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^{1}H-nmr Study on the Preferred Conformations of Chiral Pyridine-Dinucleotide Models

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The synthesis of four chiral NAD^+ models **1** and their 1,4-dihydro analogs **2** is described. From the temperature dependence of the ^{1}H-nmr spectra it is concluded that for these compounds two preferred conformations I and II, differing slightly in energy, exist. Both conformations are "folded" with the more or less parallel *p*-anisyl and pyridine groups mutually *gauche*, but in I the pyridine group is rotated by about 180° as compared with II, thus leading to a conspicuous difference in orientation of the substituent *Z* (NH_2CO, $C_6H_5NHSO_2$, $(CH_2)_4NSO_2$, or $(C_4H_8ON)SO_2$) in the pyridine ring toward the anisyl group. The most stable conformation (I) has *Z* closest to the center of the *p*-anisyl group. In 360-MHz spectra of the dihydropyridines at low temperature (−10°C), slow interconversion of I and II leads to the observation of an *XY* pattern for the C-4 methylene protons of the 1,4-dihydropyridine system. The anisochronity in this methylene group is caused mainly by the anisotropy of the neighboring *p*-anisyl group.

INTRODUCTION

The conformation of pyridine dinucleotides, both in solution and in their dehydrogenase bound form, has been the subject of many investigations.

In dilute aqueous solution below 25°C, 220-MHz ^{1}H-nmr spectra (*1a*) of NADH and some of its analogs display an *XY* pattern[2] for the C-4 nicotinamide protons ($^2J_{XY} \approx 17$ Hz, $\Delta\delta_{XY} \approx 0.97$ ppm). This has originally been interpreted (*1b*, *1c*) to result from the presence of rapidly interconverting stretched and helical (folded) conformations, the latter with the adenine moiety approaching preferentially the *B*-side of the nicotinamide ring. Recent measurements (*2*) of ^{1}H and ^{13}C spin–lattice relaxation times have shown, however, that—at least for the oxidized form—the conformation of pyridine dinucleotides in solution is highly mobile without any pronounced preference for a single helical conformation. Thus it could be proven (*2b*) that conformations, if any, in which the aromatic rings of NAD^+ approach one another closer than 4.5 Å must have a lifetime considerably shorter than the reorientational correlation time (2.6×10^{-10} sec) of this molecule.

For a number of pyridine dinucleotide-dependent dehydrogenases, the crystal structure of the enzyme/coenzyme complex has been resolved (*3*, *4*). In these complexes the coenzyme is found to adopt a stretched conformation with one side of the nicotinamide group shielded from substrate access. Thus, in the *B*-specific glyceraldehyde 3-phosphate dehydrogenase/NAD^+ system, the *A*-side of the nicotinamide ring

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[2] The notation *XY* instead of *AB* is used here to avoid confusion with the H_A, H_B notation which refers to C-4 protons on the diastereotopic sides of the dihydronicotinamide ring.

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faces (*4*) the aromatic side chain of a tyrosine (Tyr-317) residue with a plane separation of 4 Å in all four subunits.

We have set out to prepare simple model compounds showing a preferential shielding of one side of the nicotinamide group from access by (model) substrates and to investigate their physical and chemical properties.

The present paper describes the synthesis and the ^{1}H-nmr conformation analysis of some of such compounds.

RESULTS AND DISCUSSION

Compounds Studied

We have recently reported (*5*) on the temperature dependence of the nmr spectrum for **1a**. It was shown (*5*, *6*) that the bulky t-Bu group enforces the exclusive population

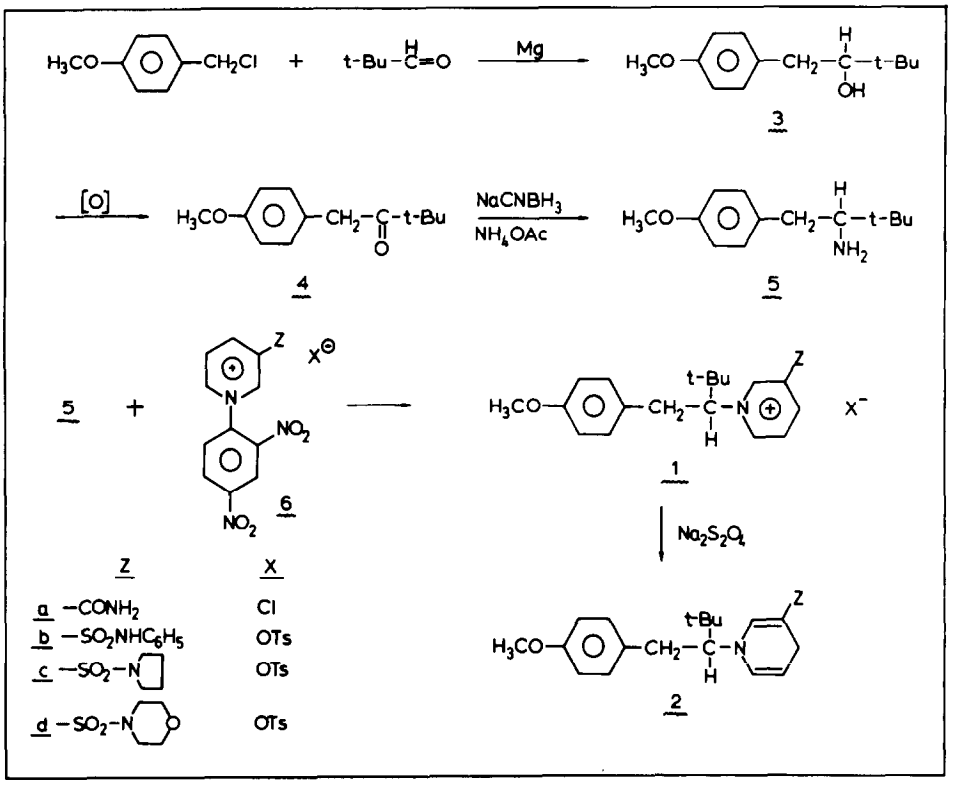

FIG. 1. Syntheses and structures of model compounds **1a–1d** and **2a–2d**.

of a single staggered conformation around the C(α)–C(β) bond, *i.e.*, the one where the t-Bu and *p*-anisyl groups are in the *anti* position. Furthermore, hindered rotation around the C(β)–N bond ($\Delta G\ddagger \approx 60$ kJ/mol) was observed (*5*). In addition to **1a**, **1b–1d**, and the 1,4-dihydropyridine analogs **2a–2d** were synthesized according to Fig. 1 (cf. Experimental section), and their ^{1}H-nmr spectra studied at various temperatures.

Conformational Preference

For all compounds doubling of various ^{1}H-nmr signals occurs at low temperature, which can be attributed (*5*) to a hindered rotation around the C(*β*)–N bond, i.e., to a

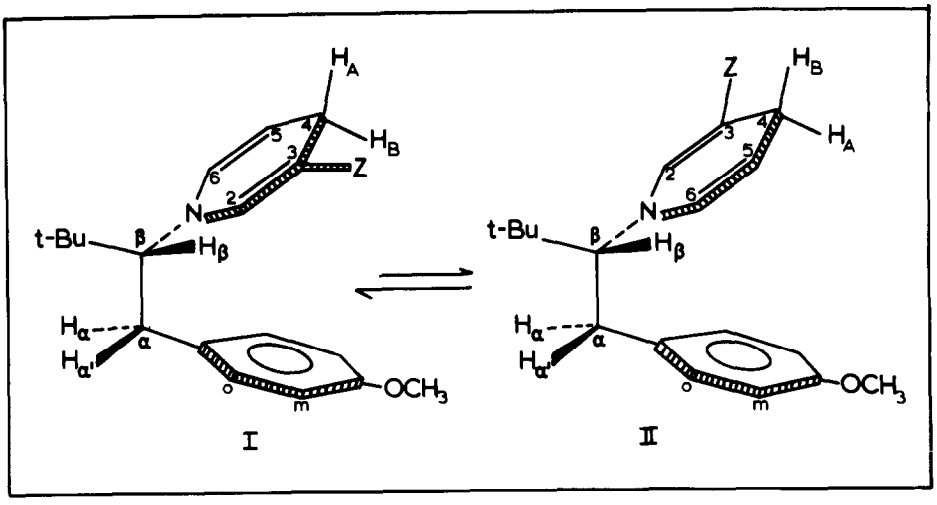

FIG. 2. Schematic representation of the rotamers I and II for the *R*-enantiomer of compounds **2**.

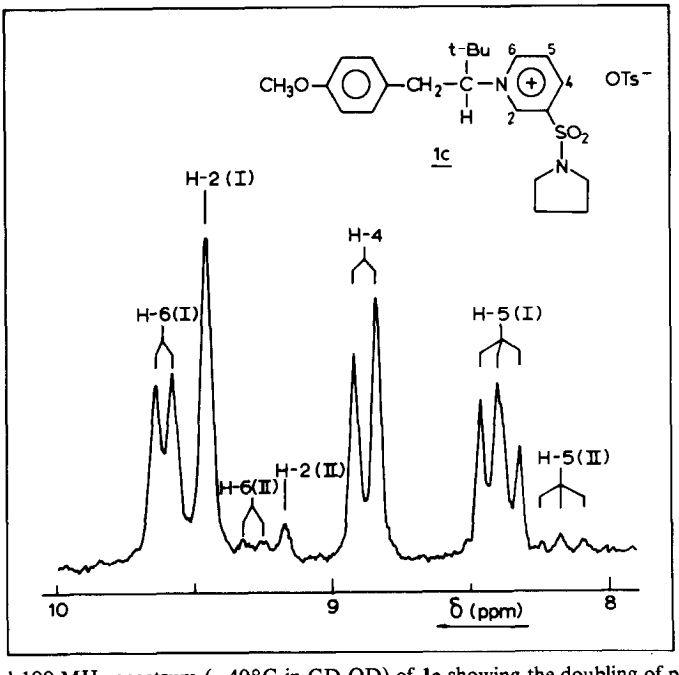

FIG. 3. Partial 100-MHz spectrum (–40°C in CD_3OD) of **1c** showing the doubling of pyridinium-ring-proton signals H-2, H-5, and H-6 due to slow interconversion of rotamers I and II (cf. Fig. 2).

slow interconversion of the rotamers I and II (cf. Fig. 2). This doubling of signals is exemplified in Figs. 3 and 4 for **1c** and **2c**, respectively, while quantitative data on the chemical shift differences between rotamers I and II are compiled in Tables 1 and 2. It

should be noted that these rotamers are diastereomers. Therefore, all nuclei in I can have a chemical shift different from the diastereotopic nuclei in II.

The largest chemical shift differences between I and II are observed for protons at the 2,5- and 6-positions of the pyridine ring system. This is apparently due to their different orientation relative to the shielding cone of the *p*-anisyl group in I and II. For the C-4 proton in **1**, which is located on the axis of rotation, no shift difference between I and II could be detected. Small shift differences are observed for protons connected to the *p*-anisyl group. Thus, doubling of the methoxyl signal is observed in several cases as well

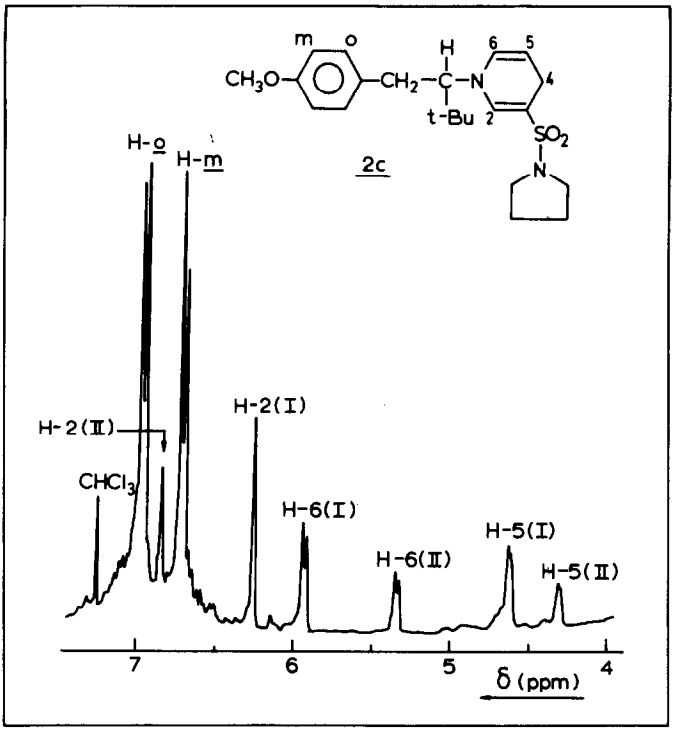

FIG. 4. Partial 360-MHz spectrum of **2c** (−10°C in $CDCl_3$) showing the doubling of dihydropyridine-ring-proton signals H-2, H-5, and H-6 due to rotamers I and II.

as doubling of the aromatic proton signals. This is most pronounced for **1b** and **2b**, probably due to the presence of an aromatic group in *Z*. For **2a** the pattern generated by the three protons attached to the C(α)–C(β) bond is shown in Fig. 5.

At 25°C the 360-MHz pattern is broadened while at −10°C a well-resolved but complicated pattern arises. This pattern could be simulated (cf. Fig. 5) by assuming it to be the sum of two three-spin patterns with an intensity ratio of 1.65 : 1 (*vide infra*) generated by conformations I and II, respectively. The analysis indicates (cf. Fig. 5) that these two spin patterns contain the same set of coupling constants (but different chemical shifts), which supports the view that conformations I and II involve the same relative orientation about the C(α)–C(β) bond, i.e., the orientation with the t-Bu group *anti* to the *p*-anisyl group. This explains the large $^3J_{\beta\alpha} = 11$ Hz (*anti*-coupling) and the

small $^3J_{\beta\alpha'}$ = 2 Hz (*gauche*-coupling) values. In all cases a population difference between rotamers I and II is apparent from the low-temperature spectra (cf. Figs. 3, 4). Rotamer II is concluded to be less populated since the weaker H-5 signal is always found at high field, as expected from the orientation of H-5 relative to the shielding cone (*7*) of the *p*-anisyl group in rotamer II (cf. Fig. 2).

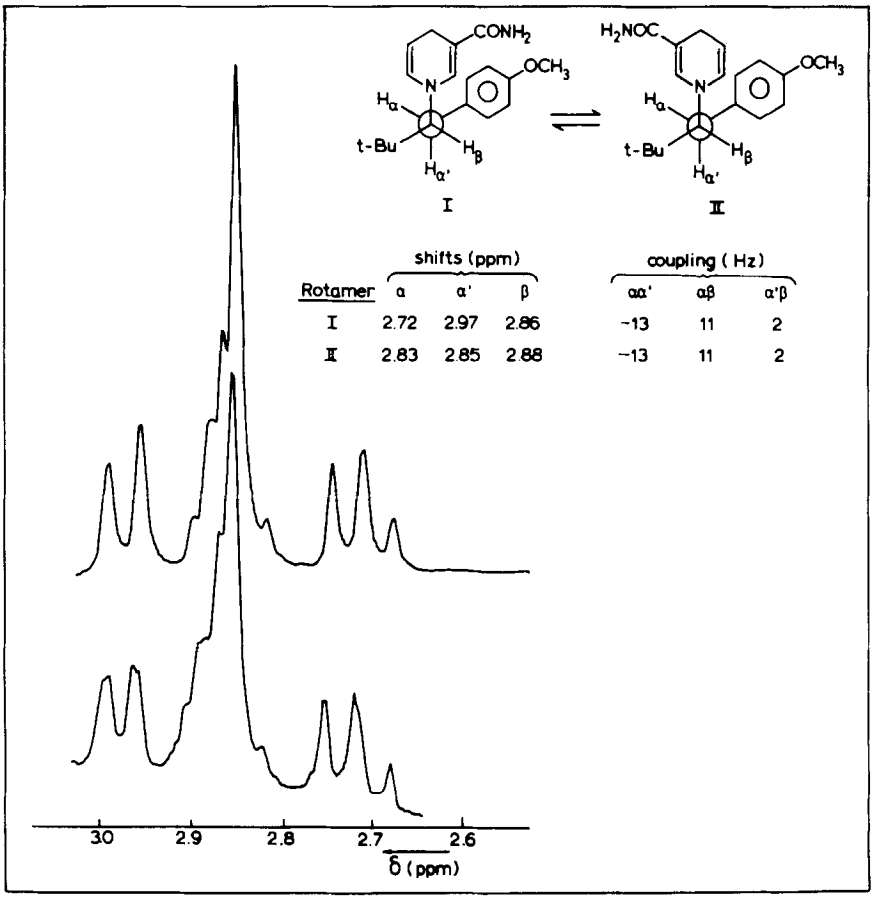

FIG. 5. Lower trace: splitting pattern generated by protons H-α, H-α', and H-β of **2a** (360 MHz, −10°C in $CdCl_3$). Upper trace: simulated pattern with parameters indicated, assuming an intensity ratio of I:II = 1.65 (cf. text).

The observed ratio of the populations for I and II (p_I/p_{II}) is indicated in Tables 1 and 2. In the range of −10 to −60°C this ratio showed no detectable changes. An influence of changes in the pyridine ring substituent *Z* on the p_I/p_{II} ratio is apparent however (cf. Tables 1 and 2). The influence of *Z* appears to be larger for compounds **1** (Table 1) than for the reduced analogs **2** (Table 2). This might be attributable to a different stabilization of rotamers I and II by the intramolecular donor–acceptor interaction known to occur (*6*) between the *p*-anisyl group and the pyridinium group in **1**. Such donor–acceptor interaction has recently been proposed (*8*) to influence rotational equilibria. In **2** reduction has destroyed the electron-acceptor properties of the pyridinium system. The

TABLE 1

CHEMICAL-SHIFT DIFFERENCES ($\Delta\delta = \delta_I - \delta_{II}$) AND POPULATION RATIO ($p_I/p_{II}$) AS OBSERVED FOR THE STABLE ROTAMERS I AND II OF COMPOUNDS **1**[a]

			$\Delta\delta$ (ppm)[c] ± 0.02					
Compound[b]	Solvent	p_I/p_{II}	H-2	H-4	H-5	H-6	t-Bu	OCH_3
1a	CD_3OD	1.7 ± 0.1	0	0	0.17	0.37	0	0
	D_2O	1.5 ± 0.1	0.17[d]	0	0.24	0.45	0	0
1b[e]	$CDCl_3$	1.3 ± 0.1	—[f]	0	0.07	—[f]	0.05	0.05
1c	CD_3OD	8.7 ± 0.1	0.27	0	0.22	0.28	0	0
1d	CD_3OD	6.5 ± 0.5	0.27	0	0.22	0.37	0	0

[a] Measured at −40°C, 100 MHz unless stated otherwise.

[b] See Fig. 1 for structure.

[c] Positive values indicate that the signal attributed to I lies to the low-field side of the corresponding signal of II.

[d] Sign uncertain (cf. Ref. 5).

[e] Measured at 360 MHz and −10°C.

[f] Strongly overlapping signals.

TABLE 2

CHEMICAL-SHIFT DIFFERENCES ($\Delta\delta = \delta_I - \delta_{II}$) AND POPULATION RATIO ($p_I/p_{II}$) AS OBSERVED FOR THE STABLE ROTAMERS I AND II OF COMPOUNDS **2**[a]

		$\Delta\delta$[c] (ppm) ± 0.01							
Compound[b]	p_I/p_{II}	H-2	H-4[d]	H-5	H-6	t-Bu	OCH_3	H-*o*	H-*m*
2a	1.65 ± 0.05	−0.65	0.08	0.32	0.68	0	0.01	0.02	0
2b	1.25 ± 0.10	—[e]	0	0.34	0.75	0	0.04	—[e]	—[e]
2c	1.80 ± 0.05	−0.58	0.08	0.31	0.59	0	0	0	0
2d	2.60 ± 0.10	−0.58	0.08[f]	0.30	0.57	0	0	0	0

[a] Measured at −10°C, 360 MHz, in $CDCl_3$.

[b] See Fig. 1 for structure.

[c] Positive values indicate that the signal attributed to I lies to the low-field side of the corresponding signal of II.

[d] $\Delta\delta_{XY}$ of the XY pattern observed (cf. text).

[e] Strongly overlapping signals.

[f] Tentative value; XY pattern strongly overlapped by signals from protons of the morfoline system.

general preference for I in both **1** and **2** is thought to arise from steric repulsion between Z and t-Bu in II.

For compounds **2** the $\Delta\delta = \delta_I - \delta_{II}$ values of H-2 and H-6 are almost equal but of *opposite* sign (cf. Table 2), as would be expected when I and II are related by rotation over ~180° around the C(β)–N bond.

For compounds **1** such a "mirror-symmetry" between the $\Delta\delta$ values for H-2 and H-6 is not found. Furthermore, these $\Delta\delta$ values for compounds **1** seem to depend strongly on the solvent and the counterion (X). This can be explained by sterically imposed differences in solvent and counterion accessibility of H-2 (and H-6) in I and II. Such

differences are known (*9*) to have a marked influence on the ^{1}H-nmr shifts of H-2 and H-6 in pyridinium ions.

The C-4 Methylene Protons of the Dihydropyridine Ring in **2**

In high-temperature (>25°C) 100-MHz spectra of **2a**, **2b**, and **2c**, the C-4 methylene protons appear as a narrow multiplet reflecting coupling with H-5 ($^3J_{4,5} \approx 3.5$ Hz) and with H-6 ($^4J_{4,6} \approx 1.5$ Hz); for **2d** this multiplet is hidden by other resonances in a 100-MHz spectrum. Thus, the diastereotopic C-4 protons behave isochronously in a 100-MHz spectrum at temperatures where rapid interconversion of rotamers I and II occurs.

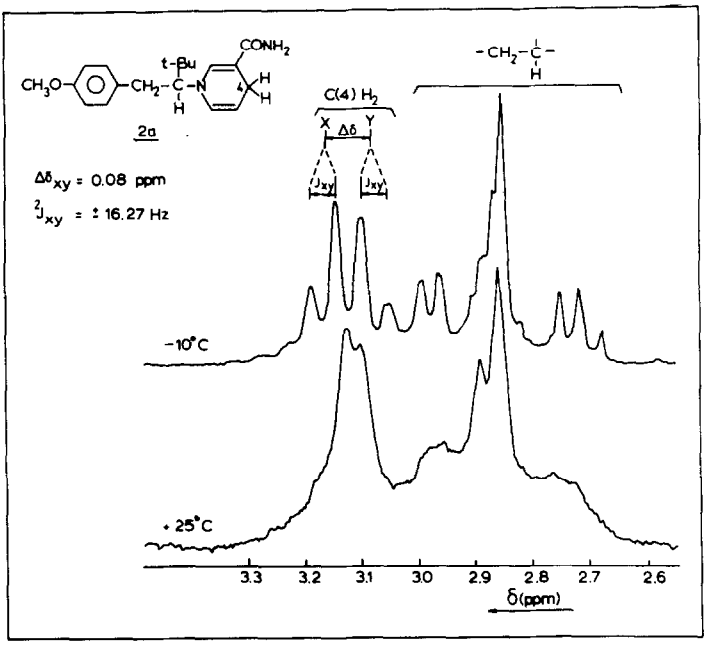

FIG. 6. Temperature dependence of the C-4 methylene proton signal for **2a** (360 MHz in $CdCl_3$).

At lower temperatures broadening of the C-4 methylene signal occurs in a 100-MHz spectrum while at 220 MHz, and especially at 360 MHz, the low-temperature spectra of **2a**, **2c** and **2d** display *XY* patterns for the C-4 protons with $^2J_{XY} = 16.27$ Hz for **2a** (cf. Fig. 6) and $^2J_{XY} = 15.6$ Hz for **2c** and **2d**. For **2d** the C-4 protons appear as a single broad line, even at −20°C and 360 MHz.

From the data above it seems reasonable that the *XY* patterns observed for the C-4 protons are in fact a superposition of two almost identical *XY* patterns generated by the rotamers I and II, respectively. Additional support for this view comes from the spectra of a sample of **2a** obtained by reduction of **1a** with sodium dithionite in deuterium oxide leading to substitution of one of the C-4 protons[3] by deuterium. The 360-MHz spectrum of this **2a** (4-d_1) sample shows two broad lines for the C(4)HD group at low

[3] The present data do not allow any conclusion about an eventual preferential deuteration (**1b**) of the *A* or *B* side of the pyridine ring!

temperature and one broad line at high temperature (cf. Fig. 7). These signals are all shifted upfield by about 0.01 ppm relative to nondeuterated **2a** due to a deuterium-isotope effect.

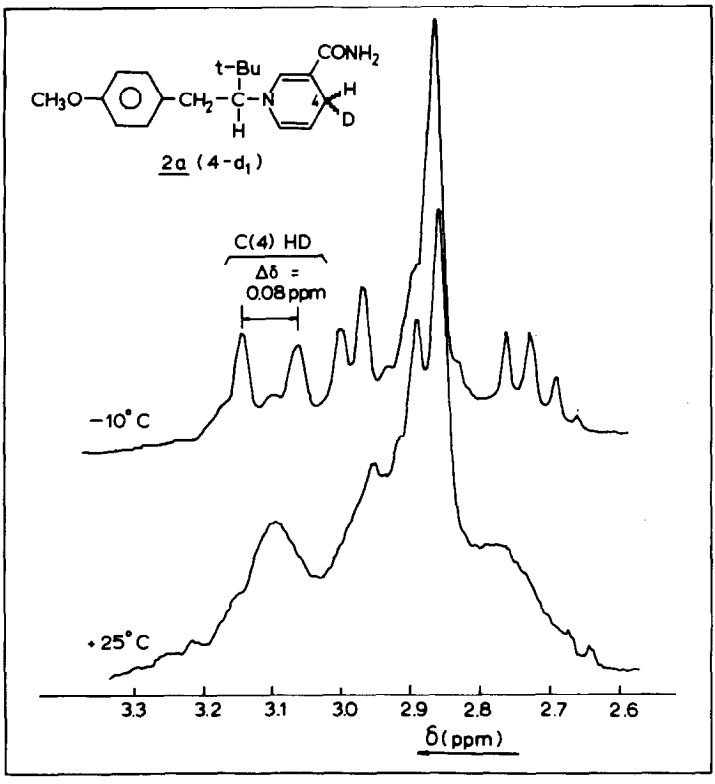

FIG. 7. Temperature dependence of the H-4 signal for **2a** (4-d_1) (360 MHz in $CdCl_3$).

Thus the four diastereotopic positions available for a C-4 methylene proton (i.e., two in rotamer I and two in rotamer II) can approximately be described by two chemical shifts:

$$\delta_A(\mathrm{I}) = \delta_B(\mathrm{II}) = \delta(\mathrm{exo}),$$

$$\delta_B(\mathrm{I}) = \delta_A(\mathrm{II}) = \delta(\mathrm{endo}),$$

where δ_A(I) refers to the shift of H_A in rotamer I while δ(endo) and δ(exo) refer to shifts attributed to a proton pointing toward the *p*-anisyl group and away from it, respectively, for the enantiomer of **2** specified in Fig. 2.

In the low-temperature limit, this leads to a single *XY* pattern with $\Delta\delta_{XY} = \delta(\mathrm{endo}) - \delta(\mathrm{exo})$. As indicated in Table 2 a $\Delta\delta_{XY}$ value of ~0.08 ppm is observed for **2a**, **2c**, and **2d**. This difference is thought to arise mainly from differential shielding of the *endo* and *exo* positions by the *p*-anisyl group. For **2b** the $\Delta\delta_{xy} \approx 0$ value probably indicates a mutual cancellation of shielding effects exerted by the *p*-anisyl and the sulfonanilide

groups. In the high-temperature limit (rapid exchange), the chemical shifts of H_A and H_B become (for the enantiomer of Fig. 2)

$$\delta_A = p_{II}\delta(\text{endo}) + p_I\delta(\text{exo}),$$
$$\delta_B = p_{II}\delta(\text{exo}) + p_I\delta(\text{endo}).$$

Combination leads to $\delta_A - \delta_B = [\delta(\text{endo}) - \delta(\text{exo})][p_{II} - p_I]$. Thus an XY pattern should still be observable when p_I and p_{II} are sufficiently different. In the high temperature 360-MHz spectrum of **2a**, a small shift difference between the C-4 protons appears to be observable (cf. Fig. 6).

CONCLUSION

Compounds **1** and **2** constitute systems in which one side of the pyridine ring is preferentially shielded by the *p*-anisyl group. This situation resembles the specific shielding of the nicotinamide *A*-side by a tyrosine residue in the lobster D-glyceraldehyde 3-phosphate dehydrogenase/NAD^+ complex (*4*). We are currently investigating whether such preferential shielding is reflected in a differentiation of chemical reactivity between the two pyridine ring sides.

EXPERIMENTAL

Spectra

^{1}H-nmr spectra were recorded on Varian HA-100, HR-220, and Bruker HX-360 (Fourier transform) spectrometers. Chemical shifts (δ) are given in parts per million relative to $\delta_{TMS} = 0$ or (for the 360-MHz spectra) relative to $\delta_{CHCl_3} = 7.27$ ppm. Simulation of the spin system of **2a** (Fig. 5) was achieved with a Varian 620-i spectrosystem using the SIMEQ II-16 program written by C. W. F. Kort and M. J. A. de Bie.

Syntheses

1-(4-Methoxyphenyl)-3,3-dimethyl-butane-2-ol (**3**) (Ref. *10*). A solution of 4-methoxybenzylchloride (0.56 mol) in 500 ml of dry ether was added over a period of 3 hr to a stirred and refluxed suspension of magnesium powder (1.4 mol) and magnesium turnings (1.4 mol) in 600 ml of dry ether. Then pivaldehyde (0.3 mol) in ether (300 ml) was introduced over a period of 1.5 hr followed by 1.5 hr of reflux. Hydrolysis with ammoniumchloride/ice-water removal of ether and extraction with *n*-pentane yielded a pentane soluble product which was purified by fractional distillation *in vacuo* to give 39 g (66%) of pure **3**; bp 106–112°C/0.05 mm Hg. nmr (CCl_4): 0.9 (9*H*, *s*), 2.0–3.4 (3*H*, *m*), 3.7 (3*H*, *s*), 6.8 (4*H*, *m*).

1-(4-Methoxyphenyl)-3,3-dimethyl-butane-2-one (**4**). To a solution of pyridinium-chlorochromate (*11*) (0.23 mol) in dichloromethane (200 ml) a solution of **3** (0.19 mol) in dichloromethane (20 ml) was added rapidly at room temperature. After stirring for 2 hr the reaction mixture was extracted with ether (1.5 liter). The ethereal solution was

filtered over a short Florisil column and then fractionated *in vacuo*. This yield 30 g (76%) of pure ketone **4**.

bp 95–105°C/0.1 mm Hg.
nmr ($CDCl_3$): 1.2 (9*H*, *s*), 3.8 (5*H*, *s*), 6.9 (4*H*, *m*).
ir (capture) C=O: 1700 cm^{-1}.

1-(4-Methoxyphenyl)-3,3-dimethyl-2-butylamine (**5**). A solution of **4** (0.145 mol), ammonium acetate (1.45 mol), and sodium cyanoborohydride (*12*) (0.1 mol) in dry methanol (300 ml) was stirred for 48 hr at room temperature. After acidification with concentrated HCl (pH $\approx$ 1), methanol was removed *in vacuo*, and the residue was dissolved in H_2O (100 ml) and extracted three times with 100-ml portions of ether.

The water layer was then made alkaline (pH $\approx$ 11) with KOH and saturated with NaCl.

Extraction with ether yielded the crude amine. After evaporation of the ether this crude amine was dissolved in dry methanol. From the solution the HCl salt of **5** was precipitated by saturation with dry HCl gas. Yield, 30 g (HCl salt = 85%).

nmr (HCl salt in CD_3OD): 1.1 (9*H*, *s*), 2.5–3.5 (3*H*, *m*), 3.7 (3*H*, *s*), 4.9 (3*H*, broad singlet of NH_3^+), 7.1 (4*H*, *m*).

Anal. Calcd. for $C_{13}H_{23}NOCl$ (MW = 230.5): C, 64.2; H, 9.1; O, 6.6; N, 5.75; Cl, 14.5%. Found: C, 62.33; H, 9.31, O, 7.04; N, 5.94; Cl, 15.15.

Zincke-salts **6a**, **6b**, **6c**, *and* **6d**. These were prepared by heating of the appropriate pyridine derivative with excess of 2,4-dinitrochlorobenzene (for **6a**) or 2,4-dinitrophenyl-toluene-*p*-sulfonate (for **6b**, **6c**, and **6d**) as described by de Gee (*13*).

Pyridinium salts **1a**, **1b**, **1c**, *and* **1d**. These were prepared by "Zincke reaction" of **5** with the appropriate Zincke salt **6** in boiling methanol according to the procedure described by de Gee (*13*) and purified by repeated precipitation with dry ether from methanol.

Dihydropyridines **2a**, **2b**, **2c**, *and* **2d**. The appropriate pyridinium salt (3 mmol) dissolved in water (30 ml) was gradually added under N_2 atmosphere to a stirred solution of sodium carbonate (6.9 mmol) and sodium dithionite (7.5 mmol) in water (45 ml) at 55°C.

The dihydropyridine, which generally separates as a yellow oil, was isolated by extraction with chloroform. Evaporation of the neutralized and dried chloroform extract *in vacuo* at ~10°C yielded the dihydropyridines in a form sufficiently pure for the nmr investigation.

ACKNOWLEDGMENTS

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