tions. It may be that the generic group (hydroxamic acids), due to their peculiar molecular structure such as the R—C(=O)—N(—H)—OH moiety, are a group of oxidative phosphorylation uncoupling agents and compete for the energy available within the cellular contractile mechanism.

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NMR Study on 1,2,4,5-Tetrasubstituted 3,3,6,6-Tetradeuterated Cyclohexanes: Conformational Contributions and 1,3-Diaxial and Vicinal Deshielding Effects of Amino and Alkylated Amino Groups and Their Protonated Forms

BARTLETT D. WHELTON*, BETTY R. LOWRY, JOHN B. CARR†, and ALAIN C. HUITRIC▲

Abstract ☐ The partially deuterated compounds trans-2-o-tolyl-cis-4-hydroxy-trans-5-aminocyclohexanol-3,3,6,6-d_4 and trans-2-o-tolyl-cis-4-amino-trans-5-hydroxycyclohexanol-3,3,6,6-d_4, the corresponding N-methyl and N,N-dimethyl derivatives, the trimethylammonium iodide salts, the C-1 benzoyloxy esters of all these compounds, and a few C-4 acetoxy derivatives were investigated by NMR for conformational changes brought about by alkylation and protonation of axially oriented amino groups and for 1,3-diaxial and vicinal deshielding effects of amino and protonated amino groups. In these compounds, hydrogens H-1 and H-2 and hydrogens H-4 and H-5 make up two separate AX systems where, in a given chair conformation, the hydrogens of one pair have trans-diaxial orientations and those of the other pair have trans-diequatorial orientations. Changes in coupling constants J_{12} and J_{45} give a sensitive measure of conformational changes. The most pronounced conformational changes occurred with protonation of the dimethylamino derivatives in formic acid and in chloroform, where the equilibrium highly favors the inverted chair conformations with an axially oriented o-tolyl group. The 1,3-diaxial deshielding of the primary amino group was found to be somewhat

comparable to that of the hydroxyl group, with little change in the effect upon protonation. The effects of N-methylation are discussed. Geminal and vicinal deshielding by the amino group and the role of N-methylation and protonation on these effects are given. Further data supporting the long-range deshielding by the carbon–carbon double bond and the epoxy group are also presented.

Keyphrases ☐ Cyclohexanes, 1,2,4,5-tetrasubstituted 3,3,6,6-tetradeuterated—NMR study, conformation contributions and deshielding effects of amino groups and protonated forms ☐ Coupling constants, 1,2,4,5-tetrasubstituted 3,3,6,6-tetradeuterated cyclohexanes—conformation changes produced by alkylation and protonation of axial amino groups ☐ Deshielding effects, 1,3-diaxial and vicinal—1,2,4,5-tetrasubstituted 3,3,6,6-tetradeuterated cyclohexanes, conformation changes produced by alkylation and protonation of axial amino groups ☐ NMR spectroscopy—tetrasubstituted cyclohexanes, conformation changes produced by alkylation and protonation of axial amino groups, vicinal deshielding effects of amino groups, coupling constants

A number of partially deuterated six-membered ring compounds have been prepared in this laboratory in recent years for NMR conformational analysis studies (1–4) and for study of long-range 1,3-diaxial deshielding of ring hydrogens by the hydroxyl group (5). On the basis of this previous work, the substituted 3,3,6,6-tetradeuterated cyclohexene (III) was easily available as an ideal intermediate for the synthesis of the tetradeuterated compounds of series VI–IX, of known stereochemistry, through the epoxides IV and V. The stereochemistry for IV and V was established for the nondeuterated epoxides (6). The deuterated compounds of series VI through IX were of special interest for the study of possible conformational changes as a function of solvent resulting from successive alkylation of the amino group and/or from protonation of the primary, secondary, and tertiary amines. The tetradeuterated compounds also provide useful systems for the study of the relative 1,3-diaxial deshielding properties of primary, secondary, and tertiary amino groups and the effect of protonation on this property. The series further proved useful in demonstrating the deshielding effect of protonation of the amino groups on the chemical shifts of vicinal hydrogens.

A very important feature of the tetradeuterated compounds, VI–IX, is that the four hydrogens on the cyclohexane ring form two separate AX systems, whose coupling constants give a very sensitive NMR probe for the study of conformational changes. Since each ring hydrogen is adjacent to only one other hydrogen, with the exception of the hydrogen on the amino-bearing carbon, the NMR signal of each gives a simple doublet which allows accurate measurements of coupling constants and chemical shifts from first-order treatment of the spectra. The components of each doublet are broadened slightly by weak coupling with the deuterium atoms on adjacent carbons, but this does not interfere with the measurement of coupling constants between hydrogens of individual AX pairs. Occasionally, there was coupling with the hydroxyl hydrogen, especially in dimethyl sulfoxide-d_6, but this could be circumvented by addition of a small quantity of D_2O.

RESULTS AND DISCUSSION

Conformational Study—Coupling constants between axial hydrogens or adjacent carbon atoms (J_{aa}) are normally around 11 Hz., and those between equatorial hydrogens (J_{ee}) are usually in the neighborhood of 3 Hz. (1). In the 1,2,4,5-tetrasubstituted 3,3,6,6-tetradeuterated cyclohexanes under consideration, there are two separate AX systems of *trans*-hydrogens. In structures having the chair conformation, the hydrogens of one vicinal pair have axial–
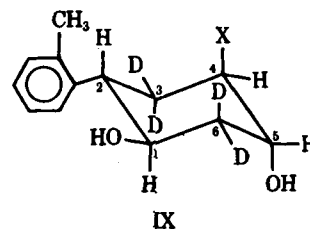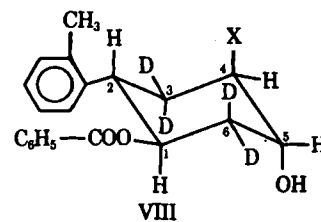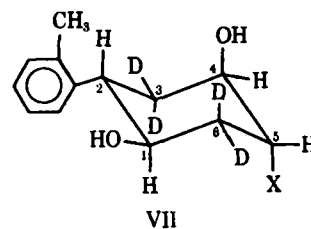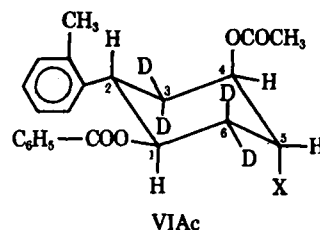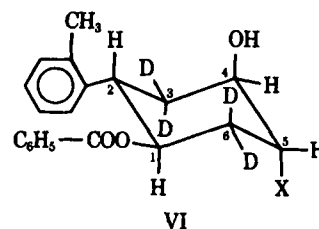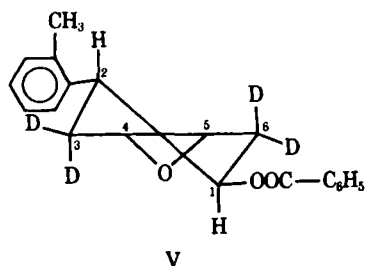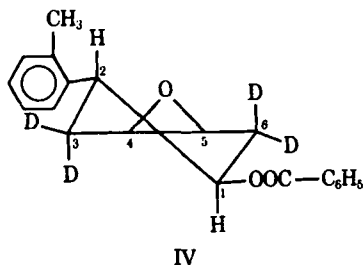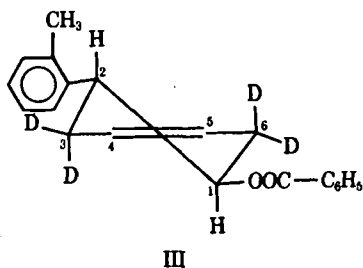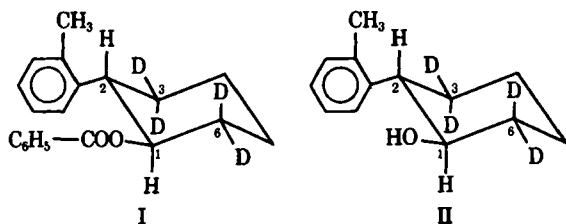
VI–IXa: X = NH₂
VI–IX b: X = NH—CH₃
VI–IX c: X = N(CH₃)₂
VI–IX d: X = Ñ(CH₃)₃Cl⁻
VI–IX e: X = deuterium
VI–IX f: X = OH
VI–IX g: X = N=C⟨CD₃ CD₃⟩

I

II

III

IV

V

VI

VIAc

VII

VIII

IX

X

XI

CH₃
H
OCOCH₃
H
C₆H₅—COO
H
H
N(CH₃)₂

XII

CH₃
H
N(CH₃)₂
H
C₆H₅—COO
H
H
OCOCH₃

XIII

CH₃
H
OCOCH₃
H
CH₃COO
H
H
N(CH₃)₂

XIV

CH₃
H
N(CH₃)₂
H
CH₃COO
H
H
OCOCH₃

XV
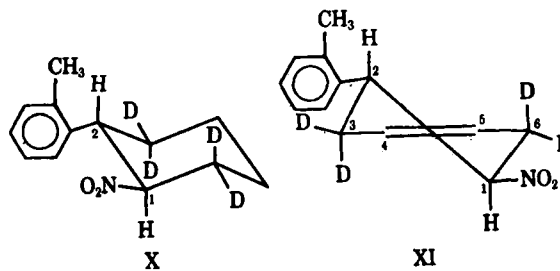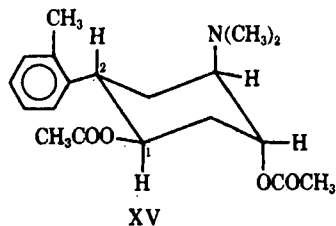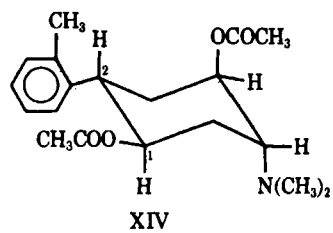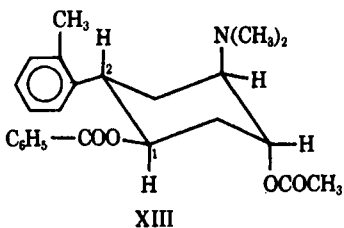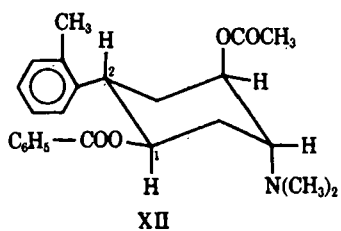
methiodides of Compounds XII–XV are included

axial orientations while those of the other pair are equatorial–equatorial. Since the assignment of signals was possible in all cases, the coupling constants J_{12} and J_{45} provide a unique NMR probe for determining the preference between the two chair conformers in systems where the equilibrium favors a large predominance of one of the two possible chair conformations. In such a system, either J_{12} or J_{45} will be the normal J_{aa} value, while the other one will be the normal J_{ee} value. In cases where J_{12} and J_{45} are essentially equal, the system does not give a direct differentiation between an equilibrium involving nearly equal populations of the two chair conformers with the exclusion of other conformers and a situation where the equilibrium involves partial or total contribution from boat and other flexible conformations, because in the flexible conformations the average dihedral angle between H-1 and H-2 will be the same as between H-4 and H-5. It is important to realize that unequal J_{12} and J_{45} values indicate a predominance of one chair conformation over the other; but in cases where neither coupling constant approaches the normal J_{aa} value, the system does not provide a direct differentiation between an equilibrium involving only the two chair conformers in unequal populations and an equilibrium involving one or both chair conformers and flexible forms.

The normal J_{aa} value is seen from Table I to be 11.0 Hz. in the reference benzoate I and about 10.0 Hz. in the corresponding hydroxy Compound II in the various solvents investigated. The data also show conformational changes in the 1,2,4,5-tetrasubstituted compounds which vary from partial to total chair inversion. The roles of solvent, alkylation, protonation, quaternization, and intramolecular hydrogen bonding on conformational changes are also demonstrated by the J_{12} and J_{45} values in Table I.

Effects of Intramolecular Hydrogen Bonding—The J_{12} and J_{45} values indicate that in hydrogen-bonding solvents, which act as hydrogen acceptors, the equilibria for the free base forms of the primary, secondary, and tertiary amines of classes VI through IX favor a high predominance of the normal chair conformation having the o-tolyl group on C-2 and the benzoate or hydroxyl group on C-1 in equatorial orientations, with the amino and hydroxyl or acetoxy group on C-4 and C-5 in axial orientations. In deuterated chloroform, the J_{12} and J_{45} values of 7.3 and 5.9 for the dimethylamine VIc indicate a reduction in the population of the normal chair conformation. Threefold dilution did not change the J_{12} and J_{45} values. The acetoxy derivative VIcAc shows a high predominance of the normal chair form. The conformational change in VIc in chloroform is attributed to the stabilization of the inverted chair or some flexible conformation through intramolecular hydrogen bonding between the C-4 hydroxyl and C-5 amino groups. Because of intermolecular hydrogen bonding between the compounds and solvent molecules in acetone, pyridine, and dimethyl sulfoxide-d_6, intramolecular hydrogen bonding will not have the same degree of stabilization of the inverted conformation in these solvents as in chloroform. For reasons discussed later, the inverted chair is favored over flexible forms. The presence of intramolecular hydrogen bonding in chloroform was demonstrated in the nondeuterated Compounds VIc and VIIIc by IR spectroscopy. In each case, the spectra showed a nonbonded OH stretching band at 3610 cm.⁻¹ and a broader bonded OH band at 3440 cm.⁻¹. This latter band persisted when the solution was diluted to 0.01 M.

The conformation free energy values $(-\Delta G)$ for amino, methylamino, and dimethylamino groups have been studied by several investigators by different methods (7–10). The general trend, as expected, is that of an increase in $-\Delta G$: (a) with increased alkylation, (b) in going from nonhydrogen-bonding to hydrogen-bonding solvents, and (c) with protonation. Conformational equilibrium studies have also been reported for 1,2-aminocyclohexanol systems (11, 12) and, to no surprise, the combined $-\Delta G$ value for the trans-1,2-aminohydroxy groups, in trans-2-amino-trans-5-isopropylcyclohexanol in tetrachloroethylene, was found to be larger than the sum of the individual values (12). This effect is attributed to hydrogen bonding in the chair conformation with the two functional groups in equatorial orientations. Tichý et al. (11) also found a slight contribution from an intramolecularly hydrogen-bonded conformer of cis-4-tert-butyl-trans-2-dimethylaminocyclohexanol, and they favored an inverted chair conformation, with the tert-butyl group axial, in preference to contributions from flexible forms for this conformer.

Effects of Methylation—For the free bases of the primary, N-methyl, and N,N-dimethylamines of classes VI through IX, the equilibrium in every case favors a high predominance of the normal chair conformation in all hydrogen-bonding solvents that act as hydrogen acceptors. In these solvents, the combined $-\Delta G$ values of the adjacent trans-hydroxyl and amino groups are not sufficient to overcome the combined $-\Delta G$ values of the trans-o-tolyl and the C-1 substituent, and no conformational change can be observed upon successive methylation. In deuterochloroform, the combined $-\Delta G$ values of the trans-hydroxyl and amino groups are larger, presumably because of a greater contribution from intramolecular hydrogen bonding, and the differences between the combined $-\Delta G$ values for the substituents on C-1 and C-2 versus those on C-4 and C-5 fall within the range where conformational changes can be observed in VIc and VIIc. Comparison of J_{12} and J_{45} values between VIa, VIb, and VIc in chloroform shows that there is a considerable reduction of the normal chair in going from the N-methyl to the N,N-dimethyl derivative but that there is very little difference in conformation between the primary and secondary amines.

Effects of Protonation—Protonation brings about changes in conformation which vary with degree of alkylation and which are highly solvent dependent in the limited solvents investigated. The most pronounced changes are found with the trifluoroacetate salts in chloroform and the formate salts in formic acid-D₂O solutions. With the latter, the changes seem more pronounced in solutions containing 5% D₂O than in those with 25% D₂O. Very little difference was seen between formate and hydrochloride salts in formic acid-D₂O solutions, and the data for the hydrochloride salts in those solutions are not included in Table I.

Of special interest is the high predominance of the inverted chair conformation with the o-tolyl group in axial orientation for the salts of the N,N-dimethyl derivatives VIc, VIIc, and VIIIc in formic acid and for the trifluoroacetate of VIc in chloroform-d. In dimethyl sulfoxide the equilibrium favors a high predominance of the normal chair conformation, with the o-tolyl group in equatorial orientation for the hydrochloride salts of VIc and VIIIc and the trifluoroacetate of VIc. A comparison of the data on the salts of the primary and secondary amines shows that in dimethyl sulfoxide-

Table I—NMR Data for Tetradeuterated Six-Membered Ring Compounds

Compound	Solvent	Coupling Constants, Hz. J_{12}	J_{45}	Chemical Shifts in δ Units, p.p.m. N—CH₃	H-1	H-2	H-4	H-5	Deshielding Shifts $(\nu - \nu°)^a$, Hz. at 60 MHz. $\nu_1 - \nu_1°$	$\nu_2 - \nu_2°$
I	Carbon tetrachloride	11.0	—	—	5.21	3.09	—	—	—	—
	Acetone-d_6	11.0	—	—	5.29	3.23	—	—	—	—
	Dimethyl sulfoxide-d_6	10.9	—	—	5.25	3.17	—	—	—	—
	Pyridine	11.0	—	—	5.53	3.28	—	—	—	—
	Formic aicd-D₂O (95:5)	11.0	—	—	5.35	3.28	—	—	—	—
	Chloroform-d	10.8	—	—	5.25	3.13	—	—	—	—
II	Acetone-d_6	10.0	—	—	3.71	2.73	—	—	—	—
	Dimethyl sulfoxide-d_6	10.1	—	—	3.60	2.62	—	—	—	—
	Pyridine	10.0	—	—	3.89	2.90	—	—	—	—
	Formic acid-D₂O (75:25)	10.2	—	—	4.01	2.86	—	—	—	—
	Chloroform-d	9.8	—	—	3.66	2.67	—	—	—	—
III	Carbon tetrachloride	9.2	10.0	—	5.38	3.40	5.72[b]	5.61[b]	8	19
	Acetone-d_6	10.3	10.2	—	5.50	3.55[c]	5.83[b]	5.73[b]	12	19
	Dimethyl sulfoxide-d_6	10.2	10.2	—	5.45	3.49	5.83[b]	5.72[b]	12	19
	Pyridine	10.0	10.1	—	5.70	3.61	5.81[b]	5.71[b]	10	20
IV	Carbon tetrachloride	11.2	—	—	5.12	3.40	3.09	3.09	-5	19
	Acetone-d_6	11.0	—	—	5.30	3.49	3.23	3.23	0	16
	Dimethyl sulfoxide-d_6	11.6	—	—	5.27	3.42	3.27	3.27	1	15
	Pyridine	11.5	—	—	5.46	3.64	3.19	3.19	-5	22
V	Carbon tetrachloride	11.2	3.5	—	5.33	3.15	3.20[b]	3.06[b]	7	4
VIa	Dimethyl sulfoxide-d_6	11.0	3.2	—	5.76	3.70	3.75	3.25	31	32
	Pyridine	10.6	3.3	—	6.27	4.19	4.18	3.71	44	55
	Chloroform-d	9.3	4.3	—	5.63	3.66	3.85	3.30	22	32
VIa-HCl	Dimethyl sulfoxide-d_6	10.2	3.2	—	5.75	3.73	4.19	3.55	30	34
VIa-HCl	Pyridine[c]	9.8	3.8	—	6.17	4.13	5.00	4.41	38	51
VIa-FA[d]	Formic acid-D₂O (95:5)	5.6	7.2	—	5.71	3.83	4.66	4.11	—	—
VIa-TFA[e]	Chloroform-d	4.8	8.0	—	5.57	3.72	4.57	—	—	—
VIb	Acetone-d_6	11.2	3.0	2.44[f]	5.71	3.80	3.97	2.96	25	35
	Dimethyl sulfoxide-d_6	11.1	3.0	2.44	5.65	3.71	3.86	2.86	24	33
	Pyridine	10.5	3.0	2.49[f]	6.12	4.17	4.22	3.23	35	53
	Chloroform-d	9.9	3.7	2.51	5.57	3.65	3.95	2.91	19	32
VIb-HCl	Dimethyl sulfoxide-d_6	10.2	3.2	2.72	5.62	3.70	4.24	3.39	22	32
VIb-HCl	Pyridine[c]	9.8	3.8	3.23	6.07	4.13	4.94	4.08	32	52
VIb-FA	Formic acid-D₂O (95:5)	5.8	7.2	3.13	5.72	3.83	4.67	3.91	—	—
VIb-TFA	Chloroform-d	4.1	8.8	2.91	5.62	3.70	4.58	—	—	—
VIc	Acetone-d_6	10.9	3.1	2.39	5.62	3.83	4.26	—	19	36
	Dimethyl sulfoxide-d_6	11.0	2.8	2.37	5.53	3.72	4.12	—	17	34
	Pyridine	10.8	3.2	2.42	6.00	4.20	4.50	—	28	55
	Chloroform-d	7.3	5.9	2.35	5.53	3.62	4.15	—	—	—
VIc-HCl	Dimethyl sulfoxide-d_6	9.6	3.6	2.94	5.46	3.68	4.33	3.51	13	31
VIc-TFA	Dimethyl sulfoxide-d_6	8.0	5.0	3.00	5.41	3.67	4.31	—	—	—
VIc-FA	Formic acid-D₂O (95:5)	3.8	9.2	3.20[g] 3.15	5.74	3.79	4.69	3.97	—	—
VIc-TFA	Chloroform-d	2.7	10.2	2.88[g] 2.98	5.69	3.67	4.63	—	—	—
VIcAc	Acetone-d_6	11.5	3.2	2.42	5.58	3.63	5.26	—	20	30
	Chloroform-d	11.0	3.2	2.43	5.56	3.55	5.28	—	19	35
VIcAc-FA	Formic acid-D₂O (95:5)	6.0	7.4	3.21	5.69	3.79	5.68	4.11	—	—
VIcAc-TFA	Chloroform-d	4.5	8.0	2.96[g] 3.03	5.64	3.65	5.63	4.01	—	—
VId	Dimethyl sulfoxide-d_6	6.7	6.4	3.28	5.61	3.95	4.49	—	—	—
	Pyridine	6.5	7.1	3.79	6.12	—	5.25	4.69	—	—
	Formic acid-D₂O (95:5)	5.8	7.0	3.39	5.79	3.75	4.82	4.08	—	—
VIdAc	Pyridine	~8	~5	3.90	6.07	—	6.21	4.99	—	—
	Pyridine-D₂O (~50:50)	7.0	6.0	3.60	5.96	—	5.95	4.55	—	—
	D₂O	—[h]	—[h]	3.61	5.94	—	5.94	4.55	—	—
	Formic acid-D₂O (~50:50)	7.0	6.0	3.34	5.78	3.64	5.75	4.28	—	—
VIIa	Dimethyl sulfoxide-d_6	10.0	2.6	—	4.02	3.13	3.54	3.04	25	30
	Pyridine	10.0	3.2	—	4.61	3.82	4.04	3.58	43	55
	D₂O	10.0	3.2	—	4.15	3.24	3.78	3.19	—	—
VIIa-HCl	Dimethyl sulfoxide-d_6	9.3	3.7	—	4.08	3.19	3.98	3.33	29	34
VIIa-HCl	Pyridine[c]	8.2	4.7	—	4.85	3.89	4.91	4.33	—	—
VIIa-FA	Formic acid-D₂O (75:25)	7.6	6.0	—	4.42	3.52	4.41	3.92	—	—
VIIa-HCl	D₂O	9.0	4.3	—	4.21	3.36	4.15	3.67	—	—
VIIb	Acetone-d_6	10.0	2.9	2.36[f]	4.13	3.32	3.84	2.81	25	36
	Dimethyl sulfoxide-d_6	10.4	2.8	2.32	3.96	3.14	3.70	2.68	21	31
	Pyridine	10.2	3.0	2.47	4.62	3.88	4.22	3.19	44	59
	D₂O	10.4	2.9	2.35[f]	4.09	3.21	3.92	2.86	—	—
VIIb-HCl	Dimethyl sulfoxide-d_6	~8.3	~3.9	2.61	4.07	3.21	4.10	3.25	—	—
VIIb-HCl	Pyridine[c]	8.4	5.0	3.18	4.71	3.80	4.79	3.96	—	—
VIIb-FA	Formic acid-D₂O (75:25)	6.9	6.0	3.00	4.39	3.52	4.43	3.70	—	—

(continued)

Table I—(Continued)

Compound	Solvent	Coupling Constants, Hz.		Chemical Shifts in δ Units, p.p.m.					Deshielding Shifts $(\nu - \nu°)^a$, Hz. at 60 MHz.	
		J_{12}	J_{45}	N—CH₃	H-1	H-2	H-4	H-5	$\nu_1 - \nu_1°$	$\nu_2 - \nu_2°$
VIIb-HCl	D₂O	~8.7	~4.9	2.86	4.21	3.38	4.26	3.51	—	—
VIIc	Acetone-d₆	9.2	3.8	2.27	4.11	3.35	4.09	—	24	37
	Dimethyl sulfoxide-d₆	9.8	3.2	2.22	3.91	3.15	3.94	—	18	32
	Pyridine	9.4	3.2	2.25	4.50	3.81	4.35	—	36	54
	Chloroform-d	8.2	4.5	2.29	4.12	3.27	4.05	2.41	—	—
VIIc-HCl	Dimethyl sulfoxide-d₆	6.7	6.7	2.80	4.04	3.23	4.16	3.38	—	—
VIIc-FA	Formic acid-D₂O (75:25)	3.8	9.4	3.10ᵈ 3.00	4.44	3.53	4.49	3.79	—	—
VIIc-HCl	D₂O	5.0	8.6	2.99	4.27	3.43	4.39	3.63	—	—
VIId	Dimethyl sulfoxide-d₆	5.8	7.2	3.19	4.11	—	4.32	3.69	—	—
	Formic acid-D₂O (75:25)	5.9	7.6	3.30	4.58	—	4.64	3.89	—	—
	D₂O	~6.5	~7.3	3.33	4.56	—	4.65	3.82	—	—
VIIIa	Pyridine	10.6	3.2	—	6.29	4.07	3.51	4.30	45	48
VIIIa-HCl	Dimethyl sulfoxide-d₆	10.2	3.5	—	5.64	3.75	3.41	4.24	23	35
VIIIa-HCl	Pyridine	9.2	3.8	—	6.26	4.28	4.24	5.19	44	60
VIIIa-FA	Formic acid-D₂O (95:5)	5.8	7.0	—	5.71	3.75	4.02	4.64	—	—
VIIIb	Pyridine	11.0	3.0	2.52	6.35	4.03	3.09	4.31	49	45
VIIIb-HCl	Dimethyl sulfoxide-d₆	10.0	3.0	2.69	5.62	3.77	3.33	4.38	22	36
VIIIb-HCl	Pyridine	9.3	3.7	3.10	6.23	4.33	3.93	5.18	42	63
VIIIb-HCl	Formic acid-D₂O (95:5)	6.2	7.2	3.13	5.74	3.78	—	4.70	—	—
VIIIc	Acetone-d₆	10.7	3.1	2.33	5.75	3.70	—	4.42	27	28
	Dimethyl sulfoxide-d₆	11.0	3.0	2.27	5.64	3.56	—	4.25	23	24
	Pyridine	10.7	3.3	2.30	6.24	3.89	—	4.66	42	37
VIIIc-HCl	Dimethyl sulfoxide-d₆	9.0	3.9	2.93	5.59	3.88	3.47	4.51	20	43
VIIIc-FA	Formic acid-D₂O (95:5)	3.8	9.5	3.16	5.69	3.84	3.80	4.69	—	—
VIIId	Dimethyl sulfoxide-d₆	6.2	8.0	3.33	5.42	3.59	—	4.61	—	—
	Pyridine	5.8	8.4	3.88	5.88	—	4.66	5.24	—	—
	Formic acid-D₂O (95:5)	4.6	9.2	3.42	5.63	3.82	4.01	4.87	—	—
IXa-FA	Formic acid-D₂O (75:25)	8.1	4.8	—	4.45	3.36	3.78	4.47	—	—
IXb	Pyridine	10.2	2.8	2.45	4.79	3.64	2.98	4.38	54	44
IXb-HCl	Dimethyl sulfoxide-d₆	~8.9	~3.6	2.60	4.05	3.15	3.11	4.21	—	—
IXb-HCl	Formic acid-D₂O (75:25)	8.2	4.8	2.99	4.46	3.36	3.58	4.56	—	—
IXc	Pyridine	9.9	3.4	2.23	4.67	3.57	—	4.53	46	40
IXc-HCl	Dimethyl sulfoxide-d₆	6.0	6.5	2.82	4.01	3.29	3.33	4.29	—	—
IXc-FA	Formic acid-D₂O (75:25)	4.6	8.5	3.03	4.42	3.47	3.57	4.53	—	—
	D₂O	6.0	7.4	2.96	4.28	3.32	3.44	4.47	—	
VIIe	Acetone-d₆	10.6	3.0	—	3.81	3.34	4.02	1.83	6	37
	Dimethyl sulfoxide-d₆	10.4	2.8	—	3.64	3.17	3.87	1.71	3	33
	Pyridine	10.4	3.0	—	4.15	3.85	4.30	2.10	15	57
	Formic acid-D₂O (75:25)	10.4	3.1	—	4.06	3.35	4.23	1.97	3	30
	Chloroform-d	10.2	2.9	—	3.79	3.27	4.07	1.88	8	36
VIIf	Acetone-d₆	10.4	3.1	—	4.22	3.32	4.00	3.82	30	36
	Dimethyl sulfoxide-d₆	10.2	3.2	—	3.99	3.11	3.79ᵇ	3.61ᵇ	24	29
	Pyridine	10.2	3.2	—	4.93	3.98	4.65ᵇ	4.44ᵇ	62	65
	Formic acid-D₂O (75:25)	10.4	3.4	—	4.43	3.36	4.28	4.09	25	30
VIf	Acetone-d₆	11.4	3.4	—	5.82	3.83	4.18ᵇ	4.02ᵇ	31	36
	Dimethyl sulfoxide-d₆	11.0	3.2	—	5.74	3.74	4.03ᵇ	3.88ᵇ	29	34
	Pyridine	11.2	3.5	—	6.45	4.30	4.68ᵇ	4.51ᵇ	55	61
	Formic acid-D₂O (95:5)	10.7	3.7	—	5.79	3.83	4.49ᵇ	4.35ᵇ	26	33
VIg	Acetone-d₆	11.2	3.4	—	5.88	3.90	3.92	3.74	35	41
VIIg	Acetone-d₆	10.0	3.6	—	4.26	3.38	3.76	3.60	33	39
VIIIg	Acetone-d₆	10.8	3.4	—	5.89	3.91	3.69	3.88	35	41
X	Carbon tetrachloride	11.0	—	—	4.71	3.40	—	—	—	—
XI	Carbon tetrachloride	11.0	—	—	4.93	3.69	5.71	5.71	13	18

ᵃ The symbols ν₁° and ν₂° refer to the chemical shifts of H-1 and H-2 in the appropriate reference Compounds I, II, and X, and ν₁ and ν₂ are the chemical shifts of H-1 and H-2 for the compound under consideration. ᵇ Differentiation between H-4 and H-5 is not certain. ᶜ From 2 to 5 drops of D₂O was added to solubilize the salts in pyridine. ᵈ FA signifies the formate salt, obtained by dissolving the amine in formic acid. ᵉ TFA signifies the trifluoroacetate salt. This was obtained by the addition of a few drops of trifluoroacetic acid to a solution of the amine in the given solvent. ᶠ Differentiation between the NCH₃ and ArCH₃ signals is not certain because the two signals are very close. ᵍ Two methyl signals. ʰ The coupling constants cannot be determined. All signals are very broad and poorly resolved, suggesting micellar aggregation of the quaternary salt in D₂O. Addition of a few drops of formic acid causes sharpening of all signals.

d₆, and in pyridine containing a few drops of D₂O, the equilibrium favors the normal chair conformation. In chloroform and in formic acid–D₂O solutions, the equilibrium is shifted predominantly toward the inverted chair conformation. It is also noteworthy that the formate and trifluoroacetate salts of VIc, in formic acid and chloroform-d, respectively, exhibit a higher population of the inverted chair conformation than the corresponding salts of the acetoxy derivative VIcAc.

Figure 1 shows the spectra and predominant conformers of the hydrochloride salt of VIIIc in dimethyl sulfoxide-d₆ and formic acid–D₂O solutions. The inversion of the values of J_{12} and J_{45} in the two solvent systems is obvious. On the basis of the extreme shifts in equilibrium positions between the two chair conformers for the salts of VIc and VIIIc with a change of solvent, it seems reasonable to conclude that the observed intermediate J_{12} and J_{45} values for the primary, secondary, and tertiary amines and their salts in certain

solvent systems represent equilibria of different proportions of the two conformers, but the possibility of contributions from twist and boat forms is not ruled out.

There is no doubt that protonation of the amino groups, especially the dimethylamino group, increases the steric requirements of the groups by forcing the nitrogen into the sp^3 hybrid form and preventing strain release by nitrogen inversion. Undoubtedly this factor contributes in part to the conformational differences seen between VIa, VIb, VIc, and their respective trifluoroacetate salts in, for example, chloroform-d. But since one is dealing with thermodynamic equilibria, it is the overall free energy differences between the various conformers in the given medium that affect the relative populations at equilibrium and it would be an oversimplification to attribute the observed differences strictly to steric factors of the protonated amino groups. The significant differences seen between solvent systems indicate that in the salts, as was the case in the free amines, intermolecular hydrogen bonding with the solvent molecules *versus* intramolecular hydrogen bonding between the hydroxyl and the amino function at the C-4 and C-5 positions in the inverted chair contributes significantly to the relative free energy states of the systems. A comparison of the data for the quaternary ammonium salts with those for the protonated dimethylamines also lends support to this contention.

In the case of the free amines, the intramolecular hydrogen bonding will be mostly between the hydroxyl hydrogen and the nitrogen; in the salts, it is the oxygen that is the hydrogen acceptor. The strength of these hydrogen bonds is not necessarily comparable. The differences in energy barriers to rotation of the amino group upon alkylation and/or protonation will also affect the strength of hydrogen bonds. The apparent importance of intramolecular hydrogen bonding in formic acid is noteworthy. There is a possibility of a bridging of the two equatorially oriented functional groups by the formate anion or a formic acid molecule. If this plays any role in the conformational state of the system, the resulting free energy differences would involve differences between two intermolecular hydrogen-bonding states rather than competition strictly between intermolecular and intramolecular hydrogen bonding. The same phenomenon could be operative with the trifluoroacetates in chloroform and dimethyl sulfoxide-d_6, but in dimethyl sulfoxide-d_6 the intermolecular association with solvent molecules is expected to play a much more important role than in chloroform.

Another factor which must not be overlooked in considering factors that affect the relative thermodynamic stabilities of conformers of the salts is the stronger basicity of an equatorial than an axial amino group, as shown in *cis*- and *trans*-4-*tert*-butylcyclohexylamines (7, 13).

Effects of Quaternization—With the quaternary ammonium salts, none of the systems shows a high predominance of chair conformers with the o-tolyl group equatorial and the trimethylammonium group axial. This is understandable because the trimethylammonium group probably has steric requirements that are as demanding as those of the tertiary butyl group. What is of greater interest is the much lower population of the inverted chair with the quaternary salts VId and VIId in formic acid in comparison to the salts of the corresponding dimethylamines VIc and VIIc in the same solvent. This again suggests the importance of intramolecular hydrogen bonding in the secondary amines, but the possibility of a greater contribution from twist or other flexible conformers with the quaternary salts is not ruled out. The contributions to the overall equilibria from flexible forms cannot be assessed from the present data, but it is noteworthy that almost all systems of the quaternary salts show unequal populations of the two chair conformers, showing that the systems cannot consist of flexible forms only. The highest population of the inverted chair form is seen with VIIId in formic acid-D_2O solutions.

A deformation involving a flattening of the chair ring was invoked by Tichý *et al.* (11) for strain release in *trans*-4-*tert*-butyl-*cis*-2-dimethylaminocyclohexanol and *cis*-5-*tert*-butyl-*cis*-2-dimethylaminocyclohexanol, where the dimethylamino group is axial in each case. A flattening of this type about the C(2)—C(3) and C(3)—C(4) bonds, with little change in the geometry of the rest of the molecule, was found by Shefter and Smissman (14) in 3(a)-dimethylamino-2(a)-acetoxy-*trans*-decalin methiodide in the solid state by single crystal X-ray crystallography. The results with the systems in solution in the present study give no evidence of significant deformation of this type. The effect of such a deformation would be to increase the dihedral angle between H-4 and H-5, bringing this angle closer to
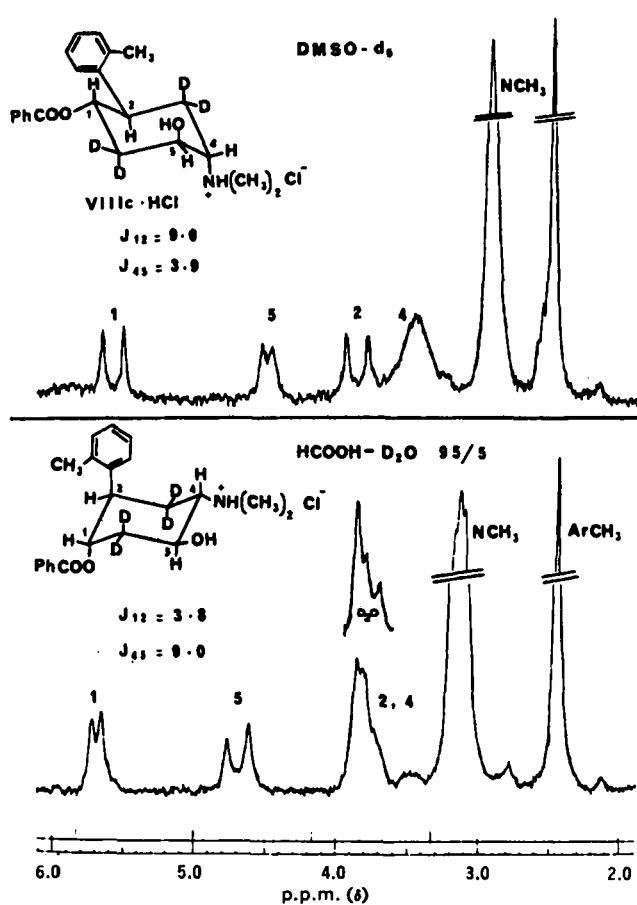

Figure 1—*The 60-MHz. NMR spectra and predominant conformations of* trans-2-o-tolyl-cis-4-N,N-*dimethylamino*-trans-5-hydroxy-cyclohexyl-3,3,6,6-d₄ *benzoate hydrochloride in dimethyl sulfoxide-d₆ and in formic acid–D₂O (95:5) solution.*

90° from its normal value of 60°, thus causing a decrease in the J_{45} value with little if any change in the value of J_{12}. No significant change of this type is seen with increased steric requirements of the amino group either by methylation to the dimethylamino or by protonation. In fact, the sum of J_{12} and J_{45} remains quite constant throughout the series. Assessment of contribution from this type of deformation in the systems is more direct where J_{12} has the normal J_{aa} value, and it would be hazardous to eliminate such contributions in the quaternary salts on the basis of the consistencies of the sums of the J_{12} and J_{45} values.

Half-Chair Conformations of Cyclohexene and Epoxides—The data in Table I also show a very high predominance of the half-chair conformations, with the aromatic and benzoate substituents in equatorial orientations in the cyclohexenes III and XI and the epoxides IV and V.

Equatorial–Equatorial Coupling Constants—The data in Table I also show that for those systems having the chair conformation with H-4 and H-5 in equatorial orientations the J_{45} values are not very sensitive to changes in substituents at C-4 and C-5, as seen by comparison of the various aminohydroxy compounds, the dihydroxy Compounds VIf and VIIf, and the deuterated hydroxy Compound VIIe.

Deshielding Effects—*1,3-Diaxial Deshielding*—The spatial 1,3-diaxial deshielding effect of a hydroxyl group on ring hydrogens is a well-documented phenomenon in NMR spectroscopy of six-membered ring compounds (5, 15–20). It is also known that the effect varies with solvents. There is enhanced deshielding in pyridine over chloroform and many other solvents (5, 19). Acetylation to the acetoxy group reduces the deshielding effect (15, 16, 18, 20). The tetradeuterated compounds of series VI–IX are well suited for the investigation of similar deshielding effects by primary, secondary, and tertiary amino groups and for a comparison of the effects in protonated and nonprotonated forms. This has been done by comparison of the chemical shifts of H-1 in compounds of series VI and VII and
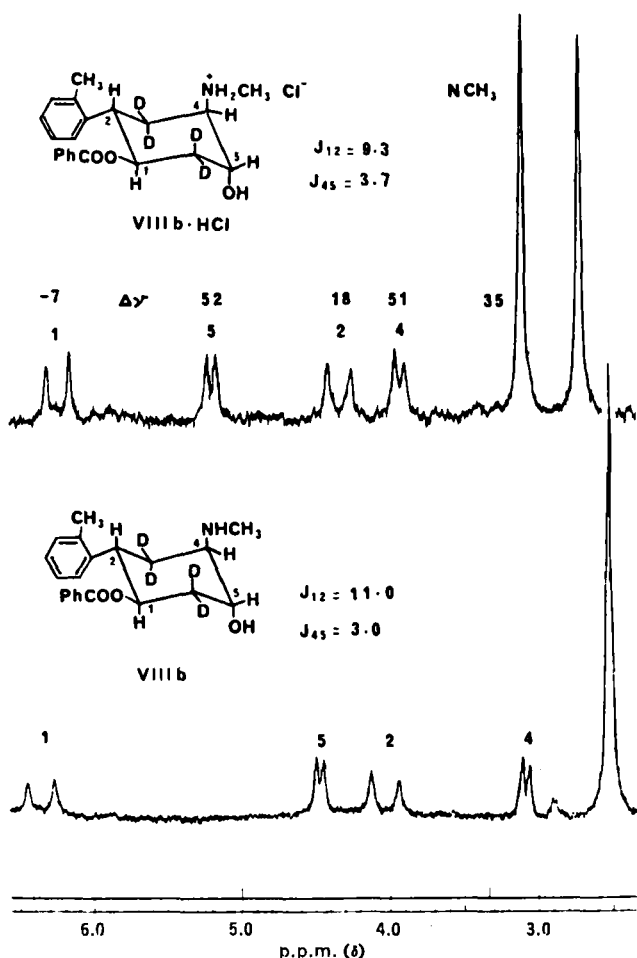
Figure 2—*The 60-MHz. NMR spectra of* trans-2-o-tolyl-cis-4-N-methylamino-trans-5-hydroxycyclohexyl-3,3,6,6-d_4 benzoate and the hydrochloride salt in pyridine. The shifts upon protonation are designated in hertz.

of H-2 in compounds of series VIII and IX with those of H-1 and H-2 in the appropriate reference Compound I or II in the same solvent. Obviously, the same spectra also provide information on the deshielding effects of the hydroxyl group in the same compounds. The comparison could not be done in acetone with the primary amines because of rapid imine formation, but there was no evidence of any imine formation between acetone and the secondary amines. The observed shifts are given in hertz at 60 MHz. in Table I.

The 1,3-diaxial deshielding by the amino and hydroxyl substituents has meaning only in those systems known to have a high predominant population of the chair conformer with the amino and hydroxyl groups in axial orientations. Therefore, the deshielding shifts have been listed in Table I only for those systems where the J_{12} values are 9 Hz. or above. In systems where the inverted chair predominates, the downfield shifts of H-1 and H-2, compared to the reference Compounds I and II, are mostly the result of a change of the orientation of these hydrogens from axial to equatorial.

The pertinent data for the 1,3-diaxial deshielding by amino groups are more complete for the compounds of series VI and VII, those compounds in which H-1 is deshielded by the amino groups and H-2 is deshielded by the hydroxyl group. It is important to note the consistency of the deshielding ($\nu_2 - \nu_2°$) by the hydroxyl group in a given solvent for those systems having a high predominance of the normal chair conformation (tolyl group equatorial). These observed $\nu_2 - \nu_2°$ deshielding values are generally in excellent agreement with the corresponding values in the reference Compound VIIe, having a deuterium at C-5, as well as for those of the 4,5-dihydroxy Compounds VIf and VIIf and the previously published values (5) of 38 Hz. in acetone and 58 Hz. in pyridine for trans-2-o-tolyl-cis-4-hydroxycyclohexanol-3,3,6,6-d_4. This information reinforces the conclusion arrived at from observed coupling constants

that there is no evidence for a significant deformation involving a flattening of the chair ring about the C(5)—C(4) bond in these compounds in solution.

1,3-Diaxial deshielding of H-1 by the NH$_2$ group at C-5 in Compounds VIa and VIIa is comparable to the deshielding of H-2 by the C-4 hydroxyl group in dimethyl sulfoxide-d_6. In pyridine, however, a slightly lower deshielding effect by the NH$_2$ compared to the OH group is observed. Alkylation to methylamino and dimethylamino derivatives reduces the deshielding in the two solvents. In acetone the deshielding of H-1 by the secondary and tertiary amino groups is somewhat comparable to the results in dimethyl sulfoxide-d_6. There are undoubtedly several factors involved in the reduction of the 1,3-diaxial deshielding effect upon methylation. Changes in the time-average orientations of the amino group will affect any deshielding resulting from the anisotropies of the unshared electrons and the N—H and N—CH$_3$ bonds, and there are undoubtedly differences in the anisotropic properties of the N—H and N—CH$_3$ bonds. Successive methylation should also affect solvation of the amino group, and differences in solvation can affect the time-average orientations of the anisotropic solvent molecules associated through hydrogen bonding with the NH hydrogens. The role played by solvation in the decreased deshielding in going from primary to secondary to tertiary amines in the free bases is difficult to assess from the available data. If orientation of the solvent molecules through hydrogen bonding with N—H hydrogens played a dominant role in 1,3-diaxial deshielding by the amino group, a larger difference should be expected in pyridine than in the other solvents between primary, secondary, and tertiary amines. It is interesting that there is very little difference in the 1,3-diaxial deshielding effects of the protonated versus nonprotonated amino groups in VIa, VIb, VIc, and VIIa in dimethyl sulfoxide-d_6 and in VIa and VIb in pyridine containing slight amounts of D$_2$O. Surprisingly, a larger difference is not found in pyridine for VIa and VIb upon protonation, where hydrogen bonding of the protonated form with pyridine is expected to play a larger role. Such a difference is seen in pyridine for the protonated versus nonprotonated forms in Compounds VIIIa and VIIIb, where the deshielding is on H-2. Comparisons of 1,3-diaxial deshielding effects of protonated and free bases are limited to those compounds that have a high predominance of the normal chair conformation under both conditions. The conformational changes brought about by protonation restricts the available data for such comparisons. The overall results suggest important contributions from the anisotropic properties of the ring C—N and the N—CH$_3$ bonds to the observed long-range 1,3-diaxial deshielding effects caused by free amino groups and aminium cations. If this is the case, the C(5)—N bond contributes to deshielding of H-1 while the N—CH$_3$ bond contributes to shielding of H-1.

Table I shows that in the imines VIg, VIIg, and VIIIg in acetone, the 1,3-diaxial deshielding by the N=C(CD$_3$)$_2$ group is of about the same magnitude as the deshielding caused by an NH$_2$ or an OH group. When the primary amines are added to acetone-d_6 at an NMR concentration of about 100 mg. in 0.5 ml. of solvent, the equilibrium is reached within a few minutes, and at equilibrium the NMR spectra show no evidence of any amine. Dropwise addition of D$_2$O shifts the position of the equilibrium, and the NMR spectra now show the presence of the free amine by the appearance of second H-1 and H-5 signals. After the addition of 10 drops of D$_2$O to the NMR sample of VIg in acetone-d_6, the ratio of the imine to amine was about 3 to 1. The chemical shifts of H-1 and H-5 of the primary amine were δ 5.68 and 3.38, respectively. The difference in 1,3-diaxial deshielding of H-1 by the N=C(CD$_3$)$_2$ and NH$_2$ groups in this medium is only 4 Hz. at 60 MHz.

Geminal Deshielding—A comparison of the chemical shift of H-5 in VIIe with those of H-5 in VIIa and VIIb gives an indication of the geminal deshielding effects of the amino group in dimethyl sulfoxide and pyridine and of the methylamino group in acetone, dimethyl sulfoxide, and pyridine. Accurate chemical shifts could not be obtained for H-5 of VIIc in these solvents because the signal is overlapped by the signals of the aromatic and amino methyl groups. The deshielding is more pronounced with the primary amino group and decreases successively with the methyl and dimethylamino groups. At 60 MHz. there is a geminal deshielding of 80 Hz. in dimethyl sulfoxide-d_6 and 89 Hz. in pyridine by the NH$_2$ group in going from VIIe to VIIa; a deshielding of 59 Hz. in acetone, 58 Hz. in dimethyl sulfoxide-d_6, and 65 Hz. in pyridine by the NHCH$_3$ group in going from VIIe to VIIb; and a deshielding of 32 Hz. in CDCl$_3$ in going from VIIe to VIIc. A comparison of the chemical

shifts of N-methyl hydrogens in the monomethyl and dimethyl compounds shows that the signal of the monomethyl group is further downfield by from 3 to 14 Hz., with the largest differences occurring in pyridine.

Protonation of the primary amino group of VIa and VIIa causes a downfield shift of H-5 (geminal deshielding) of 17 or 18 Hz. in dimethyl sulfoxide-d_6 and of 42–45 Hz. in pyridine. With the secondary amines VIb and VIIb, the downfield shift of H-5 upon protonation is of the order of 32–34 Hz. in dimethyl sulfoxide-d_6, while in pyridine it is from 46 to 52 Hz. Thus, there is a significant difference in the geminal deshielding of H-5 between the primary and secondary amines upon protonation in series VI and VII in dimethyl sulfoxide-d_6 but not in pyridine. However, the protonation geminal deshielding effect is considerably larger in pyridine than in dimethyl sulfoxide-d_6. The downfield shift of the N—CH$_3$ group upon protonation in series VI and VII in dimethyl sulfoxide-d_6 is about twice as large for the dimethylamino as for the corresponding monomethyl compounds.

Vicinal Shielding and Deshielding—A comparison of the chemical shift of H-4 in VIIe with those of H-4 in Compounds VIIa, VIIb, and VIIc gives information on the vicinal shielding effects of the NH$_2$, NHCH$_3$, and N(CH$_3$)$_2$ groups where the dihedral angle between H-4 and the C(5)—N bonds is essentially 60°. The data show an upfield shift of 19 and 16 Hz. (at 60 MHz.) for H-4 in dimethyl sulfoxide and pyridine, respectively, in the primary amine VIIa compared to the reference Compound VIIe. The methylamino group causes a shielding of H-4 in VIIb of 11, 10, and 5 Hz. in acetone, dimethyl sulfoxide-d_6, and pyridine, respectively. The dimethylamino group causes a deshielding of H-4 by 4, 4, and 3 Hz. in acetone, dimethyl sulfoxide-d_6, and pyridine, respectively, and practically no change in chloroform-d.

Protonation of the amino group brings about a significant downfield shift of vicinal H-4 in compounds of series VI and VII in comparison to the chemical shifts of H-4 in the free amines. There is little difference in this effect between the primary and secondary amines of series VI and VII in a given solvent, but there is a significant difference between dimethyl sulfoxide-d_6 and pyridine for a given compound. The shifts in VIa and VIb in dimethyl sulfoxide-d_6 are 26 and 23 Hz., respectively, but they are 49 and 42 Hz. in pyridine. For VIIa and VIIb the shifts are 26 and 24 Hz., respectively, in dimethyl sulfoxide-d_6 and 52 and 35 Hz. in pyridine. For the dimethyl Compounds VIc and VIIc, the shift of H-4 in dimethyl sulfoxide-d_6 upon protonation is 13 Hz. in each case, but only VIc-HCl of the two salts is shown to have a high predominance of the normal chair form.

The shifts upon protonation are demonstrated for VIIIb in pyridine in Fig. 2.

Although there is a significant vicinal deshielding upon protonation in these compounds, the net vicinal deshielding effects of the aminium cation, as seen by comparison of the chemical shift of H-4 in VIIe with those of H-4 in the protonated amines, is not as large as the shift observed in going from the free amine to the protonated form with the primary and secondary amines. This results from the fact that the free NH$_2$ and NHCH$_3$ groups cause a vicinal shielding of H-4 in VIIa and VIIb.

Demarco *et al.* (19) compiled data on deshielding effects in pyridine relative to chloroform for geminal and vicinal deshielding by the hydroxyl group, and they pointed to the dihedral angle dependency for the solvent difference of the vicinal deshielding by the hydroxyl group. The data on the chemical shifts of H-4 and H-5 of Compound VIIe are of special interest, because they provide information on these effects in a system where the hydroxyl function and the deshielded hydrogens are remote from other functional groups. The observed downfield shift differences of 0.23 and 0.22 p.p.m. for H-4 and H-5, respectively, in pyridine relative to CDCl$_3$ are in good agreement with differences listed by Demarco *et al.* (19). The differences in these two solvents are even greater for the chemical shifts of H-1 and H-2 in VIIe, but in this AX system the presence of the aromatic group may affect the chemical shift of H-1 differently in the two solvents. With respect to H-2, in addition to the solvent difference on the vicinal deshielding by the OH group is added the difference of these solvents on the 1,3-diaxial deshielding by the C-4 hydroxyl group. There is also a possibility that the geminal deshielding of H-2 by the aromatic group could be different in the two solvents. The observed difference of 0.58 p.p.m. in the chemical shift of H-2 in the two solvents is the result of a summation

of these effects. The differences in the other three solvents are also of interest.

Deshielding by the Double Bond—The long-range deshielding effect of the double bond is seen by comparison of the chemical shifts of the hydrogens on the nitro- and aromatic-bearing carbons, respectively, between Compounds X and XI. Deshielding values of 0.22 p.p.m. for H-1 and 0.29 p.p.m. for H-2 in XI, measured in carbon tetrachloride, are in agreement with reported values in an analogous system in chloroform-d (20). Similar results are seen by comparison of the chemical shifts of H-1 and H-2 between I and III in carbon tetrachloride, acetone-d_6, dimethyl sulfoxide-d_6, and pyridine. The deshielding of H-1 varies from 0.17 to 0.21 p.p.m. in the four solvents, and the deshielding of H-2 varies between 0.31 and 0.33 p.p.m. The larger deshielding of H-2 could be associated with a difference in the average orientation of the *o*-tolyl group in the saturated *versus* the unsaturated compounds, causing a slight difference in the deshielding of H-2 by the magnetic anisotropy of the tolyl group. It is also possible that the magnetic anisotropy of the benzoate functional group has different effects on H-2 and/or H-1 in the unsaturated and saturated compounds.

Deshielding by the Epoxy Group—The anisotropic effects of the epoxy group in substituted epoxycyclohexanes were reported previously (20). In the half-chair conformation of *trans*- and *cis*-4,5-epoxy-*trans*-2-(*p*-chlorophenyl)nitrocyclohexane, the epoxy group was found to cause a deshielding of the axial hydrogen *cis* to the epoxy group, and two carbons removed from it, by about 0.2–0.26 p.p.m. when measured in chloroform. Little effect was found on the chemical shift of the *trans*-axial hydrogen two carbons removed from the epoxy group. For epoxides IV and V, again the J_{12} values indicate a large predominance of the expected half-chair conformation. A comparison of the chemical shifts of H-1 and H-2 in epoxide IV with the chemical shifts of H-1 and H-2 in the reference ester I shows a deshielding of H-2 ranging from 0.25 to 0.36 p.p.m. in the four solvents, with little change in the chemical shift of H-1. This substantiates the previous results (20). Epoxide V was investigated only in carbon tetrachloride. It shows a deshielding of H-1 by 0.12 p.p.m. and of H-2 by 0.06 p.p.m.

EXPERIMENTAL[1]

Compounds III, IV, V, and XI—These compounds were prepared by the methods previously described for the corresponding hydrogen-containing compounds (6, 21), except that *m*-chloroperbenzoic acid was used instead of perbenzoic acid for the epoxidation. The selective incorporation of deuterium on carbons 3 and 6 was accomplished by using butadiene-1,1,4,4-d_4 (1, 22) instead of butadiene in the Diels–Alder condensation step of the synthetic scheme (6).

Compound I—This compound was obtained by catalytic hydrogenation of III using 5% palladium-on-carbon in ethyl acetate, m.p. 71–73°. Mass spectral analysis for the molecular ion gave *m/e* 298.1880 (calc. for C$_{20}$H$_{18}$D$_4$O$_2$: 298.1866).

Compound II—This compound was obtained by basic hydrolysis of I. The corresponding hydrogen-containing compound was reported previously (22).

Compound VIIe—This compound was obtained by lithium aluminum deuteride reduction of IV. The corresponding hydrogen-containing compound was reported previously (6).

Compound VIf—This compound was obtained by acid-catalyzed hydrolysis of a mixture of IV and V by warming for 5 min. in a solution of 7% sulfuric acid in a mixture of 60% dioxane in water. The corresponding hydrogen-containing compound was also prepared and recrystallized from a mixture of dioxane, benzene, and hexane, m.p. 173.5–174°.

Anal.—Calc. for C$_{20}$H$_{22}$O$_4$: C, 73.60; H, 6.79. Found: C, 73.43; H, 6.80.

Compound VIIf—This compound was obtained from basic hydrolysis of VIf. The corresponding nondeuterated analog was also prepared and recrystallized from water. The compound under-

Table II—Physical Constants and Analytical Data for *trans*-2-*o*-Tolyl-*cis*-4-hydroxy-*trans*-5-methylaminocyclohexanol, *trans*-2-*o*-Tolyl-*cis*-4-methylamino-*trans*-5-hydroxycyclohexanol, and the Corresponding Dimethylamino and 1-Benzoate Derivatives

Compound	Amine	Melting Point Hydrochloride	Methiodide	Analysis[a], % Calc.	Found
VI*b*	162.5–164°	276–279°	—	C — H — N —	— — —
VII*b*	148–149°	263–264.5°	—	C 61.85 H 8.16 N 5.16	62.01 7.79 5.20
VIII*b*	—	240–241.5°	—	C — H — N —	— — —
IX*b*	—	211–213°	—	C 61.85 H 8.16 N 5.16	61.94 8.17 4.99
VI*c*	157–158°	214–217°[c]	202.5–204°	C 74.75 H 7.70 N 3.96[b]	74.79 7.45 3.85[b]
VII*c*	163–163.5°	260–263.5°	205–207°	C 63.04 H 8.46 N 4.90	63.14 8.20 4.85
VIII*c*	142–143.5°	240.5–245.5°	234–234.5°	C 74.75 H 7.70 N 3.96[b]	74.56 7.91 4.01[b]
IX*c*	51–74°[d]	248–250°	89–162°[e]	C 63.04 H 8.46 N 4.90	63.22 8.37 5.15

[a] The analyses are on the hydrochloride salts unless stated otherwise. [b] Analysis on the free amine. [c] Appears to undergo polymorphic changes, melts at about 210°, resolidifies, and remelts between 214–217°. [d] Crystallizes as a hydrate from an acetone–water mixture. [e] Crystallizes as a 1:1 dioxane complex from dioxane–methanol solution. The ratio of dioxane was determined by NMR.

goes polymorphic changes upon heating: melts at 150–153°, resolidifies at 156–160°, and remelts at 177.5°.

Anal.—Calc. for C₁₃H₁₈O₂: C, 70.24; H, 8.16. Found: C, 69.97; H, 8.15.

Compound X—This compound was reported previously (1).

trans-2-*o*-Tolyl-*cis*-4-hydroxy-*trans*-5-aminocyclohexyl-3,3,6,6-*d₄* Benzoate (**VI***a*)—This compound and the corresponding *N*-methyl (**VI***b*) and *N,N*-dimethyl (**VI***c*) derivatives were obtained from the epoxide IV; likewise, the *trans*-2-*o*-tolyl-*cis*-4-amino-*trans*-5-hydroxycyclohexyl-3,3,6,6-*d₄* benzoate (**VIII***a*) and the corresponding *N*-methyl (**VIII***b*) and *N,N*-dimethyl (**VIII***c*) derivatives were obtained from epoxide V by heating the appropriate epoxide with either ammonia, methylamine, or dimethylamine in peroxide-free tetrahydrofuran at temperatures ranging from about 100 to 120° in a stainless steel pressure bomb for 1–4 days (usually for 2 days). The method is analogous to that described by Mousseron *et al.* (23) for opening of epoxides with amines. The yields were generally quite good, mostly above 80% and often above 90%. The degree of aminolysis of the benzoate group was usually low. Treatment of the benzoates with potassium hydroxide in methanol gave the corresponding dihydroxyamino compounds, VII*a*, VII*b*, VII*c*, IX*a*, IX*b*, and IX*c*. The quaternary trimethylammonium chlorides were obtained from the corresponding dimethylamino compounds by heating with a large excess of methyl chloride in anhydrous, peroxide-free tetrahydrofuran at 90–95° for 48 hr. in a stainless steel pressure bomb.

Analytical data and physical constants for the nondeuterated *N*-methyl and *N,N*-dimethylamino derivatives of Compounds VI–IX are given in Table II. The melting points of the methiodide salts obtained from the *N,N*-dimethylamino compounds are also included. The nondeuterated primary amines were not prepared. Melting points of the deuterated primary amines and their hydrochloride salts were as follows: VI*a*, 122.5–123.5°; VI*a*-HCl, 254–256.5°; VII*a*, 139.5–141°; VII*a*-HCl, 259–263°; VIII*a*, 167–167.5°; VIII*a*-HCl, 228.5–231°; and IX*a*, 215.5–217.5°. Mass spectral analyses were obtained for the diols VII*a* and IX*a*, which, in turn, were obtained by basic hydrolysis of the benzoates VI*a* and VIII*a*. Mass spectral analysis for the molecular ion gave *m/e* 225.1654 for VII*a* and 225.1648 for IX*a* (calc. for C₁₃H₁₅D₄NO₂: 225.1660). The mass spectral analyses were carried out on the hydrochloride salts. The loss of hydrochloric acid is a normal fragmentation of salts of amines.

Nondeuterated Compounds VI*c* and VIII*c* were converted to the corresponding acetates XII and XIII by treating with acetic anhydride in anhydrous pyridine, and similar treatment of the non-

deuterated diols VII*c* and IX*c* produced the corresponding diacetates XIV and XV, respectively.

trans-2-*o*-Tolyl-*cis*-4-acetoxy-*trans*-5-dimethylaminocyclohexyl Benzoate (**XII**)—This compound had a melting point of 140.5–141.5°; NMR (pyridine): δ 5.90 (td, 1, poorly resolved, w ~ 25 Hz., H-1), 5.48 (m, 1, w ½ = 7 Hz., H-4), 3.81 (td, 1, *J* = 11.5, 4.8 Hz., H-2), 2.48 (s, 3, ArCH₃), 2.43 (s, 6, N—CH₃), and 2.17 (s, 3, Ac).

Anal.—Calc. for C₂₄H₂₉NO₄: C, 72.88; H, 7.39; N, 3.54. Found: C, 73.05; H, 7.50; N, 3.65.

The hydrochloride had a melting point of 197–202.5°, and the methiodide had a melting point of 215–217.5°.

trans-2-*o*-Tolyl-*cis*-4-dimethylamino-*trans*-5-acetoxycyclohexyl Benzoate (**XIII**)—This compound had a melting point of 85.5–87°; NMR (pyridine): δ 5.92 (td, 1, partially overlapped by H-5, H-1), 5.67 (m, 1, overlaps H-1, H-5), 3.83 (dt, 1, *J* = 11.0, 4.8 Hz., H-2), 2.50 (s, 3, Ar—CH₃), 2.30 (s, 6, N—CH₃), and 2.13 (s, 3, Ac).

The hydrochloride salt melts at about 208°, resolidifies, and remelts at 214–217°.

Anal.—Calc. for C₂₄H₃₀ClNO₄: C, 66.73; H, 7.00; N, 3.24. Found: C, 66.46; H, 6.90; N, 3.16.

The methiodide had a melting point of 208–211°.

trans-2-*o*-Tolyl-*cis*-4-acetoxy-*trans*-5-dimethylaminocyclohexyl Acetate (**XIV**)—This compound had a melting point of 88.5–91°; NMR (pyridine): δ 5.62 (dt, 1, *J* = 11.0, 4 Hz., H-1), 5.40 (m, 1, H-4), 3.58 (dt, 1, *J* = 11.2, 5 Hz., H-2), 2.37 (s, 3, ArCH₃), 2.34 (s, 6, N—CH₃), 2.13 (s, 3, Ac), and 1.72 (s, 3, Ac).

Anal.—Calc. for C₁₉H₂₇NO₄: C, 68.44; H, 8.16; N, 4.20. Found: C, 68.13; H, 7.97; N, 4.31.

The hydrochloride had a melting point of 220.5–224.5°. The methiodide melts around 237°, resolidifies, and remelts at 246–248°.

trans-2-*o*-Tolyl-*cis*-4-dimethylamino-*trans*-5-acetoxycyclohexyl Acetate (**XV**)—This compound had a melting point of 90–91°; NMR (pyridine): δ 5.59 (td, 1, overlapped by H-5, H-1), 5.56 (m, 1, overlaps H-1, H-5), 3.60 (dt, 1, *J* = 10.5, 4.8 Hz., H-2), 2.42 (s, 3, Ar—CH₃), 2.26 (s, 6, N—CH₃), 2.08 (s, 3, Ac), and 1.68 (s, 3, Ac).

Anal.—Calc. for C₁₉H₂₇NO₄: C, 68.44; H, 8.16; N, 4.20. Found: C, 68.10; H, 8.10; N, 4.24.

The hydrochloride had a melting point of 185–188.5°, and the methiodide had a melting point of 210.5–211.5°.

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Excretion of *trans*-Δ9-Tetrahydrocannabinol and Its Metabolites in Intact and Bile Duct-Cannulated Rats

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Abstract ☐ Tritiated Δ9-tetrahydrocannabinol (I) was administered both orally and intravenously to groups of bile duct-cannulated rats and those with their bile duct intact. From 55.8 to 66.9% of the total radioactivity was excreted during the 96-hr. period following administration. The excretion of radioactivity was minimal in each group beyond 48 hr. after drug administration. The major route of excretion following the intravenous administration in bile duct-cannulated rats was by way of bile (59.4%; feces, 2.7%), whereas more radioactivity was excreted in feces (41.5%) than bile (21.5%) when the drug was given orally. The radioactivity excreted in the feces of the orally medicated rats was mainly extractable in petroleum ether. This extract was found to contain I by TLC and GLC analysis. After intravenous administration, the radioactivity in the feces was not extractable in petroleum ether but appeared in ether, methanol, and water extracts. TLC confirmed that the radioactivity in these solvents was associated with metabolites of I. Bile contained mainly metabolites of I, as did the urine. Less than 10% of the radioactivity was excreted in the urine of each group of rats.

Keyphrases ☐ *trans*-Δ9-Tetrahydrocannabinol, radiolabeled, and metabolites—excretion kinetics after oral and intravenous administration, intact and bile duct-cannulated rats ☐ Excretion kinetics—radiolabeled *trans*-Δ9-tetrahydrocannabinol and metabolites after oral and intravenous administration, intact and bile duct-cannulated rats ☐ Cannulation *versus* noncannulation of bile duct—excretion kinetics of radiolabeled tetrahydrocannabinol after oral and intravenous administration, rats

The earliest reports describing the distribution of Δ9-tetrahydrocannabinol (I) were made by Miras (1) and Joachimoglu *et al.* (2). These investigators described the distribution of 14C-Δ9-tetrahydrocannabinol, isolated from marijuana grown in a 14CO2 atmosphere, in the rat. The findings of King and Forney (3) using a fluorometric method and those of Turk (4) using TLC were consistent with the earlier reports. Human studies by Miras and Coutselinis (5) indicated that only 5.2% of smoked radiolabeled hashish was secreted by the bile and 16.9% was excreted through the urine.

Due to the recent synthesis of highly purified tritium- and carbon-labeled I and their availability to the research community, more progress has been made concerning the metabolism and excretion of I. Lemberger *et al.* (6, 7) showed that radiolabeled I is mainly excreted by man in the feces and to a less extent in the urine, mainly as polar metabolites. Agurell *et al.* (8, 9) studied the metabolism and excretion of radiolabeled I in the rat and the rabbit. More recently, Klausner and Dingell (10) also reported the excretion of I in the rat. Their data agree with the excretion data reported by