. Allinger and L. A. Freiberg, *J. Amer. Chem. Soc.*, **82**, 2393 (1960).

Scheme I[a]



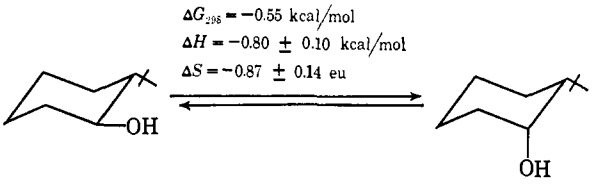
[a] ΔG and ΔH values in kcal/mol; ΔS values in eu.

± 1.0 eu. The twist-boat conformation of the *trans* isomer is more stable than the chair conformation with an axial *t*-butyl group, the mean enthalpy difference having been estimated to be 0.37 ± 0.20 kcal/mol.[3]

Johnson and coworkers[4] have measured the energy difference between the chair and twist-boat forms of a cyclohexane contained in a polycyclic structure to be about 5.5 kcal/mol. From an infrared study of hydrogen bonding in a number of *cis,cis,cis*-2,5-dialkyl-1,4-cyclohexanediols Stolow and coworkers[5] concluded that the *sec*-alkyl and *t*-alkyl systems exist ~80 and >98% in nonchair conformations.

The availability of the four stereoisomeric 2,5-di-*t*-butylcyclohexanols[6] provided an intriguing system for conformational analysis. The four alcohols can be equilibrated over Raney nickel in cyclohexane at temperatures between 78.5 and 258°.[7,8] Plots of log K *vs.* $1/T$ were quite nearly linear up to 200°; however, considerable deviation from linearity became apparent at temperatures above 200°. The enthalpies and entropies for the various possible equilibria were calculated and are given in Scheme I.

Of the two isomers with *trans*-*t*-butyl groups, the *cis,trans* isomer with the axial hydroxyl surprisingly is more stable than the *trans,cis* isomer ($\Delta G_{298} = -0.26$ kcal/mol). Equilibration of the *cis*- and *trans*-2-*t*-butylcyclohexanols similarly shows that the *cis* isomer with the axial hydroxyl is more stable than the *trans* diequatorial isomer ($\Delta G_{298} = -0.55$ kcal/mol). It



is interesting to note that in both the mono- and di-*t*-butyl systems the isomer with axial hydroxyl is enthalpically favored, whereas the isomer with equatorial hydroxyl is entropically favored.

These results indicate that the net repulsion between the equatorial *t*-butyl and hydroxyl groups (t-Bu_{eq}-OH_{eq}) in *trans,cis*-2,5-di-*t*-butylcyclohexanol and *trans*-2-*t*-butylcyclohexanol is greater than the net repulsion in the t-Bu_{eq}-OH_{ax} systems. A minimum value for this extra diequatorial interaction can be calculated by adding to the ΔG values for the two equatorial $\rightleftarrows$ axial equilibria discussed above the ΔG's for hydroxyl in the 4- and 3-*t*-butylcyclohexanols (0.64 + 0.55 or ~0.9 + 0.26 kcal/mol, respectively),[9] giving a free energy difference of $\gtrsim$1.2 kcal/mol. This observation may be contrasted with the finding of Sicher and Tichy[10] that the CH_{3eq}-OH_{eq} interaction (0.38 kcal/mol) is less than CH_{3eq}-OH_{ax} (0.66 kcal/mol) and CH_{3ax}-OH_{eq} (0.83 kcal/mol).

(3) N. L. Allinger, J. A. Hirsh, M. A. Miller, I. J. Tyminski, and F. A. Van-Catledge, *J. Amer. Chem. Soc.*, **90**, 1199 (1968).

(4) W. S. Johnson, V. J. Bauer, J. L. Margrave, M. A. Frisch, L. H. Dreger, and W. N. Hubbard, *ibid.*, **83**, 606 (1961).

(5) R. D. Stolow, P. M. McDonagh, and M. M. Bonaventura, *ibid.*, **86**, 2165 (1964).

(6) D. J. Pasto and F. M. Klein, *Tetrahedron Lett.*, 963 (1967).

(7) E. L. Eliel and S. H. Schroeter, *J. Amer. Chem. Soc.*, **87**, 5031 (1965).

(8) The rates of attainment of equilibrium were quite slow, requiring 15 days at 78.5°. The equilibrium mixtures were approached starting with at least two of the four stereoisomeric alcohols at each temperature. The analyses were carried out by glpc using a 45-ft column of 5.0% Carbowax 20M on Chromosorb G with a flame ionization detector.

(9) E. L. Eliel, S. H. Schroeter, T. J. Brett, F. J. Biros, and J. C. Richer, *J. Am. Chem. Soc.*, **88**, 3327 (1966).

(10) J. Sicher and M. Tichy, *Collection Czech. Chem. Commun.*, **37**, 3687 (1967).

The ΔH and ΔS values for the *cis,cis* ⇄ *trans,cis* equilibrium are consistent with a twist-boat ⇄ chair interconversion. The possibility that the *cis,cis* isomer might exist in a chair ⇄ twist-boat equilibrium was investigated by variable-temperature infrared techniques (3% solution in carbon disulfide). No changes in relative intensities were noted, indicating that the *cis,cis* isomer must exist almost exclusively in a single conformation. As the *cis,cis* isomer exists essentially only in the twist-boat conformation, one can readily calculate ΔG_{298}, ΔH, and ΔS for the 1,4-di-*t*-butylcyclohexane twist-boat ⇄ chair interconversion by removing the effects of the hydroxyl. Subtracting the appropriate values of ΔG_{298}, ΔH, and ΔS for $OH_{ax} \rightleftarrows OH_{eq}$ interconversion[11] in the cyclohexane system from the data given in Scheme I gives $\Delta G_{298} = -5.7$ kcal/mol, $\Delta H = -7.7 \pm 0.29$ kcal/mol, and $\Delta S = -8.0 \pm 0.7$ eu.

The thermodynamic parameters for the various equilibria involving the *trans,trans* isomer, as well as the lack of linearity of the log K *vs.* $1/T$ plots at higher temperatures, indicate that the *trans,trans* isomer is not conformationally homogeneous. The infrared spectra of the *trans,trans* isomer (3% solution in carbon disulfide) showed significant changes in band intensities with changes in temperature. Using the variable-temperature infrared data,[12] values for ΔH and ΔS for the $\text{chair}_{t,t} \rightleftarrows \text{twist-boat}_{t,t}$ equilibrium were calculated to be -0.42 kcal/mol and -1.30 eu, respectively, with $\Delta G_{298} \approx -0.05$ kcal/mol. The unexpectedly low value for ΔS for this chair ⇄ twist-boat equilibrium can be rationalized on the basis that the expected dipseudoequatorial *t*-Bu–OH interaction in the twist-boat conformation restricts the twist-boat to a small part of its ordinary pseudorotational circuit resulting in a reduction in entropy. A more detailed discussion of these results and those of related systems will appear at a later time.

Acknowledgment. The authors wish to thank Professor Ernest L. Eliel for helpful discussions concerning this work.

(11) E. L. Eliel, D. G. Nielson, and E. C. Gilbert, *Chem. Commun.*, 360 (1968).

(12) The temperature range covered was -90 to $+65°$ in carbon disulfide solution. Although several sets of peaks changed in relative intensity, two reasonably well-resolved, medium–weak peaks at 1032 and 1016 cm^{-1} were used in the determination of the thermodynamic parameters. The extinction coefficients of the two peaks were assumed to be equal.

(13) Alfred P. Sloan Research Fellow, 1967–1969.

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The Chemistry of the Modification of Tryptophan with 2-Hydroxy-5-nitrobenzyl Bromide

Sir:

The increasing use of the reagent 2-hydroxy-5-nitrobenzyl bromide for the modification and quantitative estimation of tryptophan in proteins[1,2] dictates the necessity of determining the structures of the products of the modification reaction. Recently, Spande, Wilchek, and Witkop[3] and Schellenberg, Chan, and McLean[4] made significant contributions to this end by elucidating the structures of some products of the reactions of model indole compounds with 2-hydroxy-5-nitrobenzyl bromide. In this work we wish to report the products formed during modification of tryptophan ethyl ester to produce 1:1 adducts. These and previous studies allow us to define quite completely the possible modes of reaction of 2-hydroxy-5-nitrobenzyl bromide with tryptophan in proteins to produce the monosubstitution products which are usually observed.

A complex mixture resulted when 500 mg of L-tryptophan ethyl ester hydrochloride (**1**) was allowed to react with 500 mg of 2-hydroxy-5-nitrobenzyl bromide (**1**) in aqueous acetone at pH 4.7. Unreacted **1**, 2-hydroxy-5-nitrobenzyl alcohol, and its methyl ether were identified by conventional methods. Two components, **3** and **4**, in a ratio of 57:43, were identified as monosubstitution products, and the two remaining components, **5** and **6**, were shown to be disubstitution products derived from **3** and **4**, respectively, by further reaction with 1 equiv of 2-hydroxy-5-nitrobenzyl bromide.

Compounds **3** (mp 189–190°) and **4** (mp 198–199°) had identical elemental analyses, identical infrared spectra, and identical electronic spectra: λ_{max} (95% ethanol) 240 mμ (ε 11,800), 310 mμ (8900); λ_{max} (2 *N* NaOH) 422 mμ (19,800).[5] When **3** and **4** were refluxed in 3:1 (v/v) ethanol–concentrated HCl, both yielded the same compound, **7**, which upon acetylation gave an N,O-diacetyl derivative (**7**-Ac_2) possessing characteristic indole uv absorption. The similarity of the two nonidentical compounds **3** and **4** can be explained by the formation of a new asymmetric center when the hydroxynitrobenzyl group adds at the 3 position of the indole ring;[6-8] this addition would yield diastereomeric adducts, because the asymmetric α carbon of the tryptophan ethyl ester is present as the L enantiomer. The transformation of **3** and **4** to **7** is evidently an example of an indolenine rearrangement.[7,8]

The 220-MHz nmr spectra confirmed these ideas, except that there was no signal in the aromatic region attributable to the 2-proton of an indolenine ring. This problem was resolved when it became clear that the initial indolenine products, **3′** and **4′**, had undergone nucleophilic attack at the 2 position of the ring by either the side-chain amino group of tryptophan or the *o*-hydroxyl group of the hydroxynitrobenzyl moiety to create a third asymmetric center.[3,7,8] The singlet resonance at δ 5.2 in the spectra of both compounds supports these alternatives.[3,8-10] The presence of a

(1) (a) D. E. Koshland, Jr., Y. D. Kharkhanis, and H. G. Latham, *J. Amer. Chem. Soc.*, **86**, 1448 (1964); (b) H. R. Horton and D. E. Koshland, Jr., *ibid.*, **87**, 1126 (1965).

(2) T. E. Barman and D. E. Koshland, Jr., *J. Biol. Chem.*, **242**, 5771 (1967).

(3) T. F. Spande, M. Wilchek, and B. Witkop, *J. Amer. Chem. Soc.*, 90, 3256 (1968).

(4) (a) K. A. Schellenberg, T. Chan, and G. W. McLean, *Federation Proc.*, **27**, 453 (1968); T. Chan and K. A. Schellenberg, *J. Biol. Chem.*, **243**, 6284 (1968).

(5) Because of the importance of the value of the extinction coefficient of these compounds in the assay and modification procedures, this number was determined in strong base from a Beer's law plot to be ϵ_{410} 18,450, essentially identical with the value for the free alcohol (*cf.* ref 2).

(6) E. H. Rodd, Ed., "The Chemistry of Carbon Compounds," Vol. IV, Elsevier Publishing Co., New York, N. Y., 1951, p 41 ff.

(7) A. H. Jackson and A. E. Smith, *Tetrahedron*, **21**, 989 (1965).

(8) P. L. Julian, E. W. Meyer, and H. C. Printy in "Heterocyclic Compounds," Vol. III, R. C. Elderfield, Ed., John Wiley & Sons, Inc., New York, N. Y., 1952, p 103 ff.