

Electron Spin Resonance Study of Hydrogen Adduct Radicals generated from Indole and its Derivatives in γ -Irradiated Methanolic Glasses at 77 K

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The structures of radiation-induced radicals generated from indole and a selected number of its derivatives in methanolic glasses at 77 K have been investigated by e.s.r. spectroscopy. From the spectral characteristics [tt. a_{H}^{OH} (2) 38.5, and a_{H}^{OH} (2) 12.0 G for indole], which are identical in almost all instances to the parent compound, it can be deduced that the radicals are formed primarily by hydrogen addition at the benzene nucleus. The α -carbon position, C-7, is favoured from the e.s.r. results obtained for selective blocking of reactive sites and by comparison with e.s.r. spectra obtained for benzene, indoline, and pyrrole in irradiated methanolic glasses and pyrrolidine irradiated in the pure state at 77 K. In the polycrystalline state only indole exhibits an e.s.r. spectrum which is indicative of the formation of a hydrogen adduct. Although there is some doubt about the homogeneity of these protic glasses containing $>1\text{M}$ -solute it is clear that a significant contribution to the mechanism of formation of hydrogen adduct radicals comes from a process involving solvent protonation of an intermediate indole anion.

PULSE radiolysis indicates that hydroxyl radical addition occurs at a number of sites on the indole nucleus from the complexity of the optical absorption spectra observed.^{1,2} No e.s.r. observations have been reported for adduct radicals formed by $\cdot\text{OH}$ addition to indoles. However, a number of e.s.r. and optical studies on hydrogen adduct radicals and radical cations generated from biologically important molecules in glassy and polycrystalline systems have been reported in the literature³⁻⁶ including tryptophan, a pyrrole-substituted indole derivative. E.s.r. studies on indole and methyl-substituted derivatives have been oriented towards electron adducts stabilised in aprotic matrices, *viz.* solid argon at 4 K.⁷

Our attention was thus turned to the possibility of observing hydrogen adduct radicals formed from indole compounds in γ -irradiated protic glasses at low temperatures since addition was thought likely to occur at

similar sites. Here we report an e.s.r. study of adduct radicals generated from indole and related compounds designed to probe the positions of hydrogen addition by selective blocking of probable reactive sites.

EXPERIMENTAL

Materials.—Indole and its derivatives were obtained from R. N. Emanuel and recrystallised from alcohol-water (1 : 10) before use. Indoline and pyrrole were distilled immediately prior to use. Benzene was AnalaR grade and used without further purification. Methanol was purified by the method described by Dainton *et al.*⁸

Sample Preparation and Irradiation.—Frozen clear glassy spheres were prepared by dropping small droplets of solution (*ca.* 1—2M) of indoles in methanol (2.5% w/v H_2O) from a fine capillary into liquid nitrogen. The spheres were γ -irradiated to a dose of *ca.* $9.6 \times 10^3 \text{ J kg}^{-1}$ in Pyrex tubes immersed in a Dewar containing liquid nitrogen and transferred (i) to a Dewar located in the e.s.r. cavity for observation of the e.s.r. spectra at 77 K or (ii) to quartz tubes when

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¹ R. C. Armstrong and A. I. Swallow, *Radiation Res.*, 1969, **40**, 563 and references therein.

² B. Iddon, G. O. Phillips, and K. E. Robbins, *J. Chem. Soc. (B)*, 1971, 1887.

³ R. Santus, C. Helene, and M. Ptak, *Photochem. and Photo-biol.*, 1968, **7**, 341.

⁴ F. G. Liming, jun., and W. Gordy, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **60**, 794.

⁵ M. P. Pailthorpe and C. H. Nicholls, *Photochem. and Photo-biol.*, 1971, **14**, 135; 1972, **15**, 465.

⁶ J. Moan and O. Kaalhus, *J. Chem. Phys.*, 1974, **61**, 3556.

⁷ P. H. Kasai and D. McLeod, jun., *J. Amer. Chem. Soc.*, 1973, **95**, 27.

⁸ F. S. Dainton, G. A. Salmon, and J. Tepley, *Proc. Roy. Soc.*, 1965, *A*, **286**, 1174.

using a variable temperature assembly from 77 to 150 K (also used for solid samples up to ambient temperatures). The solid samples of indoles were γ -irradiated at 77 K to a dose of *ca.* $2 \times 10^5 \text{ J kg}^{-1}$ in Spectrosil tubes in a Dewar containing liquid nitrogen after sealing off under vacuum ($>10^{-3}$ Torr). E.s.r. spectra were recorded on a Varian 4502/15 spectrometer previously described.⁹ Errors in measuring hyperfine splitting were estimated to be $\pm 0.5 \text{ G}$ in view of the rather broad e.s.r. lines observed.

RESULTS AND DISCUSSION

Hydrogen Adducts formed in γ -Irradiated Glassy Methanol.— γ -Irradiated methanolic glass (2.5% H_2O) at 77 K exhibits an e.s.r. spectrum consisting of an overlapping 1 : 2 : 1 triplet (a_{H} *ca.* 17 G) and a singlet (ΔH 15 G; g 2.001) which are characteristic of the hydroxymethyl radical, $\cdot\text{CH}_2\text{OH}$ and trapped electron (e_t^-), respectively.^{8,10} When indole is present in the glass the e.s.r. spectrum is more complicated due to the presence of another radical species. From the splitting of the outermost hyperfine lines (see Figure A) it is clear that the new resonance is characteristic of radicals formed by hydrogen addition to indole. A clearer definition of the spectrum of this radical is obtained after thermal annealing from 77 to 133 K to bleach out the less stable species, e_t^- and $\cdot\text{CH}_2\text{OH}$ (see Figure B). The spectrum consists of a triplet of triplets resulting from the hyperfine interaction of two sets of two equivalent protons, a_{H} 38.5 and 12 G.

The yield of the indole hydrogen adduct is smaller than for benzene in a methanolic glass¹¹ and for this reason concentrations $>1\text{M}$ -solute were used. The increase in hydrogen adduct resonance was paralleled by a decrease in the e_t^- absorption. Rate constants for electron reactions with indoles are in the range, 7×10^8 — $3 \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$, at room temperature determined by pulse radiolysis^{12a,b} and are higher than for benzene ($k < 7 \times 10^6 \text{ l mol}^{-1} \text{ s}^{-1}$).¹³ Thus it appears that the rate constant for electron trapping at 77 K, $k_{e_t^- + \text{T}}$, is greater for methanolic glasses containing indoles assuming $k_{e_t^- + \text{solute}}$ at 77 K to have the same order of magnitude difference as at room temperature. These observations indicate that, in view of the high solute concentration required to observe the radical, considerable solute aggregation may occur during the freezing process.⁹

Similar e.s.r. spectra were observed for 2- and 3-methylindoles after thermal annealing to 133 K (Figures C and D). The same procedure was adopted for observing the e.s.r. spectra of hydrogen adducts of the indoles listed in the Table. Almost identical e.s.r. spectra were observed in all instances. The proton coupling constants and overall spectral widths are summarised in the Table.

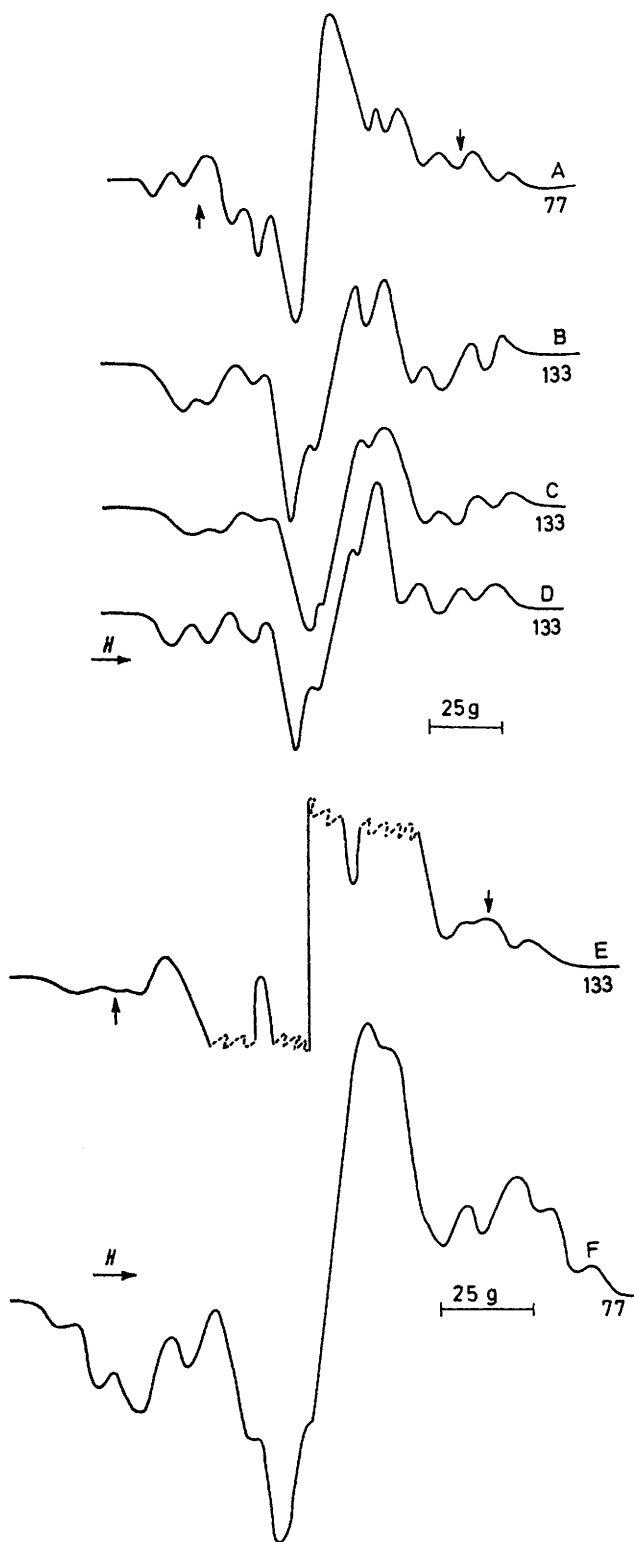
⁹ P. J. Baugh, K. Kershaw, and G. O. Phillips, *J. Chem. Soc. (B)*, 1970, 1482.

¹⁰ F. S. Dainton, G. A. Salmon, and P. Wardman, *Proc. Roy. Soc.*, 1969, *A*, **313**, 1.

¹¹ T. Shida and W. H. Hamill, *J. Amer. Chem. Soc.*, 1966, **88**, 5371.

¹² (a) J. H. Baxendale, *Nature*, 1964, **201**, 468; (b) K. E. Robbins, Ph.D. Thesis, University of Salford, 1973.

¹³ M. Anbar and P. Neta, *Internat. J. Appl. Radiation Isotopes*, 1967, **18**, 493.



E.s.r. spectra of hydrogen adduct radicals generated from indoles and related compounds (1–2M) in γ -irradiated methanolic glasses (2.5% H_2O) at 77 K and after annealing to 133 K: A, indole at 77 K, outer lines indicated by arrows; B, indole at 133 K; C, 2-methylindole at 133 K; D, 3-methylindole at 133 K; E, pyrrole at 77 K, outer lines indicated by arrows; F, indoline at 133 K

The hydrogen adducts derived from the parent compounds, benzene (0.25M) and pyrrole (1M) were also investigated. Whereas the hydrogen adduct from benzene^{11,14} was easily observed at 133 K^{12b} that derived from pyrrole could only be stabilised at 77 K where e_t^- and $\cdot\text{CH}_2\text{OH}$ resonance lines obscured the spectrum

E.s.r. data for radicals observed in γ -irradiated methanolic glasses containing indole and related compounds.

Derivative or related compound ^a	T/K	Proton coupling constants		Overall width (G)
		$a_{\text{H}^{\text{CH}_2/\text{G}}}$	$a_{\text{H}^{\text{CH}/\text{G}}}$	
Indole ^{a,b}	133	38.5 (C-7) ^c	12.0 (C-4, C-6) ^d	101
N-Methyl	133	38.0 (C-7) ^c	12.0 (C-4, C-6) ^d	100
1,3-Dimethyl	133	37.5 (C-7) ^c	12.0 (C-4, C-6) ^d	99
2-Methyl	133	39.5 (C-7) ^c	11.5 (C-4, C-6) ^d	102
3-Methyl	133	37.5 (C-7) ^c	12.0 (C-4, C-6) ^d	99
5-Methyl	133	37.0 (C-7) ^c	13.0 (C-4, C-6) ^d	100
5-Chloro	133	38.5 (C-7) ^c	12.0 (C-4, C-6) ^d	101
7-Methyl	133	37.5 (C-5?) ^c	12.0 (C-4, C-6) ^d	99
2,3-Dihydro	133	52.5 (C-4 ^c or C-7) ^c	12.5 (C-5, C-7) ^d (or C-4, C-6) ^d	142.5
Pyrrole	77	47—48 (C-2) ^c	12.0 (C-3, C-5) ^d	120
Pyrrolidine ^b	77			170

^a In methanolic glass. ^b In pure state. ^c Position of hydrogen addition. ^d Position of protons contributing.

(Figure E). Thus the hydrogen adduct derived from pyrrole is considerably less stable than that formed from indole which lends some support to hydrogen addition occurring at the benzene nucleus. In addition indoline was also investigated (see Figure F) since hydrogen addition can only occur at the benzene nucleus. The coupling constants to protons are a_{H} 52.2 and 12.5 G.

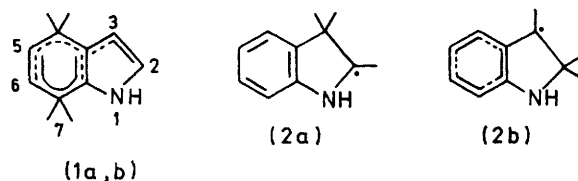
The nitro-derivatives caused a significant decrease in the intensity of e_t^- signal in the e.s.r. spectrum. The triplet spectra observed are consistent with the formation of adducts with the hydrogen located at the nitro-group (for $\text{-NO}_2\text{H}$; A_2 ca 32 G) produced by protonation of an intermediate anion.¹⁵

Hydrogen Adducts formed in γ -Irradiated Solid Indoles.—Only indole exhibited an e.s.r. spectrum consistent with formation of a hydrogen adduct. The coupling constants are identical with those observed for indole in a methanolic glass. Indole derivatives gave extremely poorly resolved e.s.r. spectra and no attempt was made to make a radical assignment.

Interpretation of E.s.r. Spectra.—For a full interpretation of the e.s.r. spectra of the adducts formed from indoles both the consequence of hydrogen addition at the pyrrole and benzene nuclei must be considered. The radicals which could give rise to the e.s.r. spectra observed can be summarised by considering structures (1) and (2).

Structures (1a and b) relate to radicals resulting from hydrogen addition at C-4 and -7 in the benzene nucleus. These radicals can be considered as substituted cyclohexadienyl radicals with some delocalisation of the un-

paired spin density into the pyrrole ring, possible for (1a). Structure (2a) relates to an alicyclic radical formed by hydrogen addition at C-3 while (2b) is a conjugated radical (hydrogen addition at C-2) in which the unpaired



spin density at C-3 is delocalised into the adjacent ring π -electron system.

Support for hydrogen addition to the benzene nucleus comes from the identical e.s.r. spectra observed for radicals generated from the N-methyl, 2-methyl, 3-methyl, 1,3-dimethyl, 5-methyl, and 5-chloro-derivatives. Kasai and McLeod⁷ found that this was also true for radical anions, stabilised after proton migration, derived from indoles in an argon matrix. They favoured hydrogen addition at C-7 rather than -4 since radical anions stabilised by hydrogen addition at C-4 and -6 appeared as a triplet of doublets. However, in our study the fact that 7-methylindole also exhibits an identical spectrum to indole cannot be completely accounted for unless hydrogen addition occurs at C-5 in this instance. The observation of a triplet of triplets for the 5-methyl and 5-chloro-derivatives favours hydrogen addition at C-7 since addition at C-4 would give rise to a triplet of doublets. Blocking C-5 does not rule out hydrogen addition at C-6. However, the fact that a triplet of doublets is not observed when C-7 is blocked tends to discount this alternative. Furthermore, hydrogen addition to α -carbon positions is favoured for adducts formed from naphthalene.¹⁶ The almost identical proton coupling constants for naphthalene (a_{H} 37 and 12.8 G) provide further support for addition occurring at the benzene nucleus. For the adduct derived from α -methylnaphthalene trapped in an irradiated β -cycloamylose matrix¹⁷ an identical e.s.r. spectrum was observed demonstrating that hydrogen addition occurs preferentially at the α -carbon position in the benzene nucleus which is unsubstituted. An analogy can be drawn here with the indoles methyl-substituted at C-1, -2, -3 and -1,3 from which identical spectra to that observed for indole were obtained.

Similar e.s.r. spectra have previously been reported for aromatic amino-acids, phenylalanine, tyrosine,^{4,18} and tryptophan.^{4,6,18} Tryptophan is an indole derivative substituted in the pyrrole nucleus and hydrogen addition at C-7 is also favoured here.^{4,6,18}

The e.s.r. spectrum observed for indoline is more complex than for indole or naphthalene. The complication may result from partially resolved coupling to the protons in the tetrahydropyrrole nucleus. These protons would not be in a planar configuration as in unsaturated structures and would thus be hyperconjugated to some

¹⁴ J. A. Leone and W. S. Koski, *J. Amer. Chem. Soc.*, 1966, **88**, 224.

¹⁵ C. Chachaty and A. Forchioni, *J. Chim. phys.*, 1968, **65**, 1649.

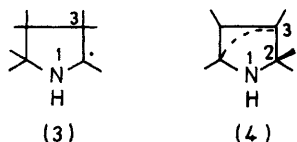
¹⁶ J. A. Leone and W. S. Koski, *J. Amer. Chem. Soc.*, 1966, **88**, 656.

¹⁷ G. O. Phillips and M. Young, *J. Chem. Soc. (A)*, 1966, 393.

¹⁸ V. T. Srinivasan and A. van de Vorst, *Internat. J. Radiation Biol.*, 1971, **2**, 133.

extent with the adjacent π -electron system. For both indole ⁷ and naphthalene ¹⁶ the proton coupling constants in rings adjacent to those in which hydrogen addition has occurred are small (1–2 G) and would not be resolved. However, the unresolved coupling would tend to broaden the hyperfine lines observed as would *meta*-proton couplings (C-5 in indole).

Support for radicals formed by hydrogen addition at the pyrrole nucleus is not nearly so strong. For $\cdot\text{OH}$ radical attack there appears to be evidence for addition at the pyrrole nucleus.^{1,2} The optical absorption spectra for hydroxy-adducts of indoles are more complex than when hydroxy-addition to the pyrrole nucleus is blocked, as in indoline ² which exhibits an absorption spectrum characteristic of benzene ¹⁹ or naphthalene.¹ Hydrogen atoms (or protons) might be expected to react with indoles to form adducts in a similar way.¹ The species may be more selective in position of attack and, particularly in a rigid glass, in view of the lower electrophilic character. A radical conforming to structure (2a) in which the additional hydrogen is attached to C-3 would exhibit a large methylene proton coupling of the order of 30–40 G consistent with β -proton couplings in alicyclic radicals ²⁰ (using $a_{\text{H}\beta}$ 38.5 G, B_0 3.5 G, and θ 30° in the equation $a_{\text{H}\beta} = B_0 + B_{\text{cos}} \cos^2 \theta$ gives B 46.7 G). The α proton coupling of 12 G is similar to that observed for tetrahydrofuran ²¹ which also shows a reduced β -proton coupling attributable to the presence of the heteroatom. The CH_2 proton coupling of 38.5 G cannot be completely accounted for since nitrogen would have a similar but reduced effect on the spin density at the α -carbon. Cronenwett and Symons ²² conclude that the NH proton broadens the spectral lines and does not contribute to the resolved hyperfine structure (a^{N} and $a_{\text{H}^{\text{NH}}}$ are small for straight chain amine radicals ²³). We attempted to generate the corresponding alicyclic radical (3) from pyrrolidine at 77 K by γ -irradiation. However, the spectral resolution was insufficient to allow assignment. The overall spectral width, 170 G, is much greater than would be expected for this radical or observed for indole adducts and is more in accordance with the radical formed from pyrrolidine by loss of a hydrogen atom from C-3.



A large CH_2 proton coupling would also be expected for a radical represented by structure (2b). This radical is somewhat similar to that (4) formed by hydrogen

addition to pyrrole at C-2. Only the smaller proton couplings, 12 G, can be directly determined for the hydrogen adduct generated from pyrrole at 77 K (Figure E). The overall spectral width is 120 G, somewhat greater than observed for indoles. Thus it can be concluded that the CH_2 proton coupling for the pyrrole adduct is larger also, 47–48 G, which is in reasonable agreement with the value reported by Kasai and McLeod ⁷ for the analogous 'stabilised' radical anion. Here also, it is difficult to propose two equivalent protons with a coupling *ca.* 12 G for the radical (2b), unless unpaired spin density is delocalised into the adjacent π -electron system thus increasing the proton coupling at C-4.

In view of the poor general spectral resolution contributions from radicals (2a and b) cannot be totally ruled out. However, e.s.r. evidence favours radicals formed by hydrogen addition at C-7 in the benzene nucleus which is in accordance with spin density calculations.^{6,7}

Mechanism of Hydrogen Addition.—Hydrogen addition to aromatic compounds including indoles is considered to occur in a number of ways as follows: hydrogen atom addition ⁴ directly, solvent hydrogen atom abstraction by an electronically excited solute molecule,⁶ hydrogen atom or proton transfer from the solvent ^{6,11,14} or indole ⁷ after formation of an intermediate anion.

Although the rate constant $k_{\text{H}\cdot + \text{CH}_3\text{OH}}$, $1.6 \times 10^6 \text{ l mol}^{-1} \text{ s}^{-1}$, is probably lower than $k_{\text{H}\cdot + \text{indole}}$ ($k_{\text{H}\cdot + \text{tryptophan}}$ has been determined, as $2 \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$)¹³ hydrogen atoms generated from the solvent will be predominantly scavenged by the excess of solvent. Thus hydrogen atom addition directly to the solute may be minimal. Addition of an electron scavenger to the glass does reduce the intensity of the hydrogen adduct resonance; complete removal of the hydrogen adduct was not observed which leads us to conclude that direct action (excitation mechanism) cannot be ruled out. Protonation of an intermediate anion can occur *via* transfer of a proton from the solvent intermolecularly ¹¹ or indole intramolecularly.⁷ We could not distinguish between these processes although irradiation of indole in CH_3OD glass does lead to a narrowing of the e.s.r. spectrum consistent with the formation of a deuterium adduct.⁶ Intramolecular proton transfer may, however, predominate in aprotic media.⁷ Thus it appears that a mechanism involving solvent protonation of an intermediate anion makes a significant contribution to hydrogen adduct formation from indoles in agreement with other work.¹¹ However, in view of the high indole concentration and probable solute aggregation the other mechanisms considered cannot be completely discarded.

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²¹ W. T. Dixon, R. O. C. Norman, and A. L. Buley, *J. Chem. Soc.*, 1964, 3625.

²² W. Cronenwett and M. C. R. Symons, *J. Chem. Soc. (A)*, 1968, 2991.

²³ P. Neta and R. W. Fessenden, *J. Phys. Chem.*, 1971, **75**, 738.