220 MHz Nuclear Magnetic Resonance Analysis and Selective Deuteriodeprotonation of Benzo[*a*]pyrene and 6-Methylbenzo[*a*]pyrene

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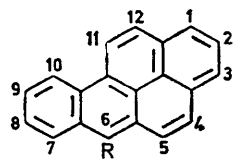
An analysis of the 220 MHz magnetic resonance spectra of the carcinogenic benzo[*a*]pyrene (1) and 6-methylbenzo[*a*]pyrene (2) is presented. A study of proton exchange in sulphuric [$^2H_2$]acid is used to determine the specific positions of electrophilic substitution. Electrophilic attack on compound (1) takes place predominantly at the 6-position, and then at the 1- and 3-positions, whereas in compound (2) the most active positions are C-1, C-3, and C-5, with C-5 the least active of the three.

COMPLETE n.m.r. analyses have been reported recently for several unsubstituted polycondensed hydrocarbons,[1] including benzo[*a*]pyrene.[2] Calculated shielding parameters were compared with experimental data obtained from the 100 and 220 MHz spectra.

The high resolution achievable with the 220 MHz



(1) R = H
(2) R = Me

spectrometer, aided by the double resonance technique and studies of specific deuteriodeprotonation with sulphuric [$^2H_2$]acid, allows us to report here some additional interpretation of the benzo[*a*]pyrene (1) ^{1}H n.m.r. spectrum, and an analysis of that of 6-methylbenzo[*a*]pyrene (2). The spectra also provide qualitative information on the positions of selective substitution indicated by such a deuterium ion exchange study. The kinetics of exchange are used for defining the relative reactivities of the various positions of the two hydrocarbons.

RESULTS

Assignments of Lines.—Benzo[a]*pyrene* (1). The 220 MHz spectrum of compound (1) is shown in Figure 1A. The peak assignments correspond to those made by Martin [2] and by Haigh and Mallion.[3] Further confirmation was achieved by the double resonance technique and selective deuteriodeprotonation studies (see later). Irradiation at the frequency of H-10 and H-11 causes the H-12 signal, identified on the basis of its coupling constant, collapses to a singlet (Figure 2A), and the complex

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[2] R. H. Martin, N. Defay, F. Geerts-Evrerol, and S. Delavarenne, *Tetrahedron*, 1964, **20**, 1073.

[3] C. W. Haigh and R. B. Mallion, *J. Mol. Spectroscopy*, 1969, **29**, 478.

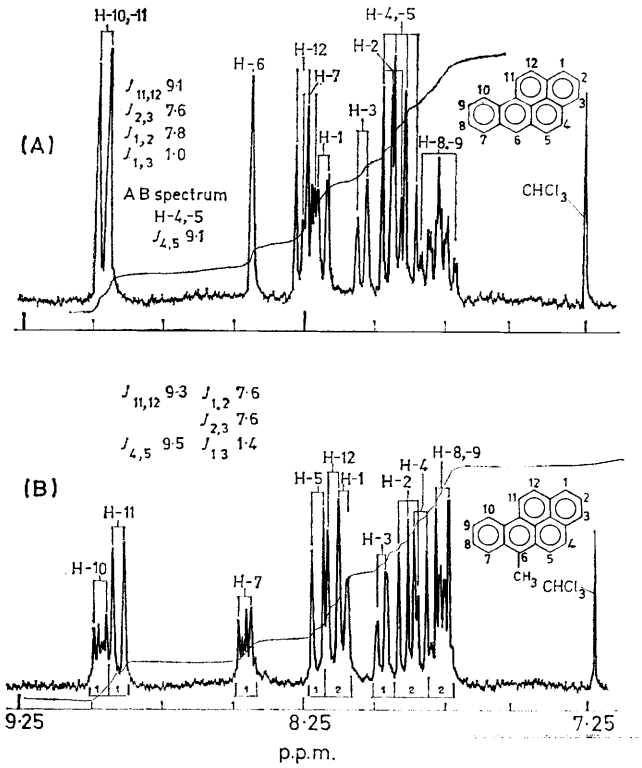

FIGURE 1 The 220 MHz n.m.r. spectra of (A) benzo[*a*]pyrene and (B) 6-methylbenzo[*a*]pyrene (2% w/v) in $CDCl_3$ at 17° (Me_4Si as internal standard). The coupling constants (Hz) were determined by expansion at 2 Hz per cm; accuracy ±0·1 Hz. The singlet methyl signal for 6-methylbenzo[*a*]-pyrene at 3·20 p.p.m. is out of the field

multiplet tentatively assigned to H-8 and H-9 * is simplified (Figure 2B). On the other hand, when the H-12 frequency is saturated, the signal of H-10 and H-11 becomes a broad singlet (Figure 2C). Irradiation of the complex multiplet from H-8 and H-9 causes

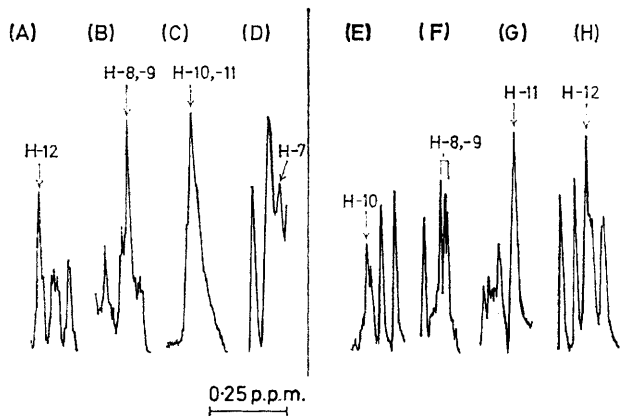

FIGURE 2 Double resonance experiments. Benzo[*a*]pyrene: (A) irradiation of H-11, decoupling of H-12; (B) irradiation of H-10, decrease of complexity of H-8 and H-9; (C) irradiation of H-12, decoupling of H-11; (D) irradiation of H-8, decoupling of H-7

6-Methylbenzo[*a*]pyrene: (E) irradiation of H-8, decoupling of H-10; (F) irradiation of H-10, decrease of complexity of H-8 and H-9; (G) irradiation of H-12, decoupling of H-11; (H) irradiation of H-11, decoupling of H-12

separation of the doublet H-11 signal from that of H-10, which appears now as a complex signal. This complexity is reduced when the frequency corresponding to H-9 is saturated.

The multiplet from H-7, partially superimposed on the H-12 and H-1 signals, is identified by the resultant decoupling when the frequency corresponding to H-8 is irradiated (Figure 2D). Thus, decoupling experiments

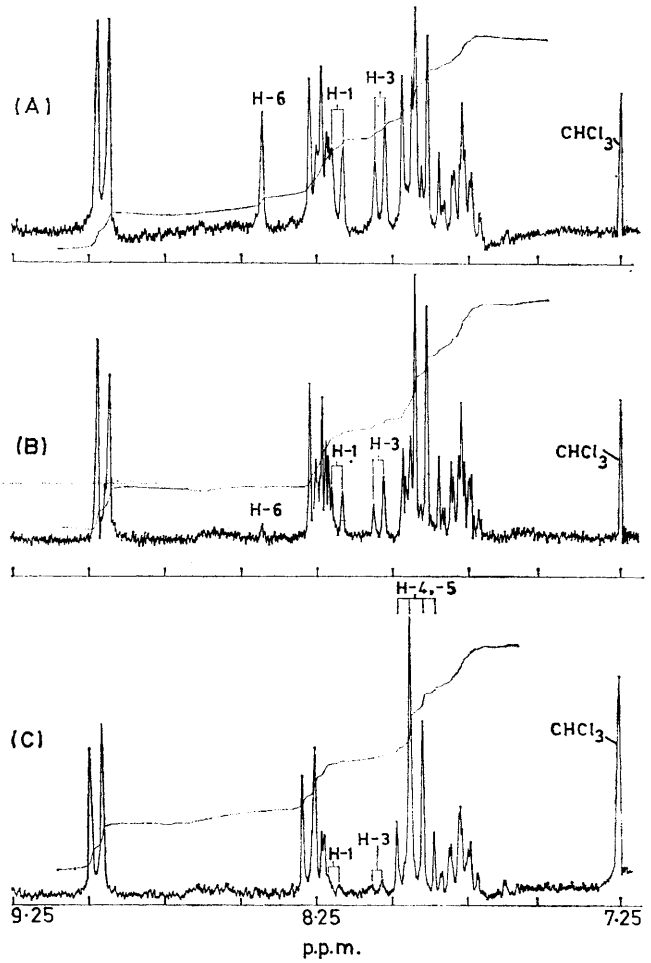

FIGURE 3 The 220 MHz n.m.r. spectra of benzo[*a*]pyrene, previously treated with sulphuric [$^2H_2$]acid (A) for 120 s at 5—10°; (B) for 60 s at room temp.; (C) for 240 s at room temp. Solutions in $CDCl_3$ at 17°; Me_4Si as internal standard

make it possible to assign unequivocally the signals from H-10, H-11, H-12, H-7, H-8, and H-9.

It was easy to see the quartet due to H-4 and H-5 when the 1- and 3-positions were selectively deuteriated (see later) (Figure 3C). In the resultant spectrum the large inner band of the quartet resulted from the superimposed singlet of the collapsed H-2 signal, arising from the disappearance of the two *ortho*-couplings (Figure 1A) with H-1 and H-3.

A direct unequivocal distinction between the H-1 and H-3 signals was not possible; we are not aware of

* The four interacting nuclei H-7, H-8, H-9, and H-10 can be considered as a ABMX System.

a valid criterion for their differentiation. Dewar's theoretical calculations predict that the reactivities for electrophilic substitution reactions decrease in the order C-6, C-1, C-3. Although the 6-position is by far the most active, a bulky Friedel–Crafts reagent would be subject to the steric hindrance typical of this type of *meso*-anthracenic position. In that case, electrophilic substitutions would be expected to take place at the 1- and 3-positions. When the hydrocarbon (1) is treated with succinic anhydride and aluminium chloride, the 1-acyl derivative is the main product.[4]

The deuteriodeprotonation experiments shown in the Table indicate that the 6-position is the most basic and

Deuteriodeprotonation (%) [a] in concentrated sulphuric [$^2H_2$]acid (isotopic purity 99·5%)

Hydrocarbon	Reaction time (s)	Positions of substitution 6	3	1	5
Benzo[*a*]pyrene	120 [b]	42	0	0	0
	60	96	55	44	0
	120	99·5	61	48	0
	180	99·5	73	68	0
	240	99·5	85	80	0
	480	99·5	99·5	99·5	0
6-Methylbenzo[*a*]pyrene [c]	120		99·5	99·5	21

[a] Calculated from the n.m.r. spectra at 2 Hz per cm by taking the ratio of the integration of the peaks corresponding to the partially substituted protons to that corresponding to the non-substituted protons. The signal of the 1-proton (Figure 1) is half-superimposed. The integration of the visible part is considered as 0·5H. The substitution reactions were carried out at ambient temperature unless otherwise specified. [b] At 5—10°. Under these conditions the hydrocarbon was not completely soluble in the acid. [c] Experiments with longer reaction times produced negative results because of the almost total decomposition of the compound under these severe conditions.

that the 1- and 3-positions differ only a little in reactivity. Of the latter two, the 1-position might be expected to be the more reactive, in view of the Friedel–Crafts reaction just mentioned. However, the assignment of Haigh and Mallion [3] is the reverse, and we accept it.

6-Methylbenzo[a]*pyrene* (2).—The spectrum (Figure 1B) clearly shows the deshielding of H-5 and H-7 in the *peri*-positions with respect to the methyl group (*peri*-effect [5]). Decoupling experiments (Figure 2E—H) allow us to designate the signals of H-8, H-9, H-10, H-11, and H-12. The downfield shift of the H-5 signal with respect to the spectrum of compound (1) (Figure 1A) leaves the H-4 signal as a doublet with about the same chemical shift and coupling constant as in (1). The characteristic triplet with two equal coupling constants defines the 2-proton signal, and while the assignment of the H-1 and H-3 signals is suggested in view of the similarity of their susceptibilities to deuteriation to the corresponding protons in (1).

Deuteriodeprotonation of Benzo[a]*pyrene* (1) *and 6-Methylbenzo*[a]*pyrene* (2).—The hydrocarbon (1) dissolved readily in concentrated sulphuric [$^2H_2$]acid. The solution was quenched with a chilled mixture of deuteriated water and chloroform after various reaction times. The partially deuteriated products were separated and their spectra (Figure 3) compared with those of the starting material (Figure 1A). The results are summarised in the Table. After 120 s at 5—10° (Figure 3A) only the 6-position had undergone exchange. The same reaction at room temperature for 60 s (Figure 3B) gave rise to almost complete exchange at the 6-position and to partial and approximately equal exchange at the 1- and 3-positions. Further, when this deuteriated hydrocarbon was reprotonated with sulphuric acid, the original spectrum (Figure 1A) reappeared. Deuteriation for longer times (*e.g.* 480 s, Figure 3C) showed the complete selective electrophilic substitution of the three active positions, leaving the other ones unaltered.

Compound (2), after treatment with sulphuric [$^2H_2$]acid for 120 s, showed complete exchange of the 1- and

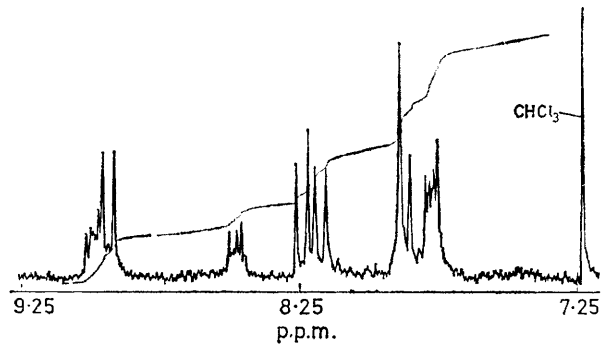

FIGURE 4 The 220 MHz n.m.r. spectrum of 6-methylbenzo[*a*]pyrene, previously treated with sulphuric [$^2H_2$]acid for 120 s at room temperature. Solution in $CDCl_3$ at 17°; Me_4Si as internal standard

3-protons (Figure 4 and Table). The 5-position seems to be active as well.

DISCUSSION

The 6-position in compound (1) is the most active one, followed by the much less active 1- and 3-positions; the latter two possess about the same reactivity. The predominant reactivity of position 6 is also substantiated by the formation of the corresponding cationic intermediate in acid medium. The spectrum of the hydrocarbon under these conditions exhibits a unique singlet in the aliphatic region at 3·78 p.p.m. corresponding to this position. These data agree with theoretical MO calculations [6,7] (see before), which indicate that positions 6, 1, and 3 have the lowest carbon localisation energies. Similarly, the deuterium exchange reactions for (2) show that the 1- and 3-carbon atoms are reactive and that the 5-position shows some reactivity.

The chemical reactivity, and presumably the carcinogenic reactivity, in aromatic hydrocarbons is induced by the electrophilic oxygen atom of the hydroxylating enzymes.[8,9] Kinetics of deuterium-ion exchange in these

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[8] P. L. Grover and P. Sims, *Biochem. J.*, 1968, **110**, 159.
[9] H. V. Gelboin, *Cancer Res.*, 1969, **29**, 1272.

compounds can thus provide information about reactive positions which might be relevant to the process of carcinogenesis.[10]

EXPERIMENTAL

The n.m.r. spectra were recorded with a Varian HR 220 MHz spectrometer at ambient temperature (17°), with deuteriochloroform as solvent and tetramethylsilane as internal standard. The additional stationary radio-frequency field for double resonance was provided by a 4204A oscillator (Hewlett-Packard). The sulphuric [$^2H_2$]-acid (99·5% isotopic purity) was obtained from Merck, Sharp, and Dohme.

Benzo[a]*pyrene* (1).—A commercial sample (Aldrich) was further purified by filtration through a column containing neutral alumina (Woelm activity I); benzene was used as the solvent. The compound was recrystallized from acetone–methanol and had m.p. 181—182°.

6-*Methylbenzo*[a]*pyrene* (2).—The compound was prepared by reduction of 6-formylbenzo[*a*]pyrene [11] according to the method of Huang-Minlon.[12]

Deuteriodeprotonation of Benzo[a]*pyrene* (1).—(*a*) Compound (1) (25 mg) was partially dissolved with stirring in conc. sulphuric [$^2H_2$]acid (1·5 ml) at 5—10° and left for 120 s. The deep red solution was then poured into deuteriated water (10 ml) and chloroform (5 ml), previously chilled, with the temperature maintained below 17°. The chloroform layer was separated and the aqueous layer was extracted again with chloroform (5 ml). The total organic solution was washed with deuteriated water (5 ml), dried (Na_2SO_4), and evaporated. The residue (*ca.* 20 mg) was dissolved in [^{2}H]chloroform (1 ml) and its spectrum was recorded.

(*b*) Compound (1) (25 mg) was dissolved in conc. sulphuric [$^2H_2$]acid (1·5 ml) at room temperature and then stirred for 60 s. The same procedure as (*a*) was then followed.

(*c*) The same conditions as (*b*) were used; compound (1) was left for 120, 180, 240, or 480 s in sulphuric [$^2H_2$]acid.

Deuteriodeprotonation of 6-*Methylbenzo*[a]*pyrene* (2).—Compound (2) (30 mg) was dissolved in sulphuric [$^2H_2$]acid (1·5 ml) and left for 120 s at room temperature with stirring. The solution became green. The same procedure as in (*a*) was followed. Results (Figure 4) show the absence of the H-3 signal at 7·95 and the H-1 signal at 8·11 p.p.m.

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