DETERMINATION OF α OR β SUBSTITUTION OF THE INDOLE NUCLEUS BY NUCLEAR MAGNETIC RESONANCE[1]

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ABSTRACT

Marked differences in chemical shift of the signal for the α proton in indole and substituted indoles occur when spectra are obtained in non-polar and polar solvents. In contrast, using polar and non-polar solvents, only small differences are observed in the chemical shift of the signal for the β proton in indoles. The utility of the solvent effect on the chemical shift to distinguish between α and β substitution of the indole nucleus is pointed out.

INTRODUCTION

Witkop *et al.* (1) have shown that nuclear magnetic resonance can be used to determine substitution at the *alpha* or *beta* position of the indole nucleus. In carbon tetrachloride or deuterochloroform solution the signal for the α protons of indole, N-methylindole, skatole, and tryptamine was at the respective τ value (referred to the internal standard, tetramethylsilane) of 3.32 (triplet), 3.18 (doublet), 3.20 (poorly resolved doublet), and 3.08 (doublet). The β proton signal in indole, N-methylindole, and 2-methylindole was at a τ value of 3.62 (triplet), 3.52 (doublet), and 3.87 (broad single peak) respectively. Thus the β proton gave a signal at higher field than that found for the α proton, the difference in chemical shift for these two protons lying between 0.3 and 0.8 τ units. The signals for the α and β protons were quite clearly discernible, well separated, and at higher field than those for the remaining protons in the indole nucleus, except for the case of quebrachamine, whose signal for the α proton was buried in the more complicated absorption due to the aromatic ring protons in the region approximately between 2 and 3 τ.

We have had occasion to determine whether the α or β position of certain indole nuclei was substituted by groups other than alkyl, and hence turned to n.m.r. for assistance in this problem. When groups more electronegative than alkyl substituents were introduced into the α position, the signal for the remaining β proton, normally found well isolated at about 3.5 to 3.9 τ (1), occurred considerably farther downfield, at approximately 2.8 to 3.2 τ, in the region where the α proton absorption is shown to occur in indole and 3-alkylated indoles (3.08–3.32 τ). Hence, observation of a signal in this region is not conclusive proof that the β, rather than the α, position has been substituted.

Among the factors listed by Roberts (2) which affect the extent of chemical shift of protons is that due to solvent. It occurred to us that, because of the obvious difference in electron environment at the α and β protons, change in solvent from one of non-polar character to one of highly polar character might affect one of the protons (α or β) to a greater extent than it did the other. Hence solvent shift or lack of solvent shift might be used as a means to determine whether we were dealing with the α- or β-substituted indole nucleus.

Accordingly a number of indole derivatives were synthesized in which substitution at the α or β position was known. These, along with indole itself, were subjected to n.m.r. analyses on a 60 Mc Varian spectrometer using a variety of solvents. Tetramethylsilane

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[1] *Taken from the thesis of R. V. Jardine to be submitted to the Faculty of Graduate Studies at the University of Alberta as part of the requirements for the degree of Doctor of Philosophy.*

was used as an internal standard and all values were expressed on the τ scale (3). To aid in determining the position of the α and β proton resonance signals, use was made of the fact that the doublet or multiplet, caused by spin–spin coupling between the proton on the nitrogen atom and the α or β proton, collapses to a singlet or to a simpler system when the >N—H is converted to >N—D (1). Where the complexity of signals for the aromatic protons interfered, the greater resolving power of the 100 Mc Varian spectrometer (HR-100) was found to be helpful in locating α or β proton resonance signals. The results of the analyses are shown in Table I.

RESULTS AND DISCUSSION

The signal for the β proton in either indole or 2-methylindole is quite well discernible in all the seven solvents employed. Its position, ranging from 3.52 τ to 3.71 τ ($\Delta\tau = 0.19$) for indole and 3.86 τ to 4.10 τ ($\Delta\tau = 0.24$) for 2-methylindole, lies well beyond and at higher field than the absorption peaks of the aromatic protons (2.0 τ to 3.0 τ). There is a slight downfield shift of the signal in polar solvents as compared with its position in non-polar solvents.

The signal for the α proton of indole is found between 2.70 and 3.46 τ, a range ($\Delta\tau$) of 0.76 τ units, whereas for 3-methylindole the signal is found between 2.94 and 3.65 τ, over a range ($\Delta\tau$) of 0.71 τ units.

It is clearly seen that the signal position of the α proton in the indole nucleus is influenced to a much greater extent by the nature of the solvent than is that of the β proton. In more polar or more basic solvents, the chemical shift of the α proton is sufficient to place it in the region assigned to the aromatic proton absorption. In some solvents (acetone, dimethylsulphoxide, dioxane, and triethylamine) the assignment of the signal for the α proton of indole was not certain and hence is tentative, as indicated by the values in parentheses in Table I. However, from the simplification of multiplets caused by the conversion of >N—H to >N—D, and with the aid of high-resolution spectra, it was quite definitely ascertained that the α proton signal must lie *below* certain τ values as indicated in the table. In only one solvent, acetone, was a similar ambiguity found for the signal position of the α proton in 3-*methyl*indole.

It is of interest to note that the methyl group in both 2- and 3-methylindole caused greater shielding of the β *and* α protons than that found for indole itself, as indicated by the shift to higher field of the signals for these protons in the alkylated indoles. This is apparent in both cases only when one *compares the spectra obtained in the same solvent.* Witkop *et al.* (1) have noted the greater shielding effect on the β proton in 2-methylindole caused by the introduction of the methyl substituent, but their data indicate that a similar increased shielding of the α proton is not found in 3-methylindole. In fact the α proton in *3-methylindole* was found to give a signal at 3.20 τ while the α proton of *indole* absorbed at 3.32 τ. However, their use of CCl_4 as solvent for indole but $CDCl_3$ for the methylindole accounts for this inconsistency. From our observations it is now clear that the more polar solvent, $CDCl_3$, will cause a significantly greater decrease in shielding for the α proton than for the β proton in indole and any increase in shielding due to the methyl substituent is not sufficient to compensate for the large downfield shift of the α proton signal due to the solvent effect.

Substitution of the α or β proton of indole by an electron-withdrawing group such as acetyl, carbonyl, carbethoxy, and benzoyl shifts the absorption of the remaining β or α

TABLE I

The effect of change of solvent on the position of the resonance signals for the α or β protons in β- or α-substituted indoles

	Position of the signals for the α and β protons in the solvents (τ)							
Compound	CCl_4	$CDCl_3$	Acetone	Dimethyl sulphoxide	Dioxane	CS_2	$(C_2H_5)_3N$	Overall $\Delta\tau$
Indole[1] (α proton)	3.46 a	3.26 a	<2.75 (2.72 b*)	<2.71 (2.6 to 2.7)	<2.89 (2.84 b*)	3.45 a	<3.12 (2.80 b*)	0.76
Indole[1] (β proton)	3.66 a	3.59 a	3.52 b	3.53 b	3.52 b	3.71 a	3.57 b	0.19
3-Methylindole[1] (α proton)	3.65 b*	3.39 b*	⩽3.0 (3.0)	2.94 b*	3.13 b*	3.63 b*	3.25 b*	0.71
2-Methylindole[1] (β proton)	4.06 b*	3.95 b	3.86 b*	3.88 b*	3.88 b*	4.10 b*	3.93 b*	0.24
Ethyl indole-3-carboxylate[2] (α proton)	Insol.	2.15 2.21 c	1.97 2.01 c	1.88 d	2.07 2.12 c	Insol.	2.22 d	0.25
Ethyl indole-2-carboxylate[3] (β proton)	Insol.	2.77 b*	2.78 b*	2.80 b*	2.80 b*	Insol.	(2.80)	0.03
3-Acetylindole[4] (α proton)	Insol.	Insol.	1.80 1.83 c*	1.66 d	Insol.	Insol.	Insol.	0.17
Indole 3-carboxylic acid[6] (α proton)	Insol.	Insol.	Insol.	1.82 1.86 c	Insol.	Insol.	Insol.	
Indole 2-carboxylic acid[5] (β proton)	Insol.	Insol.	2.77 2.80 c	2.80 2.82 c	2.72 2.76 c*	Insol.	Insol.	0.08
3-Benzoylindole[7] (α proton)	Insol.	Insol.	Insol.	(2.0)	Insol.	Insol.	Insol.	
2-Benzoylindole[3] (β proton)	Insol.	(2.54 b*)	(2.3)	?	?	?	?	
3-Bromoindole[8] (α proton)	Insol.	2.96 3.00 c	2.56 2.59 c	(2.42 c*)	2.76 2.78 c	3.10 3.15 c	2.93 2.96 c	0.54
β, β′-Diindolyldisulphide[9] (α proton)	Insol.	Insol.	2.80 2.83 c	2.62 2.66 c	2.86 2.90 c	Insol.	—	0.18

NOTE: A question mark indicates that no assignment could be made. The symbol < indicates that the signals for the α or β protons were certainly less than the values given. Figures in parentheses are tentative assignments. a — triplet; b — multiplet; c — doublet; d — singlet; the asterisk indicates poorly resolved signals. The term "insol." indicates either insoluble or insufficiently soluble to obtain an n.m.r. spectrum.

[1] Eastman White label.

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[3] See Experimental.

[4] Chem. Abstr. **5**, 2638 (1911).

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[6] Obtained from the hydrolysis of ethyl indole-3-carboxylate; cf. R. Majima and M. Kotake. Ber. **63**, 2237 (1930).

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proton as much as 0.7 to 1.2 τ units downfield from the corresponding position of the signals of indole itself. Here again one must compare spectra of compounds in the same solvent. A greater shift occurs for the α than for the β proton: The $\Delta\tau$ ranges from 0.95 to 1.2 for the α proton as compared with 0.7 to 1.1 for the β proton. Thus, for ethyl indole-3-carboxylate, the α proton signal in $CDCl_3$ is a doublet at 2.15 and 2.21 τ, while that of the α proton in 3-methylindole and indole in the same solvent occurs at 3.39 τ and 3.26 τ respectively. On the other hand, for ethyl indole-2-carboxylate, the β proton signal is found at 2.80 τ in dioxane solution while in the same solvent, the β proton signal for 2-methylindole and indole is at 3.88 τ and 3.52 τ respectively.

For compounds in which the β proton was replaced by either sulphur or halogen, the deshielding effect on the α proton due to these substituents was less than that found for the carbonyl compounds.

In dimethylsulphoxide, for the two compounds ethyl indole-3-carboxylate and 3-acetylindole it was noted that the normally expected doublet for the α proton, due to spin–spin coupling with the proton on the adjacent nitrogen atom, did *not* occur. Instead, a sharply defined singlet was observed at 1.88 τ and 1.66 τ respectively. Deuteration produced an unchanged singlet at the same position. The carboxylate exhibited the same phenomenon in triethylamine. The insolubility of the 3-acetylindole in triethylamine thwarted attempts to detect a similar observation. In other solvents (e.g. acetone and dioxane) doublets due to spin–spin coupling were clearly shown. When the spectral grade acetone solvent was treated with a small amount of water or dilute hydrochloric acid, 3-acetylindole still showed a doublet, unchanged, for the α proton. In reagent pyridine the spectrum of 3-acetylindole gave a closely spaced doublet at 1.75 τ which collapsed to a singlet when the compound was deuterated.

We have assigned the α proton absorption of 3-acetylindole to the doublet appearing at 1.80 and 1.83 τ on the basis of the collapse of this doublet to a singlet in the N-deuterated compound. Additional evidence to support the assignment was obtained from the fact that this signal disappeared completely when the α hydrogen was substituted by a methyl group.

From the data in Table I it is clear that the greater solvent dependence of the chemical shift of the α proton as compared with that of the β proton is a useful means of determining or corroborating α or β substitution in the indole nucleus. The limited solubility or the insolubility of the compounds in some of the solvents normally employed for n.m.r. spectra determinations restricts the utility somewhat. However, considering a *pair of solvents* such as acetone and dimethylsulphoxide, in which most of the compounds are soluble, the position of the β proton signal is nearly the same in either solvent, with a maximum difference of 0.03 τ. But for the α proton, a difference in signal position of 0.08 to 0.17 τ occurs. A larger difference in the chemical shift for the α proton is found when one compares signal position in a non-polar or slightly polar solvent ($CDCl_3$) and highly polar solvent (acetone or dimethylsulphoxide).

It is of interest to note that the signal position for the proton attached to the nitrogen atom of the indole ring is influenced even more strongly by the solvent employed than is the α proton. Table II summarizes the information obtained.

Where the signals are clearly discernible they are found generally to be broad, but less so in dimethylsulphoxide.

In non-polar, or relatively non-polar solvents (CCl_4, $CDCl_3$, CS_2), the N—H signals of indole and alkylated indoles are buried in the complex absorption due to the aromatic protons, roughly in the region 2.9 to 3.1 τ. Approximate locations of the signal are given

TABLE II

The effect of solvent on the position of the resonance signal for the proton on the nitrogen atom of indole and substituted indoles

	Position of the signal for the N—H proton in the solvents (τ)						
	CCl_4	$CDCl_3$	Acetone	Dimethyl sulphoxide	Dioxane	CS_2	$(C_2H_5)_3N$
Indole	(3.0)	(2.9)	0.00	−1.01	+0.48	(3.1)	−0.08
2-Methylindole	(3.1)	(3.0)	+0.33	−0.82	+0.84	(3.1)	+0.08
3-Methylindole	(3.1)	(2.9)	+0.48	−0.69	+0.91	(3.1)	−0.59
Ethyl indole-2-carboxylate	Insol.	+0.44	−0.79	−1.87	−0.51	Insol.	?
3-Bromoindole	Insol.	(2.8)	−0.61	−1.46	+0.22	(2.9)	−1.01
β,β'-Diindolyldisulphide	Insol.	Insol.	−0.46	−1.54	+0.13	Insol.	Insol.
3-Benzoylindole	Insol.	Insol.	Insol.	−2.00	Insol.	Insol.	Insol.
2-Benzoylindole	Insol.	+0.08	−1.06	?	?	?	?
3-Acetylindole	Insol.	Insol.	Insol.	−1.92	Insol.	Insol.	Insol.
3-Acetyl-2-methylindole	—	—	—	−1.8	—	—	—

NOTE: Figures in parentheses are approximate locations of the signals. The term "insol." means that the compound was either insoluble or insufficiently soluble for satisfactory n.m.r. analysis. A question mark indicates that the signal for the N—H proton could not be detected.

by the figure in parentheses, and are obtained from the decrease in integrated areas for the N-deuterated compounds. The disappearance of the signal upon N-methylation, in the case of indole, supports the assignment of this signal to the N—H proton. Electronegative substituents in the α or β positions cause a pronounced downfield shift also.

Polar solvents and those which associate with the indole compound (i.e. ethers) cause a larger chemical shift to lower field in the order dimethylsulphoxide > triethylamine > acetone > dioxane.

EXPERIMENTAL

Nuclear magnetic resonance spectra were obtained at a temperature of 40° using a Varian A-60 spectrometer. Wherever possible the concentration employed was that obtained by dissolving 100 mg of the substance in 0.35 to 0.40 ml of spectral grade solvent. In a few cases (diindolyl disulphide in dioxane, indole-2-carboxylic acid in dioxane, 3-acetylindole in actone, and ethyl indole-2-carboxylate in $CDCl_3$ and in $(C_2H_5)_3N$) saturated solutions were used unavoidably. High-resolution spectra, using a Varian HR-100 spectrometer, were obtained at 29.2° with solutions containing 20 mg of solute in 0.40 ml of the respective solvent.

Deuteration

General Procedure

The following general procedure was employed in all deuterations. About 120 mg of the indole compound was dissolved in 8 ml of reagent acetone and then 2 ml of D_2O (99.7%) was added. The resulting solution was freed of solvent in a rotary evaporator under vacuum. This treatment was repeated two or three times. Final drying was carried out in a desiccator over P_2O_5. Care was taken to protect the solutions from direct light.

The extent of deuteration was determined by infrared spectrometry and by n.m.r. Disappearance of the $>$N—H peak between 3100 and 3500 cm^{-1} in the infrared and the appearance of a new peak due to $>$N—D at a frequency of about 0.75 of that of the $>$N—H stretching gave a measure of the amount of deuteration. N-Deuteration was more rapid for the indoles containing electronegative substituents in the α or β positions. For indole and 2-substituted indoles, considerable deuteration occurred at the β position, as detected by the lower integrated area for β proton signal in the N-deuterated compounds.

*De*deuteration, both at the nitrogen atom and at the β carbon, occurred readily when the compounds were dissolved in 95% ethanol and the solvent evaporated.

N-Deutero-3-bromoindole.—Since 3-bromoindole (5) decomposes spontaneously when its solution is completely freed of solvent, the following procedure was employed. A quantity of 3-bromoindole (4) (5.0 g, 0.025 mole) was treated with 2 ml of D_2O (99.7%). The solution was concentrated at room temperature under vacuum by rotary evaporator almost to dryness, then acetone and D_2O added again and the process repeated. The concentrated solution now was poured into 100 ml of *n*-heptane to which 5 g of anhydrous magnesium sulphate was then added. After addition of decolorizing charcoal, the mixture was stirred vigorously and heated to the boiling point. As long as the 3-bromoindole was *in solution* it could be heated above its normal decomposition temperature (67°) with no ill effect. The boiling solution was quickly filtered into a *cooled* receiver. Cooling is essential since 3-bromoindole deposited at a temperature close to or above its decomposition point will spontaneously decompose. The N-deutero-3-bromoindole crystallized as plates in a yield of 80%, melting at 65–66° (decomp.).

N,β-Dideuteroindole.—A solution of indole (11.7 g, 0.1 mole) in ether (50 ml) was added slowly to an ether solution of 0.2 mole of ethyl magnesium iodide (from 4.8 g, 0.2 mole, of magnesium and 31.2 g, 0.2 mole, of ethyl iodide in 250 ml of ether). When reaction was complete the solution, protected from moisture, was cooled to 0° and then stirred rapidly while 10 ml of D_2O (99.7%) in 50 ml of anhydrous tetrahydrofuran was added dropwise. When addition was complete, the solution was refluxed overnight. The cooled mixture was then filtered rapidly to minimize admission of water since this causes rapid *de*deuteration. The solvent was then removed completely by rotary evaporator under vacuum. Sufficient hot *n*-heptane was then added to dissolve the indole which had precipitated. The resulting clear solution was dried ($MgSO_4$) and filtered. When the solution was cooled and allowed to stand overnight the dideuteroindole separated. The solid was quickly isolated by filtration, and dried under nitrogen in a desiccator. Yield, 6 g (51%). The n.m.r. analysis indicated that the β position was deuterated approximately to the extent of 50%. Infrared analysis showed that the nitrogen atom was deuterated approximately to the extent of 50%.

2-Benzoylindole

Thionyl chloride (22 ml) was slowly added to 12.3 g (0.075 mole) of solid, powdered indole-2-carboxylic acid (6). When the initial vigorous reaction had subsided, the mixture was heated for 20 minutes on a steam bath. Excess thionyl chloride was removed under vacuum. The solid product was taken up in 50 ml of dioxane and the solution then slowly added to 50 g (0.19 mole) of diphenyl cadmium (7) dissolved in 300 ml of benzene. The reaction mixture was then heated under reflux for 12 hours. The cooled solution was poured slowly, with stirring, onto 2 kg of ice, while sufficient acetic acid was added to maintain approximately a pH of 7 (hydrion paper). The benzene layer was separated and treated with ether to precipitate the 2-benzoylindole. The crude precipitate was extracted with pentane for 24 hours in a Soxhlet apparatus. The pentane extract was freed from solvent and the residue crystallized from a mixture of ethanol and water. There was obtained 5.4 g (32%) of colorless needles melting at 146–148°. The infrared spectrum in $CHCl_3$ showed N—H stretching at 3452 cm^{-1}, bonded N—H at 3325 cm^{-1}, and carbonyl stretching at 1625 cm^{-1}.

Anal. Calculated for $C_{15}H_{11}NO$: C, 81.45; H, 4.88; N, 6.33; O, 7.24. Found: C, 81.19; H, 5.37; N, 6.36; O, 7.21.

3-Acetyl-2-methylindole

The procedure for the preparation of this compound as described in the literature (8) failed to give satisfactory results. Accordingly the following method was devised. A solution of 4 g (0.033 mole) of 2-methylindole (9) in ether was added slowly to ethyl magnesium iodide (from 1.6 g, 0.067 mole, of magnesium metal and 9.6 g, 0.065 mole, of ethyl iodide) in 100 ml of ether. When addition was complete, the resulting solution was cooled to 0° and then 2.6 g (0.033 mole) of acetyl chloride in 10 ml of ether was added dropwise so that the temperature of the mixture remained below 5°. Then the mixture, continually stirred, was allowed to come to room temperature overnight. The contents of the reaction vessel was poured onto ice (200 g) along with sufficient acetic acid to react with the magnesium hydroxide produced. The precipitated 3-acetyl-2-methylindole was removed by filtration and crystallized from aqueous ethanol to yield 3.2 g (56.5%) of pure product melting sharply at 204° (lit., 195–196° (8)). An n.m.r. spectrum in dimethylformamide indicated acetyl methyl at 7.44 τ, α-methyl buried in the signal due to solvent at about 7.08 τ, and a broad signal at -2.24 τ due to N—H. In pyridine the respective τ values for acetyl methyl and α-methyl were clearly separated and discernible at 7.34, 7.22. Infrared spectra in halo oil showed N—H stretching at 3082 cm^{-1}, and carbonyl absorption at 1605 cm^{-1}.

Ethyl Indole-2-carboxylate

A solution of indole-2-carboxylic acid (5.0 g, 0.31 mole) (6) in 50 ml of anhydrous ethyl alcohol, saturated with anhydrous hydrogen chloride, was allowed to stand overnight. The mixture was poured into water and made slightly basic with dilute caustic soda. The cold, basified solution was twice extracted with 100-ml portions of ether. Removal of the ether and crystallization of the residue from aqueous ethanol gave long needles melting at 125°. Lit., 125–126° (10). Yield, 74.5%. Infrared spectra in nujol showed N—H stretching at 3310 cm^{-1} and carbonyl absorption at 1685 cm^{-1}.

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