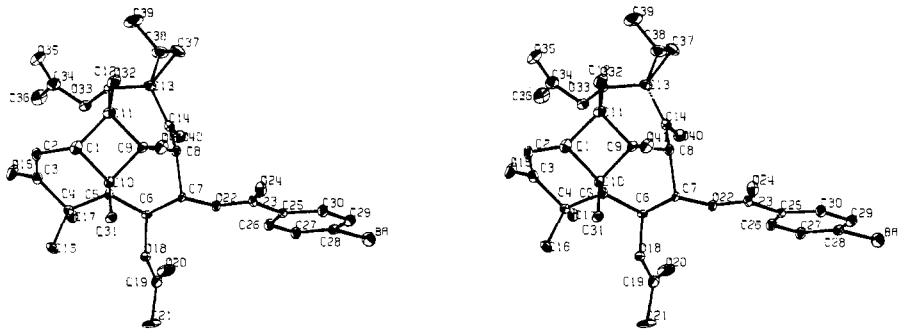

Figure 1. Stereoscopic view of the molecule IIb.

proton at 4.49 (d, $J_{7,6}$ = 2 Hz, 7β-H), one C*H*OAc proton at 4.85 (s, 12β-H), one C*H*OAc proton at 5.44 (t, $J_{6,7}$ = 2 Hz, $J_{5,6}$ = 0.5 Hz, 6α-H) (observed by decoupling experiments). The structural assignment of this compound was settled by careful oxidation with Jones reagent (60 min, 10°), whereupon barbatusin (Ia) was obtained in 80% yield.

Cyclobutatusin (IIa) and 3β-hydroxy-3-deoxobarbatusin (Ib) join the class of natural products with a cyclopropane ring, the biological and biogenetical importance of which is now fully recognized.[7] Although a number of monoterpenes and sesquiterpenes with a four-membered ring are known,[8] cyclobutatusin appears to be the first naturally occurring substance in the diterpenoid series with such a feature. The formation of cyclobutanol derivatives upon irradiation of steroids[9] and triterpenoids[10] has been extensively studied and our present findings raise the question of whether cyclobutatusin is part of a biogenetic sequence for quinonoid-type diterpenes[3] or whether it may be the product of a photochemically induced reaction of a barbatusin-type precursor. Investigations to resolve this question are now in progress. Pharmacological testing involving antitumor and antibacterial assays with barbatusin and cyclobutatusin are under way and will be reported in due course.[11]

Acknowledgments. The work was supported by National Institutes of Health Grant GM 19336 (A. H.-J. W. and I. C. P.) and by the Conselho Nacional de Pesquisas, Brazil, Grant 10468/68 (R. Z.).

Supplementary Material Available. The final atomic coordinates for IIb will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-580.

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A Model for the Proton Transfer Stages of the Biological Transaminations and Isotopic Exchange Reactions of Amino Acids[1]

Sir:

Stereospecific transamination reactions are important to the biological elaboration of amino acids.[2] The enzyme-catalyzed reactions of eq 1 are stereospecific, and the proton (or isotope) transfer occurs intramolecularly.[3,4] Pyridoxal-containing enzymes catalyze isotopic exchange of the α hydrogens of L-amino acids with a high retention of configuration.[5] The reactions of eq 2 were stereospecific and occurred partially intramolecularly.[6] The starting imine underwent isotopic

(1) This work was supported by U. S. Public Health Service Research Grant No. GM 12640-09 from the Department of Health, Education and Welfare.

(2) (a) A. E. Braunstein and M. M. Shemyakin, *Biokhimiya*, 18, 393 (1953); (b) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Amer. Chem. Soc.*, 76, 648 (1954); (c) E. E. Snell in "Chemical and Biological Aspects of Pyridoxal Catalysis," P. M. Fasella, A. E. Braunstein, and A. Rossi-Fanelli, Ed., Macmillan Co., New York, N. Y., 1963, pp 1–12; (d) E. E. Snell, A. E. Braunstein, E. S. Severin, and Yu. M. Torchinsky, Ed., "Pyridoxal Catalysis: Enzymes and Model Systems," Interscience, New York, N. Y., 1968.

(3) (a) H. C. Dunathan, L. Davis, P. G. Gray, and M. Kaplan, *Biochemistry*, 7, 4532 (1968); (b) J. E. Ayling, H. C. Dunathan, and E. E. Snell, *ibid.*, 7, 4537 (1968); (c) G. B. Bailey, T. Kusamrarm, and K. Vuttirej, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 29, 857 (1970); (d) B. S. Sukhareva, H. C. Dunathan, and A. E. Braunstein, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, 15, 241 (1971); (e) P. Besmer and D. Arigoni, *Chimia*, 23, 190 (1969).

(4) For a review of the mechanism, see W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, pp 143–145.

(5) A. F. Thomas, "Deuterium Labeling in Organic Chemistry," Appleton-Century-Crofts, New York, N. Y., 1971, pp 428–430.

(6) (a) D. J. Cram and R. D. Guthrie, *J. Amer. Chem. Soc.*, 87, 397 (1965); (b) R. D. Guthrie, D. A. Jaeger, W. Meister, and D. J. Cram, *ibid.*, 93, 5137 (1971).

(7) A. Burger, *Progr. Drug Res.*, 15, 227 (1971); C. D. Poulter, O. J. Muscio, C. J. Spillner, and R. G. Goodfellow, *J. Amer. Chem. Soc.*, 94, 5921 (1972); J. H. Law, *Accounts Chem. Res.*, 4, 199 (1971).

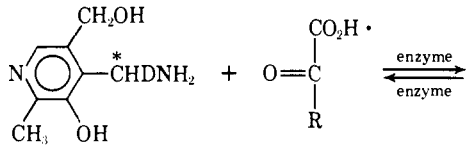
(8) See, for example, T. K. Devon and A. I. Scott, "Handbook of Naturally Occurring Compounds, Vol. II: Terpenes," Academic Press, New York, N. Y., 1972.

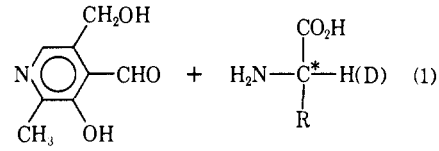
(9) J. Iriarte, K. Schaffner, and O. Jeger, *Helv. Chim. Acta*, 46, 1599 (1963); E. Altenburger, H. Wehrly, and K. Schaffner, *ibid.*, 48, 704 (1965).

(10) J. Fried and J. W. Brown, *Tetrahedron Lett.*, 1677 (1966); M. Mousseron-Canet and J. P. Chabaud, *Bull. Soc. Chim. Fr.*, 239 (1969); B. W. Finucane and J. B. Thomson, *J. Chem. Soc. C*, 1569 (1971).

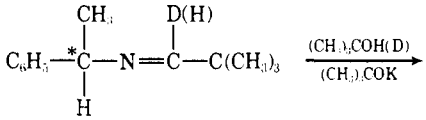
(11) See paragraph at end of paper regarding supplementary material.

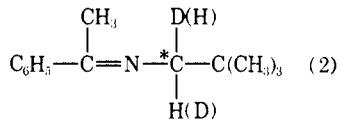
exchange with the medium with high retention.[6] The reactions of eq 3 now have been studied.



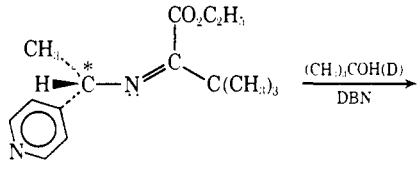


(1)

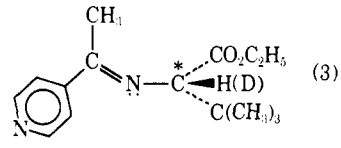




(2)



(−)-(*S*)-1



(3)

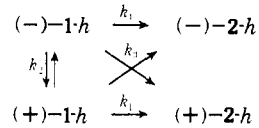
(−)-(*S*)-2

Optically pure (−)-(*S*)-α-(4-pyridyl)ethylamine[7] ((−)-(*S*)-**3**) with[8] ethyl trimethylpyruvate[9a,b] gave (51%) (−)-(*S*)-**1**[9a-d] $[\alpha]^{25}_{546}$ −93.3° (*c* 0.570, C_2H_5OH). Optically pure (+)-(*S*)-ethyl 2-amino-3,3-dimethylbutanoate ((+)-(*S*)-**4**)[9a-c] and 4-pyridyl methyl ketone[7] gave (65%) (−)-(*S*)-**2**,[9a-d] $[\alpha]^{25}_{546}$ −53.2° (*c* 0.41, $CHCl_3$). Pmr and glc analyses[9d] indicated that **1** and **2** were geometrically pure. Molecular models (Corey–Pauling–Koltun, CPK) of only these geometric isomers are able to be assembled. Hydrolysis of (−)-(*S*)-**1**[9c] (1 *N* HCl at 0°) gave optically pure (−)-(*S*)-**3**, and (−)-(*S*)-**2**[9c] gave optically pure (+)-(*S*)-**4**. Reflux (60 hr) of (±)-**3** and paraformaldehyde in D_2O–CF_3CO_2D (pD 4.5) gave (68%) **3**-*d*,[9a,b] 0.97 atom of D in the α position (pmr). This material was converted to **1**-*d*,[9a-e] >95% of one atom of D.[9e] Amino ester **4**[9a,b] was resolved (35%) to optical purity (1:1 dibenzoyl-*d*-tartarate salt) to give (+)-(*S*)-**4**,[9a-d] $[\alpha]^{25}_{546}$ +58.3° (*c* 0.64, $CHCl_3$). Control experiments demonstrated that (−)-(*S*)-**3** and (+)-(*S*)-**4** were optically stable to glc.[9c,d] Hydrolysis ($H_3O^+Cl^-$) of (+)-(*S*)-**4** gave (−)-(*S*)-*tert*-leucine, $[\alpha]^{25}_{546}$ −8.9° (*c* 1.40, H_2O), of known configuration.[10] That (+)-(*S*)-**4** was optically pure was demonstrated by its mild hydrolysis and conversion[11] to (+)-(*S*)-*tert*-leucine-*N*-tosylamide,[9a,b] mp 240–242°, $[\alpha]^{25}_{546}$ +51.3° (*c* 1.02, C_2H_5OH). Independent resolution (brucine salt) of the racemate[9a,b] gave identical material.[9a,b]

Reactions were conducted at 50° in $(CH_3)_3COH(D)$–0.5 *M* DBN–0.40 *M* **1**, or 0.10 *M* **2**. Glc analysis,[9d] separation,[9c] and polarimetric and isotopic analyses[9e] of **1** and **2** obtained after 30–60% conversions provided estimates of pseudo-first-order rate constants (one point) for **1** → **2** (k_t), (−)-**1** → (±)-**1** (k_α)[1], (−)-**2** → (±)-**2** (k_α)[2], **1**-*h* → **1**-*d* (k_e)$^{1\text{-}h}$, and **2**-*h* → **2**-*d* (k_e)$^{2\text{-}h}$. Control runs (internal standards) demonstrated ≳99% of **1** plus **2** accounted for, no changes in amounts or rotations during isolation, and no reactions without DBN. After 811 hr, **1**-*h* gave **2**-*h* with <0.5% of **1**-*h* remaining. Thus, $K = [2]/[1] \gtrsim 200$. Rate constant ratios (two–five runs each) were: $(k_t/k_\alpha)_{ROH}{}^{1\text{-}h} \simeq 1.6$–1.9; $(k_t/k_\alpha)_{ROD}{}^{1\text{-}h} \simeq 1.5$–1.9; $(k_e/k_\alpha)_{ROD}{}^{1\text{-}h} \simeq 0.23$–0.28 (DBN–DI (0.003 *M*) was present); $(k_e/k_\alpha)_{ROD}{}^{2\text{-}h} \simeq$ 7–10 (DBN–DI (0.005 *M*) was present); $(k_t)_{ROH}{}^{1\text{-}h}/(k_\alpha)_{ROH}{}^{2\text{-}h} \simeq 30$. Values for $(k_t)_{ROD}{}^{1\text{-}d}$, $(k_e)_{ROH}{}^{1\text{-}d}$, and $(k_t)_{ROH}{}^{1\text{-}d}$ were estimated, and the **2** isolated was analyzed for deuterium. Optically pure (−)-(*S*)-**1**-*h* in ROH gave (−)-(*S*)-**2**-*h* of 10% optical purity. Optically pure (−)-(*S*)-**1**-*h* in ROD gave (−)-*S*-**2** of 9–12% optical purity (several runs).

Stereochemical rate constants k_1, k_2, and k_3 of Chart I

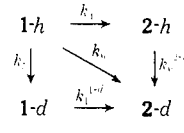
Chart I



were estimated from the values of k_t and k_α, enantiomer concentrations of **1** and **2** at times *t*, and equations previously developed.[6b] Pseudo-first-order rate constants × 10^6 sec^{-1} were: $k_1 \simeq 1.5$; $k_2 \simeq 0.68$; $k_3 \simeq 0.93$. Thus, at 50°, $k_1/k_3 \simeq 1.6$. In similar experiments at 25°, $k_1/k_3 \simeq 2.2$.

Isotopic exchange rate constants, k_4, k_5, and k_6 of Chart II, were estimated from values of $(k_e)_{ROD}{}^{1\text{-}d}$ and

Chart II



$(k_e)_{ROD}{}^{2\text{-}h}$, the concentrations of **1**-*h*, **1**-*d*, and **2**-*h* at times *t* and three simultaneous kinetic equations (steady state) derived similarly[6b] to those for Chart I. Pseudo-first-order rate constants × 10^6 sec^{-1} were: $k_4 \simeq 0.34$; $k_5 \simeq 0.28$; $k_6 \simeq 1.45$. At 50°, $k_6/k_4 \simeq 4$.

Our conclusions are: (1) *suprafacial* dominated over *antarafacial* isomerization of **1** to **2**. The *net* stereospecific component, 100% $(k_1 - k_3)/(k_1 + k_3) \simeq 23\%$, is explained by the conducted tour process, (−)-(*S*)-**1** → A → B → (−)-(*S*)-**2**. The large pyridyl

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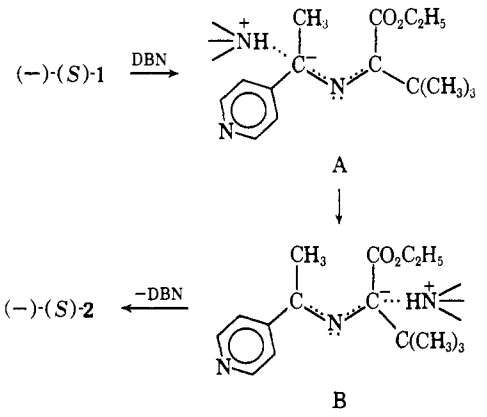
(8) (a) E. P. Kyba, *Org. Prep. Proc.*, **2**, 149 (1970); (b) K. Taguchi and F. H. Westheimer, *J. Org. Chem.*, **36**, 1570 (1971).

(9) (a) Carbon and hydrogen analyses were within 0.30 of theory. (b) The pmr spectra (100 MHz) in $CDCl_3$ were consistent. (c) Preparative glc involved a 2 ft × 0.5 in. column packed with 4% SE-30 on 40 mesh Floropak in an Areograph A-90-P machine (125°). (d) Analytical glc involved in 18 × 0.125 in. column packed with 30% SE-30 on DMCS-treated Chromosorb W (NAW) in a Perkin-Elmer Model 800 machine (125°). (e) Deuterium analyses were performed mass spectrally on an AEI MS9 with direct insertion and involved molecular ions corrected to known samples.

(10) H. Pracejus and S. Winter, *Chem. Ber.*, **97**, 3173 (1964).

(11) D. Theodoropoulos and L. C. Craig, *J. Org. Chem.*, 21, 1376 (1956).



and *tert*-butyl groups maintained geometric homogeneity in **1**, **2**, A, and B. The >NH$^+$ group is visualized as being above the plane of the azaallylic system. (2) Isotopic exchange during isomerization dominated over intramolecularity by a factor of 4. (3) Imine **1** racemized with a large isoinversion component, probably by a conducted tour mechanism involving a symmetrical >NH$^+\cdots$N$^-$C$_6$H$_4$=C– stage.[7] (4) Imine **2** underwent isotopic exchange with high retention of configuration. A likely mechanism[12] involves these stages.

$$\overset{*}{\mathrm{RH}} + \mathrm{RO^-\cdots DN^+} \lessgtr \mathrm{R^-\cdots HOR\cdots DN^+} \lessgtr \xrightarrow[-\mathrm{HOR}]{+\mathrm{DOR}}$$

$$\mathrm{R^-\cdots DOR\cdots DN^+} \lessgtr \overset{*}{\mathrm{RD}} + \mathrm{RO^-\cdots DN^+}\lessgtr$$

The structural similarities between imines **1** and **2** and their biological analogs provide similar reaction pathways. The biological[3] and model systems both possess a stereospecific and intramolecular pathway for a *suprafacial* 1,3-proton transfer across an azaallylic anion. Both possess a stereospecific pathway for an isotopic exchange reaction (retention of configuration) between the α hydrogen of a derivative of an amino acid and the medium. The model differs from the biological system by providing competing stereochemical and isotope-labeling reaction pathways.

(12) (a) W. T. Ford and D. J. Cram, *J. Amer. Chem. Soc.*, **90**, 2606 (1968); (b) K. C. Chu and D. J. Cram, *ibid.*, **94**, 3521 (1972); (c) J. Almy, D. H. Hoffman, K. C. Chu, and D. J. Cram, *ibid.*, **95**, 1185 (1973).

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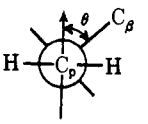
Angular Dependence of β-Carbon Atom Hyperfine Coupling Constants[1]

Sir:

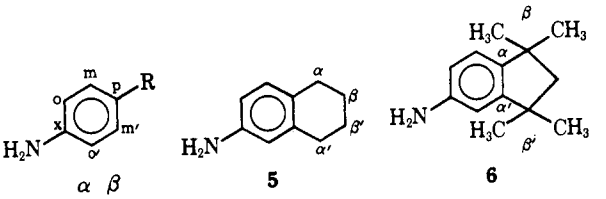
The early observations of the epr constants, a_β^C, for β-carbon atoms in free radicals were related to carbon–carbon hyperconjugation.[2] Progress in the area has been slow, however, due to difficulties in the synthesis of ^{13}C-enriched compounds and in the



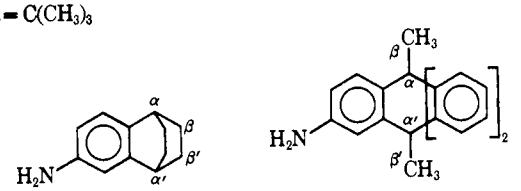
spectroscopy of ^{13}C in natural abundance. Subsequent investigators have adopted eq 1 where ρ_p^π is

$$a_\beta^C = \rho_p^\pi(B_0^C + B_2^C\langle\cos^2\theta\rangle) \quad (1)$$

the spin density in the adjacent p orbital, θ is the dihedral angle, and B_0^C and B_2^C are empirical constants for the analysis of their spectroscopic results.[2,3] In the absence of positive information, it has been assumed that B_0^C is near zero.[3] Curiously, the estimated values of B_2^C range from 10 to 20 G.[2c,3] The wide range may be due, in part, to reliance on data for molecules of uncertain conformation and difficulties in spectral interpretation. On the other hand, Russell and his associates have noted that the a_β^C data for semidiones do not conform to a simple $\langle\cos^2\theta\rangle$ relationship.[3b] The INDO theory[4] predicts that a_β^C for the *n*-propyl radical is linearly dependent on $\langle\cos^2\theta\rangle$, with B_0^C = 1.1 G and B_2^C = 13.8 G. These anomalies, the renewed interest in carbon–carbon hyperconjugation,[5] and the potential use of a_β^C for conformational analysis prompted us to study the contact chemical shifts of β-carbon nuclei resulting from the interaction of aniline derivatives, **1–8**, with nickel acetyl-



1, R = CH_2CH_3
2, R = $CH(CH_3)_2$
3, R = $CH(CH_2)_2$
4, R = $C(CH_3)_3$



acetonate[6] to establish the angular dependence in an unambiguous way.

The resonance signals for **1–8** are readily assigned on the basis of known correlations.[7] The contact chemical shifts, σ_i^C, were measured in the usual way.[6] The shifts relative to the shift, σ_m^C, for the meta carbon atom are summarized in Table I.

The results for the aryl carbon atoms correspond well with prior work with negative values for a_x^C and

(1) This research was supported by the National Science Foundation.
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(3) (a) H. Lemaire, R. Ramasseul, and A. Rassat, *Mol. Phys.*, 8, 557 (1964); (b) G. A. Russell, D. F. Lawson, H. L. Malkus, and P. R. Whittle, *J. Chem. Phys.*, **54**, 2164 (1971); (c) G. P. Laroff and R. W. Fessenden, *ibid.*, **55**, 5000 (1971).
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(7) R. K. Jensen and L. Petrakis, *J. Magn. Resonance*, 7, 105 (1972).