TRITIUM NUCLEAR MAGNETIC RESONANCE. PART V.[1] DISTRIBUTION OF TRITIUM IN LABELLED POLYCYCLIC HYDROCARBONS.

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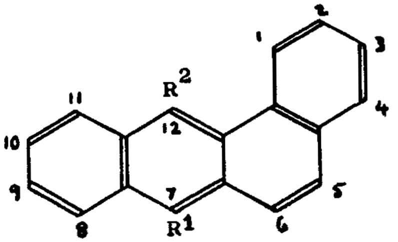
SUMMARY

The positions and extent of tritium labelling in carcinogenic polycyclic hydrocarbons are rapidly and conveniently revealed by ^{3}H n.m.r. spectroscopy.

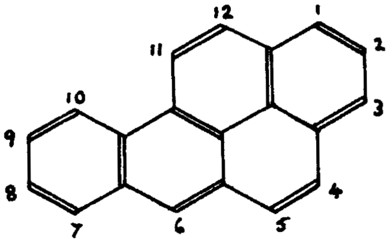
INTRODUCTION

The mode of action of carcinogenic polycyclic aromatic hydrocarbons is a problem of continuing interest and importance (2), especially as these compounds are widely distributed (3). General labelling of the hydrocarbons with tritium provides a means of tracing metabolites (4) but there is an increasing need for more specific studies requiring, e.g. knowledge of the degree of labelling in each of the many sites of the starting materials (5,6,7). Little is yet known about the tritium distribution in tritiated polycyclic aromatic hydrocarbons (8), largely because of the scarcity of specific methods of degradation or substitution (5,7,9,10). However, ^{3}H n.m.r.

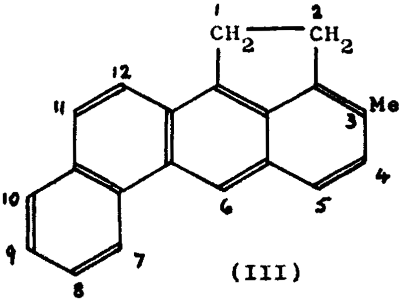
spectroscopy (1,11,12) can readily provide this hitherto inaccessible information. We illustrate the potentialities of the method by reference to tritiated benz[a]anthracenes (Ia,b,c), benzo[a]pyrene(II), and methylcholanthrene(III). General tritiation in the rings and methyl groups was effected (13) by exchange (in 70 per cent acetic acid containing tritiated water) catalysed by platinum (from reaction of Adams' catalyst in hydrogen).



(I) a, $R^1 = R^2 = H$
b, $R^1 = Me$, $R^2 = H$
c, $R^1 = R^2 = Me$



(II)



(III)

EXPERIMENTAL

Reaction conditions for the general tritiations are summarised in Table I.

Solutions of the tritiated hydrocarbons (20-50 mCi samples of high specific activity) in benzene (The Radiochemical Centre Ltd.) were frozen and lyophilised. Each sample was redissolved in perdeuteriated dimethyl sulphoxide

TABLE I

Exchange labelling of polycyclic hydrocarbons (13)

	Compound		Wt. (g)	Catalyst PtO_2 (g)	Exchange Medium 70% acetic acid (ml)	(Ci)	Temp. (°C)	Time (hr)	Specific activity (Ci/m mol)
(Ia)	Benz[a]anthracene	(i)	0.5	0.2	0.8	330	150	17	3
		(ii)	0.5	0.2	1.3	450	150	17	6
(Ib)	7-Methylbenz[a]anthracene		0.125	0.2	0.8	400	145	18	15
(Ic)	7,12-Dimethylbenz[a]anthracene		0.5	0.2	2.0	500	155	16	13
(II)	Benzo[a]pyrene	(i)	0.5	0.2	1.0	500	155	18	60
		(ii)	0.5	0.2	0.8	440	155	18	5
(III)	3-Methylcholanthrene		0.5	0.2	2.0	400	140	18	16

(to provide the field-frequency lock). A trace of tetramethylsilane (to provide the ^1H reference) was added, and the solutions were transferred to cylindrical microcells (100 μl) (Wilmad) which were sealed and inserted into standard (5 mm) n.m.r. tubes and these in turn were capped. Triton and proton spectra were recorded at 25 ± 1°C with a Bruker WH90 pulse spectrometer operating at 96 and 90 MHz, respectively. Pulse widths were 1.5 - 3 μs and the repetition interval 1.6 - 3.4 s as appropriate. Some 10^4 - $4x10^4$ transients were acquired into 8K channels and Fourier transformed to give the required display of ca. 13 p.p.m., usually at 21.31 Hz cm^{-1} for ^3H spectra and 20.00 Hz cm^{-1} for ^1H spectra, to provide identical p.p.m. scales. Triton shifts, δ_T (p.p.m.), were measured from a ghost reference $(\omega_T/\omega_H)\nu_{TMS}$ derived from the ^1H signal (ν_{TMS}) of the internal tetramethylsilane (11).

Integrated signal intensities from a ^3H n.m.r. spectrum obtained with ^1H decoupling were compared with those derived from the corresponding gated-decoupled spectrum. No differences were detected, demonstrating the absence of nuclear Overhauser effects. The maximum theoretically possible effect would be 47% (14).

In other experiments it was found that experimental longitudinal relaxation times for ^3H and ^1H nuclei at the same sites were comparable.

RESULTS AND DISCUSSION

Tritium n.m.r. spectra obtained with ^1H decoupling proved ideal for examining the distribution of tritium in tritiated polycyclic aromatic hydrocarbons, only one line per labelled site resulting. ^3H Chemical shifts in these multi-spin systems were therefore available by inspection and, being the same as ^1H shifts (11), comparison with published ^1H data at once provided the required information on regiospecificity of labelling. The integrated signal intensities gave the detailed quantitative analysis which it had not previously been possible to obtain: the reliability of the new method followed from the demonstrated absence of nuclear Overhauser effects under the operating conditions. Furthermore, where analyses by the ^{3}H n.m.r. method have been compared with the results of specific chemical degradation and counting, there is usually close agreement (1,10,15). However, the results obtained with [G-^{3}H]benzo [a] pyrene indicated about 10 per cent tritium at the 6-position compared with 34 per cent in another sample, similarly prepared, but determined using chemical substitution reactions (5). The discrepancy between the two results may be due to batch-to-batch variation in tritium distribution but this is <u>not</u> supported by the ^{3}H n.m.r. spectral data on two different batches of [G-^{3}H] benzo [a] pyrene (see Table II).

[G-^{3}H] <u>Benz</u> [a] <u>anthracene</u>. - This compound was labelled in all except the 1- and 12- positions, which are those most hindered. The results for two preparations are shown in Table II. The assignments were made by reference to the fully analysed ^{1}H spectrum (16) making allowance for the solvent change. The coincidence or near coincidence of ^{3}H chemical shifts for the 9- and 10- positions and for the 2- and 3- positions prevented individual intensity measurements at these sites, but the totals implied a similar degree of labelling to that at the other freely-accessible 4-, 5-, 6-, 8-, and 11- positions. The 7- position, however, was only lightly labelled, perhaps because of mild hindrance from the two adjacent <u>peri</u>-positions.

[G-^{3}H] 7-<u>Methylbenz</u> [a] <u>anthracene</u>. - With this compound, the method (Table I) resulted in all of the ring positions and the methyl group being labelled (see Table II). The methyl group thus activates the ring positions as expected. However, the intensity measurements showed that the hindered 1-, 12-, and 8-positions were only weakly labelled. So probably was the hindered 6- position, as suggested by the low intensity of the superimposed 6- and 11-^3H signals. The assignments were made in part from the previous ^3H spectrum but mainly from the ^1H n.m.r. data reported for 7-methylbenz[a]anthracene in carbon disulphide at 220 MHz (17). Whereas there was chemical shift coincidence between the 2- and 3- protons, between the 9- and 10- protons, and between the 6- and 11- protons under those conditions, at 96 MHz in dimethyl sulphoxide there was shift coincidence only between the last pair of hydrogen nuclei.

$[G-^3H]$7,12-*Dimethylbenz*[a]*anthracene*. - Although labelled by the same method (Table I), this product was devoid of tritium in the 1-, 6- and 11-positions (see Table II), indicating severe steric hindrance at these sites. Moreover there was

TABLE II

^3H N.m.r. (and ^1H) results for polycyclic hydrocarbons

Compound	Chemical shift δ p.p.m.	Assignment	Intensity %		
$[G-^3H]$ (Ia)	7.63	9,10	(i)	23.5	(ii) 30.1
	7.70	5		13.1	15.5
	7.76	3		21.6	12.5
	7.78	2			11.7
	7.92	6		11.3	7.1
	7.97	4		9.4	7.5
	8.16	8		10.3	10.4
	8.27	11		8.9	4.6
	8.55	7		1.9	0.6
	(8.95)	1		0	0
	(9.37)	12		0	0
$[G-^3H]$(Ib)	3.08	7-Me		18.8	
	7.66	9	*ca.* 10	26.3	
	7.68	10	*ca.* 6		
	7.71	2	*ca.* 10		
	7.76	3		11.8	

TABLE II (Cont'd)

Compound	Chemical shift δ p.p.m.	Assignment	Intensity %			
[G-^{3}H] (Ib)	7.83	5	13.4			
	8.00	4	16.4			
	8.28	6,11	7.5			
	8.41	8	3.2			
	9.04	1	0.5			
	9.41	12	1.9			
[G-^{3}H] (Ic)	3.03	7-Me	6.0			
	3.26	12-Me	1.5			
	7.66	9	ca. 11	27.1		
	7.67	10	ca. 16			
	7.72	5	43.0			
	7.73	3				
	7.74	2				
	7.97	4	17.6			
	(8.11)	6	0			
	(8.40)	11	0			
	8.44	8	4.9			
	(8.51)	1	0			
[G-^{3}H] (II)	7.90	8	(i) 9.0	(ii) 10.2		
	7.94	9	11.7	12.2		
	8.11	4	12.3	18.4		
	8.14	2	6.7			
	8.17	5	8.1	10.2		
	8.27	3	10.2	9.0		
	8.41	1	9.9	10.6		
	8.44	7	7.9	4.5		
	8.51	12	10.8	9.0		
	8.76	6	10.4	13.5		
	9.26	10	3.0	2.4		
	9.28	11				
[6-^{3}H] (II)	8.78	6	90.7,	81.0,	74.4,	94.4
	8.43	1	8.2,	14.8,	16.7,	4.0
	8.28	3	1.1,	4.2,	8.9,	1.6
[G-^{3}H] (III)	2.36	3-$C^1H^3H_2$	19.6			
	2.38	3-$C^1H_2{}^3H$				
	3.39	2-CH_2	12.9			

TABLE II (Cont'd

Compound	Chemical shift δ p.p.m.	Assignment	Intensity %
$[G-^3H]$(III)	3.68	1-CH_2	12.1
	7.34	4	7.5
	7.56	11	6.2
	7.60	9	8.3
	7.63	8	8.3
	7.76	5,12	10.4
	7.82	10	7.5
	8.79	7	0.8
	8.93	6	6.2

little label in the 8-position which also is hindered. Of the two methyl groups, the hindered 12-methyl was only lightly labelled. The assignments were made partly from our own ^1H n.m.r. spectrum of the tritiated hydrocarbon but mainly with the aid of the 220 MHz ^1H spectrum of 7,12-dimethylbenzanthracene in carbon disulphide, for which chemical shifts were given as the mid points of multiplets (17). Our ^3H n.m.r. spectrum (with ^1H decoupling) therefore provided some accurate chemical shifts for the first time. The shifts for the unlabelled 1-, 6-, and 11- positions derived from our ^1H spectrum were δ 8.51, 8.11, and 8.40 severally.

In a ^3H n.m.r. spectrum obtained without ^1H decoupling, there was a triplet signal (_J_ 16 Hz) from each methyl group, demonstrating single labelling.

$[G-^3H]$ Benzo[a]pyrene. - The assignments were made using the fully analysed ^1H n.m.r. data given by Haigh & Mallion (18) The benzopyrene sample (i), labelled by treatment at 155°C for 18 hr (Table I) gave a ^3H spectrum (Figure 1) which showed fairly uniform labelling except at the hindered 10- and 11-positions, which were only weakly labelled. Sample (ii), which had been labelled under identical conditions gave a

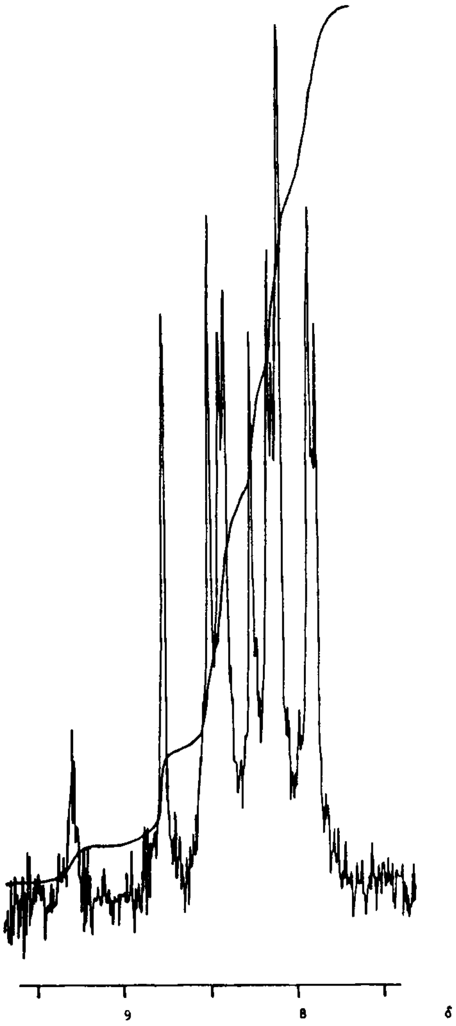


Figure 1

spectrum which showed a similar distribution of tritium. The 10 - 13 per cent tritium found at the 6-position is in agreement with the value found by Duncan and Engel (10), but the discrepancy with the 35 per cent tritium at the 6-position found by Blackburn et al (5) is not readily explained.

[6-^{3}H]Benzo[a]pyrene. - Tritium n.m.r. examination of four samples, all prepared by catalytic tritiodebromination (19) of the 6-bromo-compound, revealed some variation in labelling (Table II) depending on the sample of bromo-compound used. Two samples had over 90 per cent of the tritium in the

6-position with several per cent at the 1-position and a trace at the 3-position (Figure 2a). The others, prepared from a different batch of 6-bromo-compound, had distinctly more tritium in the 1-position (about 15 per cent) and in the 3-position with correspondingly less in the desired 6-position (Figure 2b). It is therefore important that the starting material should be pure and also specifically brominated in the 6-position.

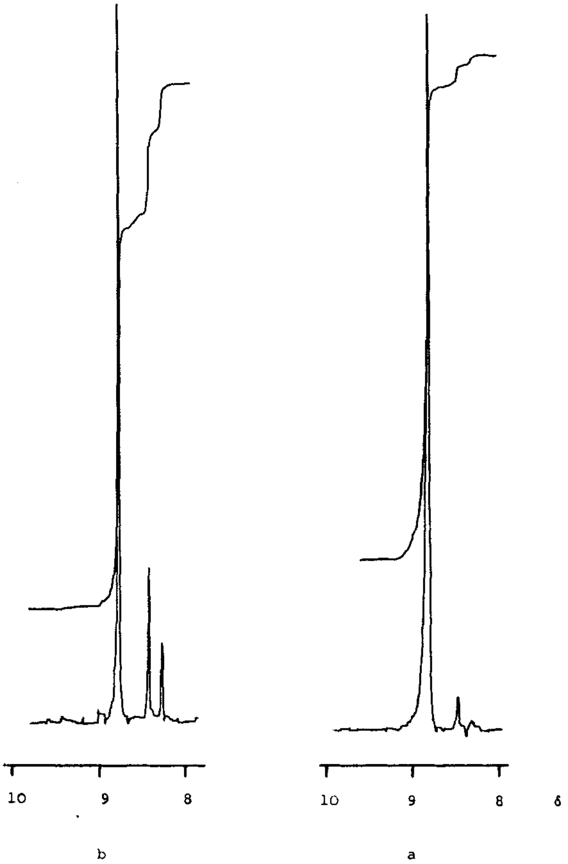


Figure 2

These results, obtained so readily and unambiguously as compared to chemical methods (5,10) underline the considerable power of ^{3}H n.m.r. spectroscopy as a tool for the analysis of tritiated compounds. The findings also stress the fact that tritium substitution reactions alone should never be relied upon to proceed with full regiospecificity or without unexpected protium-tritium exchange reactions.

[G-^{3}H]3-Methylcholanthrene. - The ^{3}H n.m.r. spectrum (with ^{1}H decoupling) was assigned with the aid of the 220 MHz ^{1}H spectrum of 3-methylcholanthrene in carbon disulphide (17). The results, given in Table II, provided accurate chemical shifts and indicated that the aliphatic groups were the most heavily labelled, particularly the 3-methyl. This showed in fact two chemical shifts, which suggested the presence of both CH_2T and CHT_2 functions. That these were indeed both present was demonstrated in the undecoupled ^{3}H spectrum by a triplet signal at δ2.38 ($\underline{J}$ 15.1 Hz) from the former and a doublet signal at δ2.36 ($\underline{J}$ 15.3 Hz) from the latter group, the high field shift resulting from the secondary isotope effect (11,20). Also noteworthy was the fact that the chemical shifts of the 1- and 2-methylene hydrogens were given directly by the ^{1}H decoupled ^{3}H n.m.r. spectrum, whereas of course these are not easily derived from the compact AA'BB' multiplet system in the ^{1}H n.m.r. spectrum. The aromatic ring positions were reasonably uniformly labelled except for the 7-position which was very weakly labelled. In contrast, the equally hindered 6-position had almost its full share of label, in agreement with a much higher reactivity there. The hydrogen nuclei at the 5- and 12-positions had accidentally coincident chemical shifts, so preventing individual determinations of tritium content, but the total signal intensity suggested both positions were labelled. Dimethyl sulphoxide was a superior solvent to carbon disulphide because use of the latter had resulted in coincidence of the chemical shifts of the 8- and 9-protons as well (17).

A summary of the labelling results is given in Table III.

TABLE III

Distribution of tritium in [G-^{3}H]polycyclic aromatic hydrocarbons

Compound														
	Position:		1	2	3	4	5	6	7	8	9	10	11	12
Benz[a]anthracene	% ^{3}H	: (i)	0	22		9	13	11	2	10	23.5		9	0
(Ia)		(ii)	0	12	12.5	7.5	15.5	7	1	10	30		5	0
7-Methylbenz[a]anthracene	Position:		1	2	3	4	5	6	11	7-Me	8	9	10	12
(Ib)	% ^{3}H	:	0.5	10	12	16	13	7.5		19	3	10	6	2
7,12-Dimethylbenz[a]anthracene	Position:		1	2	3	5	4	6	7-Me	8	9	10	11	12-Me
	% ^{3}H	:	0	43			18	0	6	5	11	16	0	1.5
Benzo[a]pyrene	Position:		1	2	4	3	5	6	7	8	9	10	11	12
(II)	% ^{3}H	: (i)	10	7	12	10	8	10	8	9	12	3		11
		(ii)	11	18		9	10	13.5	4.5	10	12	2		9
3-Methylcholanthrene	Position:		1-CH$_2$	2-CH$_2$	3-Me	4	5	12	6	7	8	9	10	11
(III)	% ^{3}H		12	13	20	7.5	10		6	1	8	8	7.5	6

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