Title
THE 220 MHZ NUCLEAR MAGNETIC RESONANCE ANALYSIS AND THE SELECTIVE DEUTERODEPROTONATION OF BENZO[a] PYRENE AND 6-METHYLBENZD[a]PYRENE

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Running Title:

The 220 MH_z NMR of Benzo[a]pyrene and 6-Methylben[illegible]

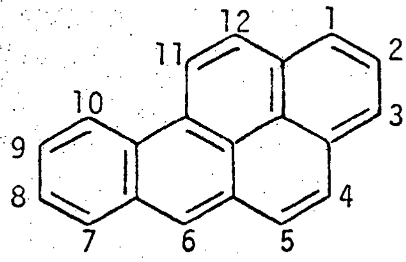
ABSTRACT: The analysis of the magnetic resonance spectra at 220 MH_z of the carcinogenic benzo[a]pyrene (1) and 6-methylbenzo[a]pyrene (2) is presented. The proton exchange study in sulfuric acid-d_2 is used to determine the specific positions of electrophilic substitution. Electrophilic attack on (1) takes place predominantly on the 6-position, followed by the 1- and 3-positions, whereas in (2), in which the 6-carbon atom is substituted, the most active positions are the numbers 1, 3 and 5, with 5 the slowest.

INTRODUCTION

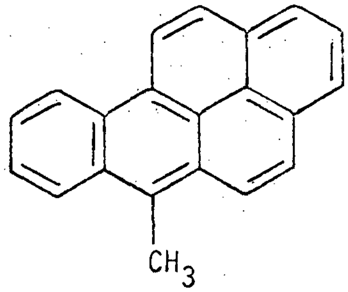
The proton nuclear magnetic resonance (NMR) study at 60 MH_z of twenty unsubstituted polycyclic hydrocarbons[1] reveals the presence of separated band-systems. The large ring-current diamagnetic effect of these molecules generates on the protons a downfield chemical shift relative to those of the benzene and naphthalene series. The particular relevance of this effect to the meso-anthracenic protons leads to their separation from the others and thus to their identification. Moreover, the angular protons exhibit an additional deshielding effect due to the non-bonded spin-spin interactions (steric compression effect)[2] and their band systems can also be easily assigned. However, the remaining protons are normally not well separated.

In the case of the polycondensed hydrocarbons, a complete NMR analysis has been achieved only for the benzo[e]pyrene.[2] This was accomplished by comparison of the theoretical calculated shielding parameters, a relatively less complicated calculation for this symmetrical molecule, with the experimental data.

The better resolution achievable with the 220 MH_z spectrometer, aided by the double resonance technique and specific deuterodeprotonation with sulfuric acid-d_2 allows us to report here the interpretation of the benzo[a]pyrene (_1_) and 6-methylbenzo[a]pyrene (_2_) proton magnetic resonance spectra. At the same time, the spectra provide qualitative information on the positions of selective substitution obtained by such a deuterium ion exchange. Finally, the kinetics of exchange are used for defining the relative reactivities of the various positions of the two hydrocarbons.



(1)



(2)

RESULTS

Assignments of Lines

BENZO[a]PYRENE (1). The 220 MH_z proton magnetic resonance of (1) is shown in Figure 1. The peaks are assigned to the corresponding protons in the following manner. The integration line provides the proton ratio in the band groups from left to right, of 2:1:3:1:3:2. A first approximate interpretation derives from Martin's empirical rules[1,3] that suggest which spectral regions are to be associated with each type of aromatic proton.

The two angular protons H_{10} and H_{11} were already characterized by Martin et al.[1] in the spectrum at 60 MH_z. The overall superposition of the two chemical shifts in this spectrum is a pure coincidence. At higher concentrations, or when the coupled protons are irradiated in the regions close to their resonance signal, using the double resonance technique (vide infra), the sharp doublet of H_{11} becomes slightly separated from the more complex coupling of H_{10}. If the two protons are irradiated with a strong radiofrequency signal at their resonance frequency, the H_{12}, postulated on the basis of the same coupling constant, collapses to a singlet (Figure 2A) and the complex multiplet, tentatively assigned to the protons* H_8 and H_9, is simplified (Figure 2B). On the other hand, when the H_{12} peak is saturated, the signal of H_{10} and H_{11} becomes a broad singlet (Figure 2C). The irradiation of the complex multiplet H_8, H_9 in the region of their resonance frequency creates a separation of the doublet H_{11} from the H_{10} which appears now as a complex signal. This complexity is reduced when the signal corresponding to the resonance frequency of H_9 is saturated.

The multiplet peak H_7, partially superimposed on H_{12} and H_3, is designated by the resultant decoupling when the signal corresponding to H_8 is irradiated (Figure 2D). Thus, the decoupling experiments make it possible to assign unequivocally the protons H_{10}, H_{11}, H_{12}, H_7, H_8 and H_9.

The characteristic peak at 8.41 ppm corresponds to the mesoanthracenic hydrogen in the 6-position. Although the two protons H_4 and H_5 possess about the same chemical shift as the H_2, the pattern of their AB spectrum can be recognized. It was easy to see the quartet of H_4 and H_5 when the H_1 and H_3 were selectively deuterated (vide infra) and Figure 4C). In the resultant spectrum the large left inner band of the quartet resulted from the superimposed singlet of the collapsed H_2, arising from the disappearance of the two ortho-coupling constants (Figure 1) with the protons 1- and 3-positions.

The precise distinction between protons H_1 and H_3 cannot be directly resolved because we are not aware of a valid criterion for their differentiation. Dewar's theoretical calculations predict that the positions for

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*The four interacting nuclei H_7, H_8, H_9, and H_{10} can be considered a ABMX system.

electrophilic substitution reactions are in decreasing order of reactivity, 6, 1 and 3. Although the 6-position is by far the most active, a bulky Friedel-Crafts reagent would manifest the steric hindrance of the meso-anthracenic type of this carbon-atom. In that case, electrophilic substitutions would be expected to take place on the 1- and 3-positions. When the hydrocarbon (1) is allowed to react with the succinate anhydride and aluminum chloride, the 1-acylated derivative is the main product.[4]

The deuterodeprotonation of (1) shown in Table 1 indicates that the 6-position is the most basic one and the 1- and 3-positions reveal only a little difference in reactivity. Of the latter two the signal exchanged slightly faster is preferentially suggested to be the proton on the 1-position. The proposed designation stems from the above reported theoretical and chemical results.

6-METHYLBENZO[a]PYRENE (2). At first glance, the NMR of this molecule (Figure 3), compared to the spectrum of (1) shows the two expected deshielded protons H_5 and H_7 in the peri-position with respect to the methyl group (peri effect[5]), and an anomalous shielding effect of one of the two angular protons as shown by the integrated spectrum. The ratio of the protons from left to right is 1:1:1:1:2:1:2:2. The complex multiplet of the two downfield shifted protons H_5 and H_7 cannot, to our knowledge, be easily explained. As a matter of fact, the "peri" coupling have been reported to be small and to produce a broadening of peaks.[6] However, strong irradiation of the methyl group (singlet at 3.2 ppm, with the linewidth at the half height of 1.6 H_z) does not affect either the broad H_5 or the broad H_7.

The results of irradiation of the H_8 and H_9 multiplet by the double resonance technique suggest that the proton centered at 8.96 ppm belongs to the 7-position (Figure 2E). On the other hand, the saturation of the latter proton affects the H_8 and H_9 signal (Figure 2F), indicating spin-spin coupling to H_8 and hence, their respective assignment. Furthermore, this finding suggests that the multiplet centered at 8.45 ppm to be, by exclusion, the proton in the 5-position. The two peri-protons, H_5 and H_7 exhibit about the same downfield shift relative to their signals in benzo[a]pyrene and this adds further evidence for their assignment. A negative result of the decoupling of the proton at 8.90 ppm by irradiation of the proton H_9 rules out the possibility that the doublet might be the proton in the 10-position and suggests this to be the proton in the 11-position.

The protons H_{11} and H_{12}, though coupled, do not show exactly the same coupling constant. However, a positive reciprocal decoupling experiment (Figures 2G, 2H) leaves no doubt about their assignments. The downfield shift of the proton H_5 with respect to its chemical shift in the NMR of (1) (Figure 1) leaves the proton H_4 as a doublet possessing about the same chemical shift and coupling constant as in (1). The characteristic triplet with two equal coupling constants defines the proton in the 2-position. While one of the protons coupled to the H_2 is directly disclosed by its having the same value for the coupling constant, the second one, partially superimposed, is disclosed after deuterium-exchange (Figure 5). They present the same patterns, the same chemical shift, and the same ease of deuteration as the protons H_1 and H_3 in (1). On this basis, their assignment is suggested. Incidentally, the three interacting protons H_1, H_2, H_3, form an ABC system as in the case of (1) (Figure 1). The spectrum of the three protons is relatively simple because the coupling constants $J_{1,2}$ and $J_{2,3}$ are practically the same. Finally, the remaining proton, namely, the doublet at 8.20 ppm, is the proton in the 10-position.

Spectrum of Benzo[a]pyrene (1) in Acid Medium

The NMR spectrum of compound (1) (39.6 mg) in a mixture of carbon tetrachloride (0.5 ml), trifluoroacetic acid (0.5 ml) and concentrated sulfuric acid (0.032 ml) reveals a singlet in the aliphatic region at δ 3.78 ppm corresponding to the two protons of the cationic intermediate. Since the 6-carbon atom is by far the most reactive one (*vide infra*), it is logical to designate that position as the predominantly protonated one. In a similar way, the NMR spectrum of (1), under the same conditions, except for trifluoroacetic acid and sulfuric acid which have been substituted with their deuterated isotopes, does not exhibit any signal in the aliphatic region.

DEUTERODEPROTONATION OF BENZO[a]PYRENE (1) AND 6-METHYLBENZO[a] PYRENE (2). The carcinogenic aromatic hydrocarbon (1) is easily dissolved in concentrated sulfuric acid-d_2. The solution is then quenched with a chilled mixture of deuterated water and chloroform at different reaction times.

After the separation of the compound, its NMR spectra (Figure 4), compared to the NMR spectrum of (1) (Figure 1), point out specific deuterated positions by the partial or total disappearance of some of the signals. The results are summarized in Table 1. When the hydrocarbon (1) is treated for 120 sec with deuterated sulfuric acid at 5-10° (Figure 4A)

Table 1

Percent[a] of deuterodeprotonation in concentrated sulfuric acid-d_2 (isotopic purity, 99.5%)

Hydrocarbon	Reaction time (sec)	Positions of substitution 6	1	3	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]The percent of the deuteration is calculated in the NMR spectra at 2 H_z per cm by the percent ratio of the integration of the peaks corresponding to the partially substituted protons with respect to the integration of the peaks corresponding to the non-substituted protons. The signal of the proton in the 3-position (Figure 1) is half-superimposed. The integration of the visible part is considered half a proton. The substitution reactions have been carried out at ambient temperature unless otherwise specified. [b]At 5-10°. In these conditions the hydrocarbon was not completely soluble in the acid. [c]Experiments with longer reaction times have produced negative results because of the almost total decomposition of the compound under these severe conditions.

only the 6-position undergoes exchange. The same reaction at room temperature for 60 sec (Figure 4B) gives rise to practically total exchange on the 6-position and to partial and approximately equal exchange on the 1- and 3-positions. Further, when this deuterated hydrocarbon is protonated with sulfuric acid, the NMR spectrum of (1) (Figure 1) reappears. Deuteration for longer times (d.g., 480 sec, Figure 4C) shows the complete selective electrophilic substitution of the three active positions, leaving the other ones unaltered.

Compound (2), also carcinogenic, shows after treatment with deuterated sulfuric acid for 120 sec a complete exchange of the protons in the 1- and 3-positions (Figure 5 and Table 1). The 5-position seems to be active as well.

DISCUSSION

The enhanced resolution of the NMR spectra at 220 MH_z in relation to the 60 MH_z has permitted the separation of the peaks of all of the different protons in (1) and (2) and, hence, to allow their assignment. In this way, it has been possible to follow the selective deuterium exchange in both compounds. 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 No. 6 is also substantiated by the formation of the corresponding cationic intermediate in the acid medium. The NMR of the hydrocarbon under these conditions exhibits a unique singlet in the aliphatic region. These data manifest a noteworthy agreement with theoretical M.O. calculations[7,8] (vide supre), which indicate that positions Nos. 6, 1, and 3 have the lowest carbon localization energies and in the same order of reactivity. Similarly, the deuterium exchange reactions for (2) show the active 1- and 3-carbon atoms and, in addition, some reactivity at the 5-carbon atom as well.

It is interesting to notice that the chemical reactivity, and presumably the carcinogenic reactivity in aromatic hydrocarbon, is induced by the electrophilic oxygen of the hydroxylating enzymes.[9-17] Therefore, the kinetics of exchange in these compounds with deuterium ion can provide information about their reactive positions, which might be relevant in the process of carcinogenesis.[18]

EXPERIMENTAL

The NMR spectra were recorded on a Varian high resolution HR 220 MH_z spectrometer at the ambient temperature (17°) in deuterochloroform as solvent with tetramethylsilane (TMS) as an internal standard. The additional stationary radio-frequency field for the double resonance was provided by a 4204A oscillator (Hewlett-Packard). The sulfuric acid-d_2 (99.5% isotopic purity) was obtained from Merck Sharp & Dohme.

Benzo[a]pyrene (1). The benzo[a]pyrene (1) was purchased from Aldrich and further purified by filtration through a chromatography 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°.

The NMR had δ 7.75 (H_8 and H_9, multiplet), 7.89 (H_4 and H_5, AB system, $J_{4,5}$ = 9.1 H_z), 7.92 (H_2, triplet, $J_{1,2}$ = 7.6 H_z, $J_{2,3}$ = 7.18 H_z), 8.02 (H_1, quadruplet, $J_{1,2}$ = 7.6 H_z, $J_{1,3}$ = 1.0 H_z), 8.16 (H_3, quadruplet,

$J_{2,3}$ = 7.8 H_z, $J_{1,3}$ = 1.0 H_z), 8.21 (H_7, multiplet), 8.24 (H_{12}, doublet, $J_{11,12}$ = 9.1 H_z), 8.41 (H_6, singlet), 8.94 (H_{10} and H_{11}, doublet).

6-Methylbenzo[a]pyrene (2). The compound was prepared by reduction of 6-formylbenzo[a]pyrene[19] according to the method of Huang-Minlon.[20] The formylbenzo[a]pyrene (0.500 g, 1.185 x 10^{-3} moles) was dissolved in the minimum amount of dioxane (10 ml). To that solution were added 0.397 g of potassium hydroxide in 0.2 ml of water, 10 ml of triethylen-glycol, and 1 ml of 100% hydrozine-hydrate. The solution was refluxed (100°) for 1.5 h. After that period hydrazine, water and dioxane were removed by distillation and the temperature was raised to 180-200° for 5 h. The cooled solution was diluted with water (40 ml) and was neutralized with 1 N hydrochloric acid. The colored precipitate was separated and dried (Na_2SO_4). It was then filtered on neutral alumina (Woelm activity I) using chloroform as solvent. The first fraction contained the yellow compound. After recrystallization from acetone-ethanol, its weight was 0.300 g (63% yield) and it had m.p. 216.2 - 216.7° (lit.[10] m.p. 216.2 - 216.7).

The NMR spectrum had δ 3.20 (CH_3 group, singlet, linewidth at half height 1.6 H_z), 7.76 (H_8 and H_9, multiplet), 7.84 (H_4, doublet, $J_{4,5}$ = 9.5 H_z), 7.89 (H_2, triplet, $J_{1,2}$ = 7.6 H_z, $J_{2,3}$ = 7.6 H_z), 7.95 (H_1, quadruplet, $J_{1,2}$ = 7.6 H_z, $J_{1,3}$ = 1.4 H_z), 8.11 (H_3, quadruplet, $J_{2,3}$ = 7.6 H_z, $J_{1,3}$ = 1.4 H_z), 8.15 (H_{12}, doublet, $J_{11,12}$ = 9.1 H_z), 8.20 (H_{10}, doublet, $J_{9,10}$ = 9.7 H_z), 8.45 (H_5, multiplet), 8.90 (H_{11}, doublet, $J_{11,12}$ = 9.3 H_z). (See Figure 3.)

Deuterodeprotonation of benzo[a]pyrene (1). (a) Compound (1) (25 mg) was partially dissolved under stirring in 1.5 ml of conc. sulfuric acid-d_2 at 5-10° and left for 120 sec. A deep red solution appeared. The acidic

solution was then poured into 10 ml of deuterated water and 5 ml of chloroform, previously chilled. Room temperature was not exceeded following the dilution. The chloroform solution after extraction was separated and the acidic aqueous-deuterated solution was extracted again with 5 ml of chloroform. The total organic solution was washed with 5 ml of chloroform. The total organic solution was washed with 5 ml of deuterated water and dried (Na_2SO_4). After evaporation of the chloroform, the approximately 20 mg of residue were dissolved in 1 ml of chloroform-d and its NMR was recorded. (b) Compound (1) (25 mg) was dissolved in 1.5 ml of conc. sulfuric acid-d_2 at room temperature and then stirred for 60 sec. After that, the same procedure as (a) was followed.
(c) The same conditions as (b) were used when (1) was left for 120, 180, 240 or 480 sec in sulfuric acid-d_2.

Deuteroprotonation of 6-methylbenzo[a]pyrene (2). Compound (2) (30 mg) was dissolved in 1.5 ml of sulfuric acid-d_2 and left for 120 sec at room temperature under stirring. The solution became green. The same procedure as (a) was followed. Results in Figure 5 show the absence of H_1 at 7.95 and H_3 at 8.11.

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FIGURE LEGENDS

Figure 1. The 220 MH_z NMR spectrum of benzo[a]pyrene (2% wt/v) in $CDCl_3$ at 17°. The scale is referred to TMS as internal standard. The coupling constants are in hertz (H_z) and have been determined by expansion at 2 H_z per cm. The accuracy of the coupling constants measurements is ± 0.1 H_z.

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, 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_7; F) Irradiation of H_7, decrease of complexity of H_8, H_9; G) Irradiation of H_{12}, decoupling of H_{11}; H) Irradiation of H_{11}, decoupling of H_{12}. Scale .25 ppm = |⟵⟶|.

Figure 3. The 220 MH_z NMR spectrum of 6-methylbenzo[a]pyrene (2% wt/v) in $CDCl_3$ at 17°. The scale is referred to TMS as internal standard. The coupling constants are in H_z and have been determined by expansion at 2 H_z per cm. The singlet signal of the methyl protons at 3.20 ppm is out of the field. The accuracy of the coupling constants measurements is ± 0.1 H_z.

Figure 4. A) The 220 MH_z NMR spectrum of benzo[a]pyrene, previously treated with sulfuric acid-d_2 for 120 sec at 5-10°. The compound is dissolved in $CDCl_3$ and the spectrum is recorded at 17°, using TMS as internal standard. B) The NMR spectrum of the same compound, previously treated

Figure 4 (continued)

with sulfuric acid-d_2 for 60 sec at room temperature, recorded in the same conditions as A). C) The NMR spectrum of the same compound, previously treated with sulfuric acid-d_2 for 240 sec at room temperature, recorded in the same conditions as A).

Figure 5. The 220 MH_z NMR spectrum of 6-methylbenzo[a]pyrene, previously treated with sulfuric acid-d_2 for 120 sec at room temperature. The compound is dissolved in $CDCl_3$ and the spectrum is recorded at 17° using TMS as internal standard. The absence of H_1 at 795 and H_3 at 811 is apparent.

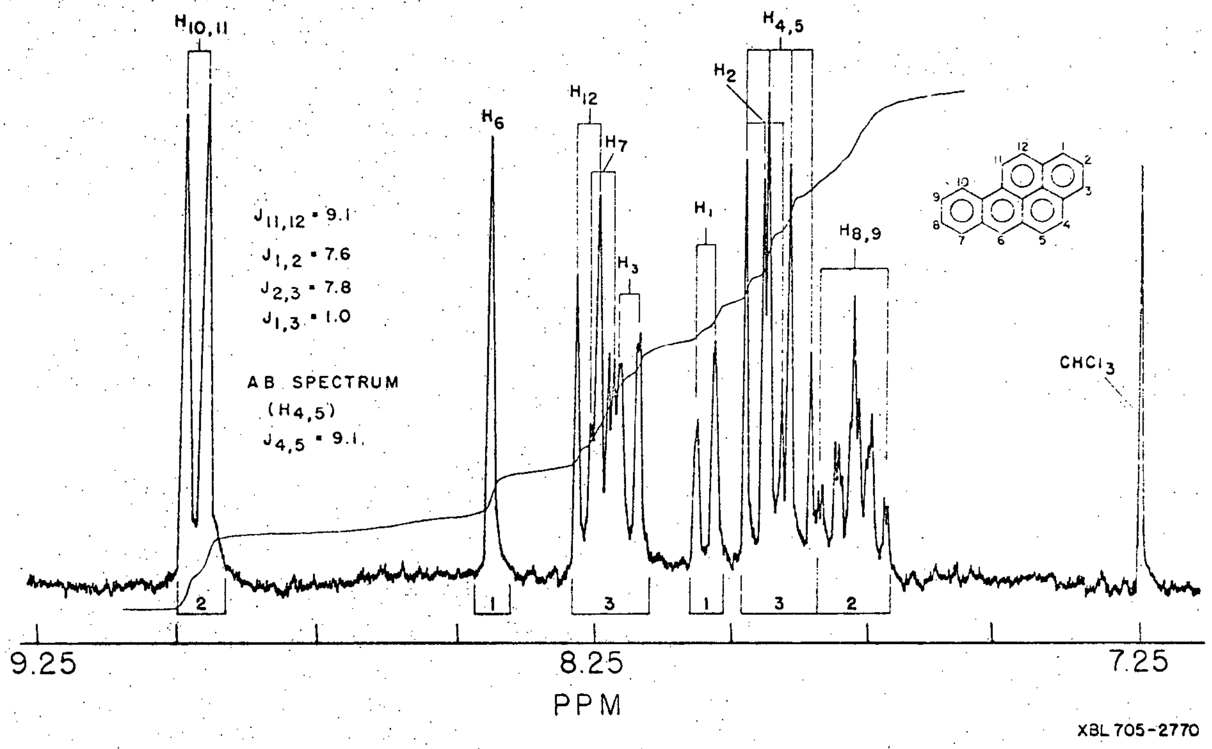

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Figure 1

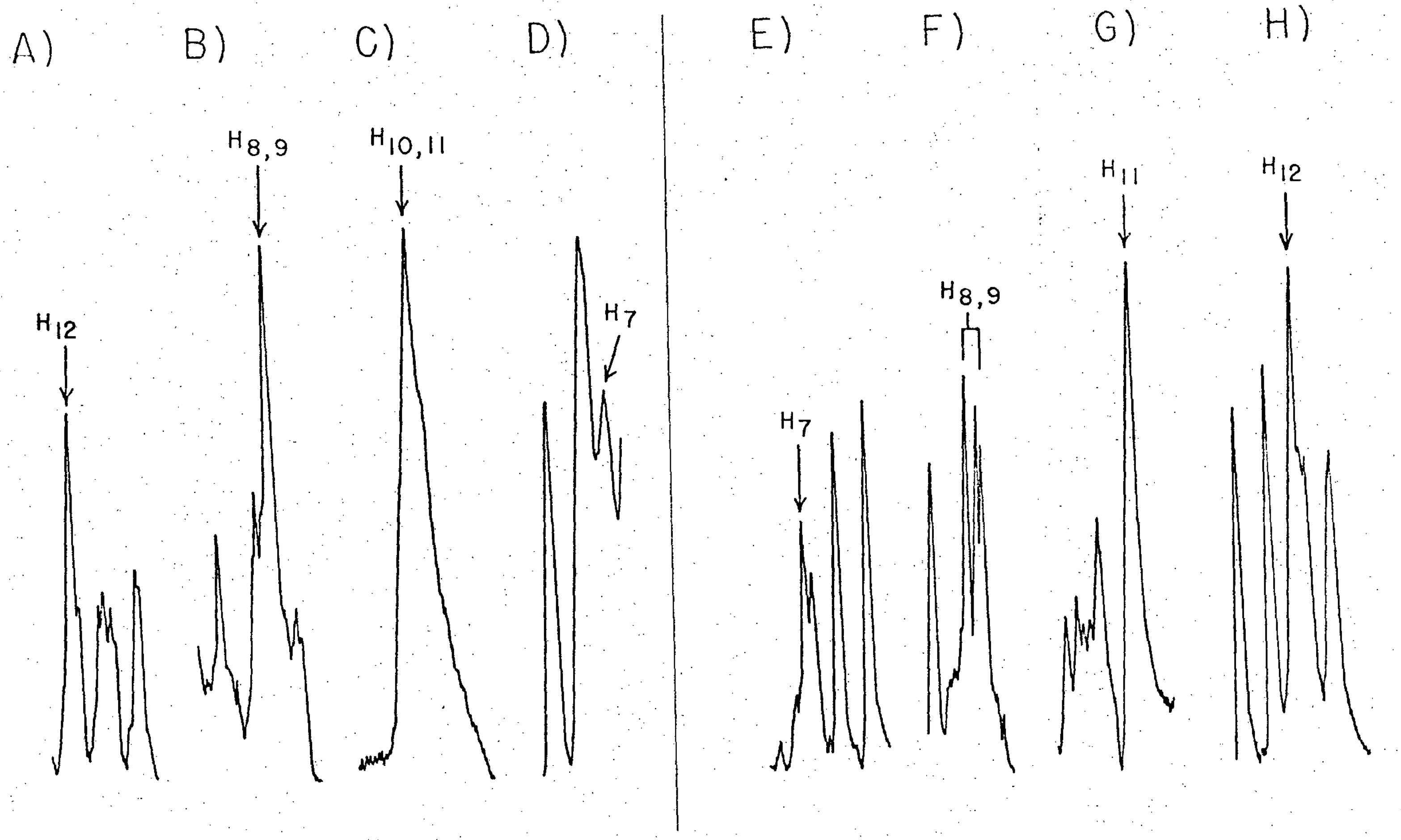

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Figure 2

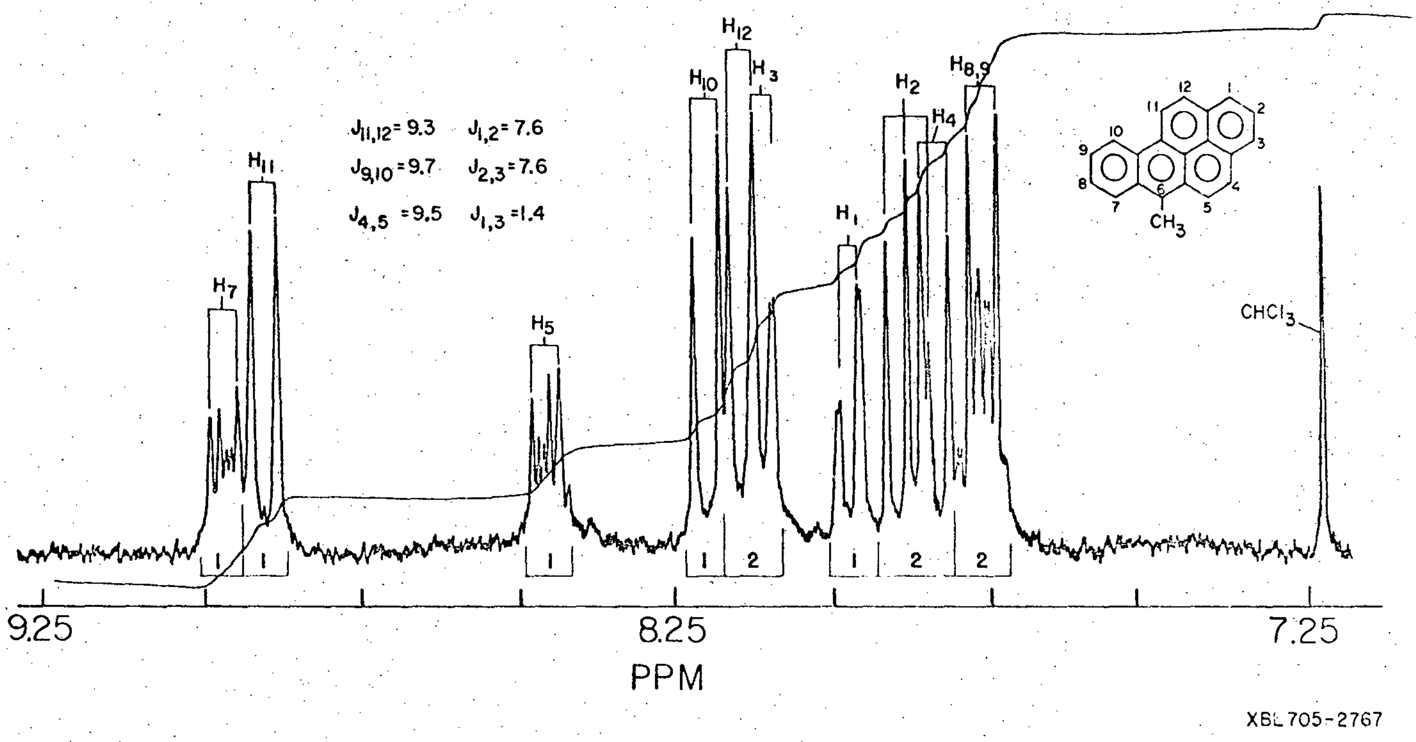

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Figure 3

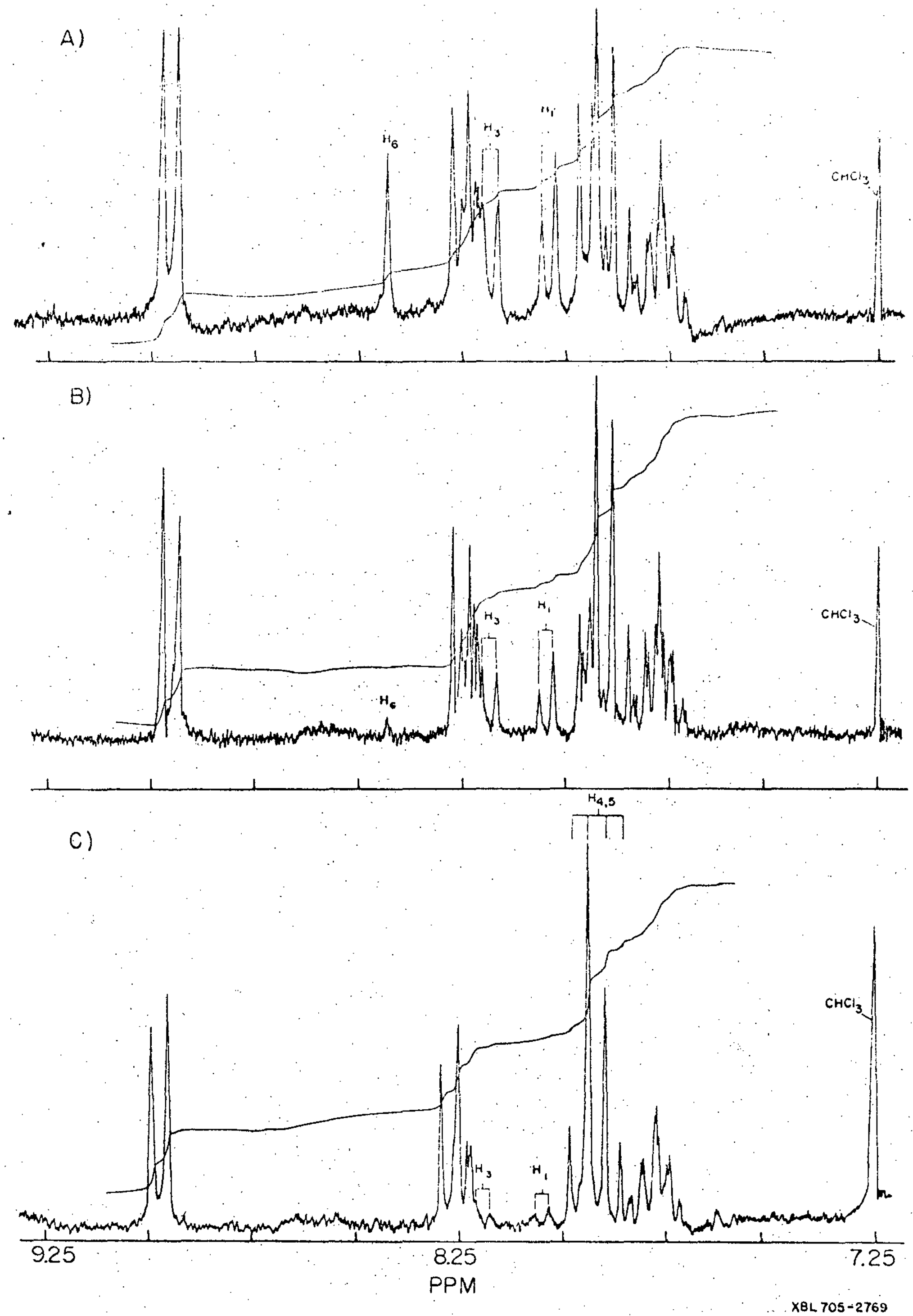

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Figure 4

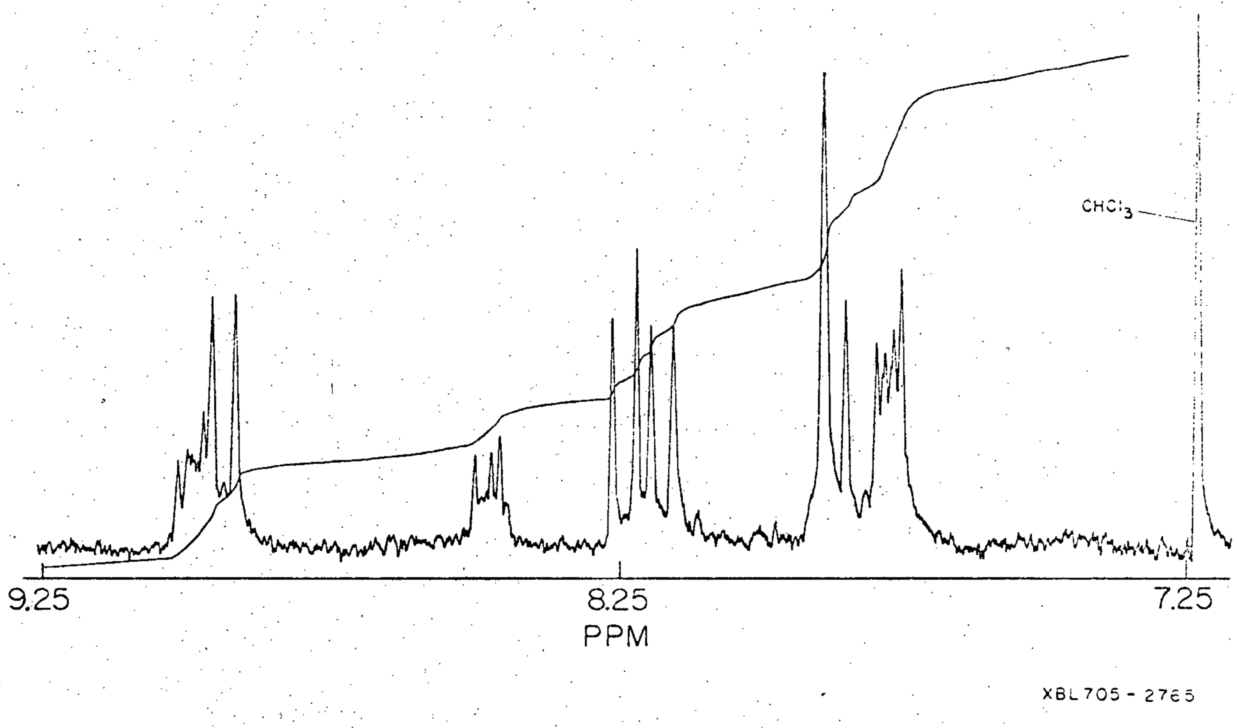

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Figure 5

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