CARBON-13 NMR SPECTROSCOPY
PART I: THIOLS, THIOLACETATES, AND LIPOIC ACID DERIVATIVES
PART II: SUBSTITUTED BIPHENYLS

by

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Approved by:

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To

my family

and

friends

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TABLE OF ^{13}C SPECTRA

INTRODUCTION

High resolution ^{13}C NMR spectroscopy has been used with great success in a variety of chemical and biochemical problems. Four NMR parameters are routinely measured by various ^{13}C NMR experiments; chemical shifts(δ), coupling constants ($^{1}J_{CH}$), multiplicities, and relaxation times(T_1). These parameters are used in combination to determine the structure of molecules. The chemical shifts are measured by ^{1}H-decoupling ^{13}C NMR experiments. In these experiments, all ^{13}C resonances appear as singlets. The coupling constants and multiplicities can be obtained from gated decoupling experiments. Off-resonance decoupling experiments also measure multiplicities, but exact coupling constants are not determined. Integrated areas, although not commonly measured in ^{13}C NMR work due to the Nuclear Overhauser Effect, may be obtained with long delay times or by introducing small quantities of a paramagnetic species. Several good texts[1,2,3] which discuss the theory and application of ^{13}C NMR spectroscopy in detail are available.

This thesis is concerned with the application of ^{13}C NMR spectroscopy to two problems. Part I is concerned with the resonance assignment of lipoic acid and its derivatives. Thiols, thiolacetates, and several octanoic acid derivatives are also covered in this first section. Part II examines the ^{13}C NMR spectra of a series of substituted biphenyls.

PART I

LIPOIC ACID DERIVATIVES

INTRODUCTION

Lipoic acid is an essential cofactor for multienzyme complexes that function in the oxidation of several α-keto acids(e.g. pyruvate, α-ketoglutarate, α-keto-isovalerate, α-ketoisocaproate, α-keto-β-methylvalerate, glyoxylate, etc.). It has been proposed that lipoic acid functions in acyl transfer to Coenzyme A and electron transfer to flavin adenine dinucleotide.[4,5]

The oxidation of pyruvate to acetyl-CoA illustrates the general role of lipoic acid in these oxidations. The overall equation is;

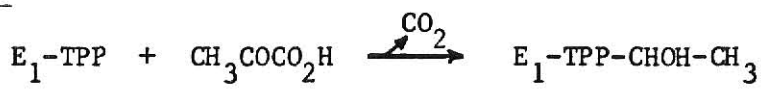
$$\text{pyruvate} + NAD^+ + CoA \longrightarrow \text{acetylCoA} + NADH + H^+ + CO_2 \ .$$

This reaction is irreversible in animal tissues ($\Delta G^{\circ\prime} = -8.0$ kcal mol^{-1}) and is necessary for the entry of all carbohydrates into the tricarboxylic acid cycle. The pyruvate dehydrogenase complex, a multienzyme complex of three different enzymes and five different coenzymes, catalyzes the reaction. The five reaction steps promoted by this complex are illustrated in Figure 1.[5]

Pyruvate dehydrogenase(E_1), whose prosthetic group is the coenzyme thiamin pyrophosphate(TPP), catalyzes step I. Pyruvate undergoes decarboxylation to yield CO_2 and the α-hydroxyethyl derivative of TPP.

In step II the hydroxyethyl group is dehydrogenated to an acetyl group, which is then transferred to the sulfur atom at carbon 6 or 8 of lipoic acid. The lipoic acid is a covalently bound prosthetic group of the second enzyme complex, lipoate acetyltransferase (E_2). Lipoic acid is attached by an amide linkage to the ε-amino group of a lysine residue in the enzyme complex (Figure 2). The transfer of a pair of equivalent electrons from the hydroxyethyl group of TPP to the disulfide bond of lipoic acid converts the latter to the reduced, dithiol form, dihydrolipoic acid.

STEP I



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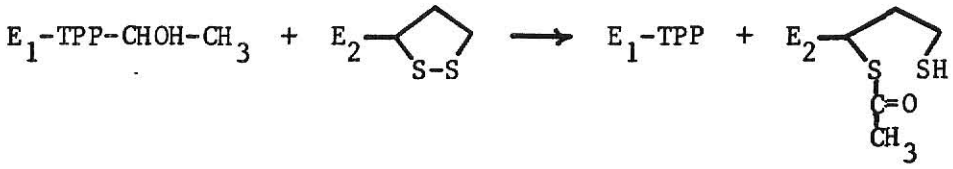

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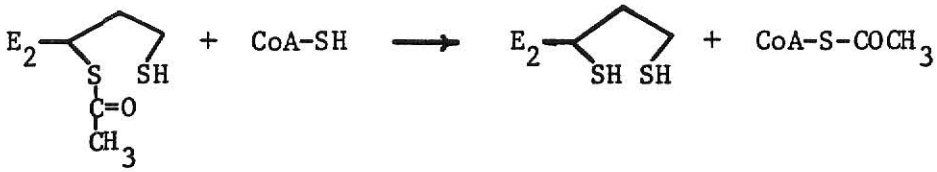

STEP IV

STEP V

Figure 1. Steps in the oxidation of pyruvate to acetyl-CoA by the pyruvate dehydrogenase complex.[4]

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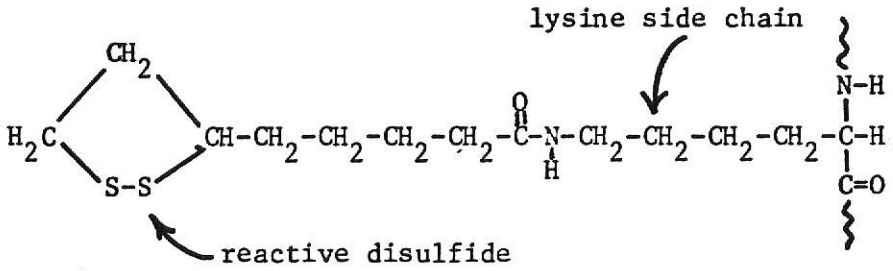


Figure 2. Lipoamide.[5]

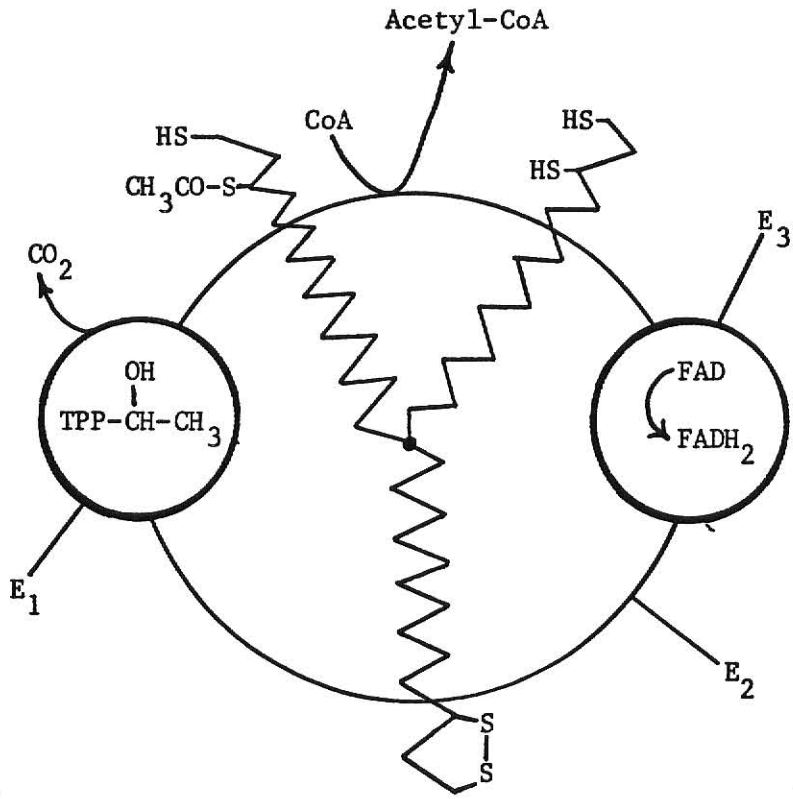


Figure 3. Role of the lipoyllysyl group in the pyruvate dehydrogenase complex.[4]

In step III the acetyl group is enzymatically transferred from the lipoyl group of dihydrolipoic acid to the thiol group of coenzyme A. The acetyl-CoA, thus formed, leaves the enzyme complex in free form.

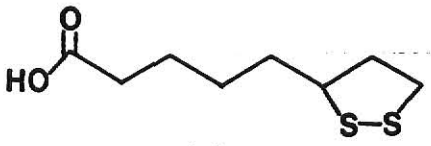
In step IV the dithiol form of the lipoyl group is reoxidized to the disulfide form by the third enzyme of the complex, lipoamide dehydrogenase(E_3). The reducible prosthetic group of E_3 is tightly bound flavin adenine dinucleotide (FAD). The resulting $FADH_2$ remains bound to the enzyme and is reoxidized in step V by NAD^+, with formation of NADH.

Figure 3 illustrates that the mobility of the long lipoyllysyl side chain of E_2 is an important feature in understanding lipoic acid's role in the pyruvate dehydrogenase complex. The lipoyllysyl side chain is free to "swing" from site to site and undergo the various reactions. In the disulfide form the chain swings to the TPP site of E_1 and undergoes a transacetylation to the dihydro form. The chain then moves to the active site of E_2 where it transfers the acetyl group to CoA. Now in its reduced form, it swings to the FAD site of E_3 and is converted to the oxidized form. The oxidized, disulfide form can now return to the TPP site of E_1 and repeat the cycle.

The question that remains to be answered concerning this "swinging arm" mechanism is whether the acetyl group obtained from TPP is attached to the 6 or 8 position of lipoic acid. Gunsalus and coworkers[6] reported that the acetyl group attaches to the 6-position. The evidence for this conclusion was the reaction of their product with N-propionyldiphenylketimine. Since N-propionyl-diphenylketimine is apparently specific for primary -SH groups, it was concluded that the enzymatic reaction results in acetylation of the secondary -SH group. Reed[7] believed that it should be kept in mind that Gunsalus' enzymatic transacetylation reactions were carried out under nonphysiological conditions involving nonbound lipoic acid. It should not necessarily follow, therefore, that these model reactions mirror transformations of the bound lipoic acid.

It may be possible to solve this dilemma by using ^{13}C NMR techniques to study the role of lipoic acid in the reductive acylation, transacylation, and reoxidation reactions catalyzed by these multienzyme complexes. For instance, in the study of the pyruvate dehydrogenase complex, it may be feasible to determine whether the acetyl group is attached specifically to the 6 or 8 position of lipoic acid, randomly to the 6 and 8 positions, or intramolecularly transfers between the two positions.

This thesis does not attempt to solve this problem. It does, however, present the ground work necessary to do a study of this nature by providing a complete analysis of the ^{13}C chemical shifts of the lipoic acid derivatives; lipoic acid [5-(1,2-dithiolan-3-yl)pentanoic acid] (I), lipoamide [5-(1,2-dithiolan-3-yl)pentanamide] (II), dihydrolipoamide [6,8-dithioloctanamide] (III), 6-S-acetyl-6,8-dithioloctanamide (IV), 8-S-acetyl-6,8-dithioloctanamide (V), and methyl 6,8-S-diacetyl-6,8-dithioloctanoate (VI).



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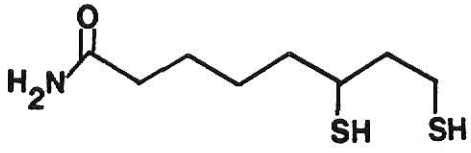

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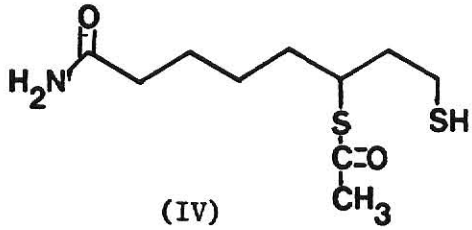

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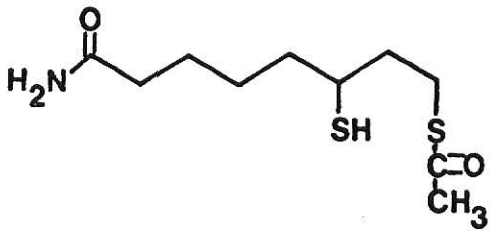

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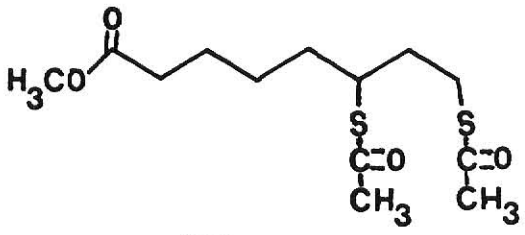


(VI)

OBJECTIVE

To assign the ^{13}C resonances of the lipoic acid derivatives (I-VI).

APPROACH

To accomplish the stated objective, some preliminary data were obtained:

1. The substituent effect parameters for primary and secondary thiol and thiolacetate groups were determined.

2. The ^{13}C resonances for n-heptane and several octanoic acid derivatives were measured and assigned.

3. The chemical shift values expected for the lipoic acid derivatives were calculated using 1. and 2.

This preliminary data, the ^{1}H-coupled and ^{1}H-decoupled ^{13}C NMR spectra of I-VI, and the relaxation studies of I-III were used to assign unambiguously the ^{13}C resonances for the lipoic acid derivatives (I-VI).

RESULTS

Thiols and Thiolacetates

The ^{1}H-coupled and ^{1}H-decoupled spectra of 1-thiobutane, 2-thiobutane, 1,3-dithiopropane, 1-thiolacetylbutane, 2-thiolacetylbutane, and 1,3-dithiolacetylpropane were measured and resonance assignments made (Table 1). Substituent effect parameters for primary and secondary thiol and thiolacetate groups were determined from these resonance assignments (Table 6).

Octanoic Acid Derivatives

The ^{1}H-coupled and ^{1}H-decoupled ^{13}C spectra of octanoic acid, octanamide, octanoyl chloride, and methyl octanoate were measured and resonance assignments made (Table 2). The T_1 relaxation times of octanoic acid and octanamide were also measured to aid in assignment (Table 5). From the resonance assignments of the octanoic acid derivatives, substituent effects for $-CO_2H$, $-CONH_2$, -COCl, and -COOMe groups were also calculated (Table 3).

Lipoic Acid Derivatives

The ^{1}H-coupled and ^{1}H-decoupled ^{13}C spectra of lipoic acid (I), lipoamide (II), dihydrolipoamide (III), 6-S-acetyl-6,8-dithioloctanamide (IV), 8-S-acetyl-6,8-dithioloctanamide (V), and methyl 6,8-S-diacetyl-6,8-dithioloctanoate (VI) were measured and resonance assignments made (Table 4). The T_1 relaxation times of lipoic acid (I), lipoamide (II), and dihydrolipoamide (III) were also measured (Table 5) to provide complete resonance assignment for the lipoic acid derivatives (I-VI).

TABLE I

^{13}C Chemical Shifts of Alkylthiols & Alkylthiolacetates[a]

compound	δ_1	δ_2	δ_3	δ_4	$\delta_{C=O}$	δ_{CH_3}
1-thiobutane	23.71	35.70	21.00	12.94	-	-
1-thiolacetylbutane	27.98	31.08	21.27	12.81	193.50	29.54
2-thiobutane	24.70	36.69	33.45	11.33	-	-
2-thiolacetylbutane	20.70	41.00	29.40	11.33	195.64	30.67
1,3-dithiopropane	22.21	36.51	22.21	-	-	-
1,3-dithiolacetylpropane	27.56	29.19	27.56	-	194.64	30.25

[a]In ppm from TMS, converted from $CDCl_3$ at 76.90 ppm (9).

TABLE 2

^{13}C Chemical Shifts of Octanoic Acid Derivatives[a]

compound	δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7	δ_8
octanoic acid	180.33	33.83	24.53	28.86	28.86	31.57	22.54	13.79
octanoyl chloride[b]	173.19	46.95	25.00	28.63	28.31	31.43	22.44	13.86
octanamide	175.93	35.87	25.48	29.10	28.87	31.55	22.48	13.93
methyl octanoate[b,c]	173.40	33.69	24.67	28.84	28.64	31.38	22.28	13.64

[a] In ppm from TMS, converted from $CDCl_3$ at 76.90 ppm (9).

[b] C-4 and C-5 may require reverse assignment.

[c] δ_{OCH_3} = 50.79 ppm.

TABLE 3

Substituent Effects[a] on ^{13}C Chemical Shifts in Octanoic Acid Derivatives

compound	$\Delta\delta_1$	$\Delta\delta_2$	$\Delta\delta_3$	$\Delta\delta_4$	$\Delta\delta_5$	$\Delta\delta_6$	$\Delta\delta_7$	$\Delta\delta_8$
octanoic acid	-	+19.75	+1.58	-3.34	-0.48	-0.63	-0.41	-0.29
octanoyl chloride	-	+32.87	+2.05	-3.63	-1.03	-0.77	-0.51	-0.22
octanamide	-	+21.79	+2.53	-3.10	-0.47	-0.65	-0.47	-0.15
methyl octanoate	-	+19.61	+1.72	-3.44	-0.70	-0.88	-0.67	-0.44

[a]Defined as δ(octanoic acid derivative) - δ(n-heptane). For n-heptane δ_1 14.08, δ_2 22.95, δ_3 32.20, δ_4 29.34. (10).

TABLE 4

^{13}C Chemical Shifts of Lipoic Acid Derivatives[a]

compound		δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7	δ_8
lipoic acid (I)		180.22	33.62	24.23	28.43	34.38	56.00	39.81	38.19
lipoamide (II)		175.09	35.49	25.07	28.72	34.52	56.26	40.13	38.37
dihydrolipoamide (III)	(obs)	175.26	35.55	24.95	26.47	38.58	39.19	42.61	22.15
	(calc)		35.87	25.22	27.23	38.59	39.24	43.16	22.57
8-acetyl dihydro-lipoamide (V)	(obs)	175.41	35.35	24.81	26.57	38.39	39.61	38.18	26.27
	(calc)		35.55	24.95	26.47	38.45	39.46	37.99	26.42
6-acetyl dihydro-lipoamide (IV)	(obs)		35.06	24.61	25.75	34.04	42.94	38.56	21.49
	(calc)		35.55	24.95	26.47	34.58	43.50	38.61	22.15
methyl 6,8-diacetyllipoate (VI)	(obs)	173.49	33.71	24.52	26.10	34.61	43.39	34.32	26.41
	(calc)		33.37	24.04	26.21	34.45	43.23	33.99	26.42

[a] In ppm from TMS, converted from $CDCl_3$ at 76.90 ppm (9).

[b] For the 8-Ac $\delta_{C=O}$ = 195.23, δ_{CH_3} = 30.36

[c] For the 6-Ac $\delta_{C=O}$ = 194.85, δ_{CH_3} = 30.52

[d] For the 8-Ac $\delta_{C=O}$ = 195.11, δ_{CH_3} = 30.44; for the 6-Ac $\delta_{C=O}$ = 194.97, δ_{CH_3} = 30.65 and δ_{OCH_3} = 51.31

TABLE 5

nT_1 Relaxation Times[a] of

Octanoic Acid Derivatives

compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
lipoic acid (I)	11.00	1.98	2.44	2.68	2.92	2.85	5.76	8.18
lipoamide (II)	20.43[b]	4.20	4.14	4.00	4.22	4.12	7.70	10.22
dihydro-lipoamide (III)		3.76	3.68	3.54	3.48	3.23	3.82	4.72
octanoic acid	15.63	2.62	3.46	3.96	4.81	6.16	7.94	13.35
octanamide	22.27[b]	3.70	4.10	4.58	5.18	6.64	8.20	13.74

[a] All measurements in $CDCl_3$. T_1's measured by the Inversion-Recovery method are reported in seconds and are accurate to $\pm$5%. (n=number of hydrogens attached to C).

[b] Determined by Homospoil Method.

TABLE 6

Substituent Effects[a] (ppm) of 1° and 2° Thiol,

Hydroxyl, Thiolacetyl, and Acetyl Groups

carbon position	1°-SH	1°-OH[b]	1°-SAc	1°-OAc[b]	2°-SH	2°-OH[b]	2°-SAc	2°-OAc[e]
α	+10.5	+48.3	+14.8	+51.6	+11.7	+44.5	+16.0	+46.5
β	+10.7	+10.2	+6.1	+6.2	+11.5[c] +8.5[d]	+9.7[c] +7.4[d]	+7.5[c] +4.4[d]	+6.6[c] +3.1[d]
γ	-4.0	-5.8	-3.7	-5.3	-1.9	-3.3	-1.9	-4.4
δ	-0.3	+0.3	-0.4	+0.9	-	+0.2	-	-

[a] Defined as δ(thiol, alcohol, thiolacetate, or acetate) - δ(n-butane). For n-butane δ, 13.20, δ_2 25.00. (10

[b] (10)

[c] C-1

[d] C-3

[e] For sec-butylacetate δ_1 18.61, δ_2 71.47, δ_3 28.11, δ_4 8.85, $\delta_{C=O}$ 175.20, δ_{CH_3} 20.33.

DISCUSSION

Thiols and Thiolacetates

Substituent effect parameters for thiol and thiolacetate groups were needed to calculate the expected δ's for several lipoic acid derivatives. Since these parameters were not available in the literature, they had to be determined for this study. Four model compounds; 1-thiobutane, 1-thiolacetylbutane, 2-thiobutane, and 2-thiolacetylbutane, were used to determine the primary and secondary -SH and -S-acetyl substituent effects.

The ^{13}C resonances of 1-thiobutane and 1-thiolacetylbutane were assigned from the ^{1}H-decoupled and ^{1}H-coupled ^{13}C spectra. Gated decoupling was used to obtain an exact $^{1}J_{CH}$ value instead of off-resonance decoupling which gives the multiplicity but not the $^{1}J_{CH}$. The C-1 resonance of each was assigned from the observed coupling constant $^{1}J_{CH}$ = 138±2 Hz. This value is typical of sulfur-substituted carbons.[8] For the C-2, C-3, and C-4 resonances of each, typical coupling constants of nonheteroatom-substituted carbons, $^{1}J_{CH}$ = 126±2 Hz, were observed.[8] The C-4 resonances were identified as quartets. The C-2 and C-3 resonances, both triplets, were distinguished by comparison to the chemical shifts of 1-butanol and 1-butylacetate.[8] The basis for this assignment was the assumption that the β, γ, and δ substituent effects of a 1° -SH and a 1° -S-acetyl group would be similar to the effects of a 1° -OH and 1° -O-acetyl group, respectively. It is also observed that the α-effects of the thiol and thiolacetate groups are in the same direction but substantially smaller than the effects of the hydroxy and acetate groups.

The α, β, γ, and δ substituent effect parameters for a primary -SH or -S-acetyl group were determined from the relationship, $\Delta\delta = (\delta_{\text{thiol or thiolacetate}} - \delta_{\text{n-butane}})$. To ensure that our substituent effect parameters were

correct, the ^{1}H-decoupled and ^{1}H-coupled ^{13}C spectra of 1,3-dithiopropane and 1,3-dithiolacetylpropane were measured. The chemical shifts obtained were compared with those calculated by adding the 1°-SH or 1°-S-acetyl substituent effects to the δ's of propane[8] and agreed within ±1.0 ppm. The 1°-SH effects were also used to calculate expected δ's for 1,2-dithiobutane and 1-thiooctane. These calculated values were found to agree within ±1.0 ppm with the observed values.[9]

The ^{13}C resonances of 2-thiobutane and 2-thiolacetylbutane were also assigned from the ^{1}H-decoupled and ^{1}H-coupled ^{13}C spectra. The C-2 resonance of both was observed as a doublet with $^{1}J_{CH}$ of 138±2 Hz. The C-3 resonance of each was the only triplet in the coupled spectrum. The C-1 and C-4 resonances, both quartets, were assigned by comparison to the chemical shifts of 2-butanol and 2-butylacetate.[8] The α, β, γ and δ substituent effect parameters for 2°-SH and 2°-S-acetyl groups were determined from the same Δδ relationship as the 1° effects.

The primary and secondary thiol and thiolacetate substituent effect parameters that were used to calculate the chemical shifts for the lipoic acid derivatives are reported in Table 6. For comparison, this table also lists the primary and secondary hydroxyl and acetyl group effects.

Octanoic Acid Derivatives

The chemical shifts of the octanoic acid derivatives were used as a basis for calculating the δ's of the lipoic acid derivatives. These values were used rather than the $-CO_2H$, $-CONH_2$, and -COMe substituent effect parameters already reported in the literature[8] for two reasons. First, the data for the octanoic acid derivatives were obtained under the same spectral conditions as the lipoic acid derivatives. Secondly, the octanoic acid derivatives are molecules of the same chain length as the lipoic acid derivatives. For these reasons, the calculated δ's for the lipoic acid derivatives were expected to be more accurate and self-consistent.

The octanoic acid resonances were assigned from T_1 measurements. The T_1 values of octanoic acid in Table 5 exhibit the segmented motion expected from a long straight-chain molecule with a hydrogen-bonded end. This requires that the nT_1 values uniformly decrease as one proceeds toward the hydrogen-bonded end. A further check on these assignments was made by comparing the observed octanoic acid δ's to δ's calculated from n-heptane chemical shifts (Table 3) and $-CO_2H$ substituent effects.[8]

The octanamide resonances were also assigned on the basis of T_1 measurements. The T_1 values of octanamide also exhibited segmented motion due to hydrogen bonding. A comparison of the octanamide assignments to the octanoic acid assignments added further verification. The C-5 through C-8 resonances of octanamide and octanoic acid were practically identical, as would be expected. The C-1 through C-4 resonances of each differed by the amount expected when substituting a $-CONH_2$ group for a $-CO_2H$ group.

The resonance assignment of octanoyl chloride and methyl octanoate followed readily from the octanoic acid and octanamide assignments. The only significant changes in δ's of these octanoic acid derivatives were observed in the C-1, C-2,

and C-3 resonances (see Table 2). It should be noted that the C-4 and C-5 resonances of octanoyl chloride and methyl octanoate are very close and may require reverse assignment.

Lipoic Acid Derivatives

With the substituent effect parameters for -SH and the complete assignment of the octanamide resonances now available, a calculation of the predicted ^{13}C spectra of dihydrolipoamide (III) could be made. The ^{1}H-coupled ^{13}C spectra of dihydrolipoamide was used to unequivocally assign the C-6 and C-8 resonances. The C-6 resonance was identified as the only doublet and also had $^{1}J_{CH}$ = 138±2 Hz. The C-8 resonance was identified as a triplet with $^{1}J_{CH}$ = 138±2 Hz. All other resonances appeared as triplets with $^{1}J_{CH}$ = 126±2 Hz. These remaining resonances were assigned by a comparison of the observed and calculated δ's. The calculated and observed values shown in Table 4 indicate clearly that all the carbons of dihydrolipoamide (III) can be assigned unambiguously.

The C-6 and C-8 resonances of 6-S-acetyl-6,8-dithioloctanamide (IV), 8-S-acetyl-6,8-dithioloctanamide (V), and methyl 6,8-S-diacetyl-6,8-dithiol-octanoate (VI) were also assigned from the $^{1}J_{CH}$ values of 138±2 Hz. The remaining resonances of the S-acetyl derivatives were assigned by a comparison to calculated δ values. These were determined by adding the appropriate $(\delta_{-SAC} - \delta_{-SH})$ factor to the observed δ values of dihydrolipoamide. The calculated δ values for the 6,8-S-diacetyl derivative were further corrected by a $(\delta_{\text{methyl octanoate}} - \delta_{\text{octanamide}})$ factor. Table 4 shows the close agreement between the assigned and calculated chemical shift values for all the S-acetyl derivatives.

The lipoic acid (I) resonance assignments were deduced from T_1 measurements. The assignments were made in accordance with the segmented motion expected from

a long-chain molecule with a hydrogen-bonded end (see Table 5). The $^{1}J_{CH}$ values of 138±2 Hz for the C-6 and C-8 resonances obtained from the ^{1}H-coupled ^{13}C spectra also aided in the assignment.

The assignment of the lipoamide (II) resonances could not be made from the T_1 values because segmented motion was not observed. The C-6 and C-8 resonances could be readily assigned from the $^{1}J_{CH}$ of 138±2 Hz. The remaining five resonances of lipoamide were assigned by comparison to both the lipoic acid and dihydrolipoamide assignments. It was anticipated that the chemical shifts of the C-2 and C-3 carbons of lipoamide would not differ much from the dihydrolipoamide δ's. For this reason, δ_C = 35.49 ppm and δ_C = 25.07 ppm were assigned to C-2 and C-3, respectively. Similarly, the change in chemical shift of C-4, C-5, and C-7 on conversion of lipoic acid to lipoamide should be negligible. Therefore, δ's of 28.72, 34.52, and 40.13 ppm were assigned to the C-4, C-5, and C-7 carbons of lipoamide, respectively, to complete the assignment. The close agreement (±0.3 ppm) of the δ values in Table 4 supports these assignments.

A comparison of the ^{13}C chemical shifts of lipoamide (II) and dihydrolipoamide (III) in Table 4 illustrates the dramatic effect that the change from a cyclic to an open chain form has on the C-6 and C-8 δ's. Upfield shifts of 17.07 and 16.22 ppm are observed in the C-6 and C-8 chemical shifts, respectively, on conversion of lipoamide to dihydrolipoamide. The effects of acetylation of the C-6 or C-8 thiol group of dihydrolipoamide on the C-6 or C-8 chemical shifts are also significant, though not as large. Acetylation of the C-6 thiol group causes a downfield shift of 3.75 ppm in the C-6 resonance. Acetylation at the C-8 thiol group results in a downfield shift of 4.12 ppm in the C-8 resonance. These changes are significant enough to distinguish between the acetyl derivatives (IV, V, and VI) of dihydrolipoamide and provide encouragement for further studies on the enzymatic interconversion between the oxidized (II), reduced (III), and acetylated (IV-VI) forms of lipoamide.

SUMMARY

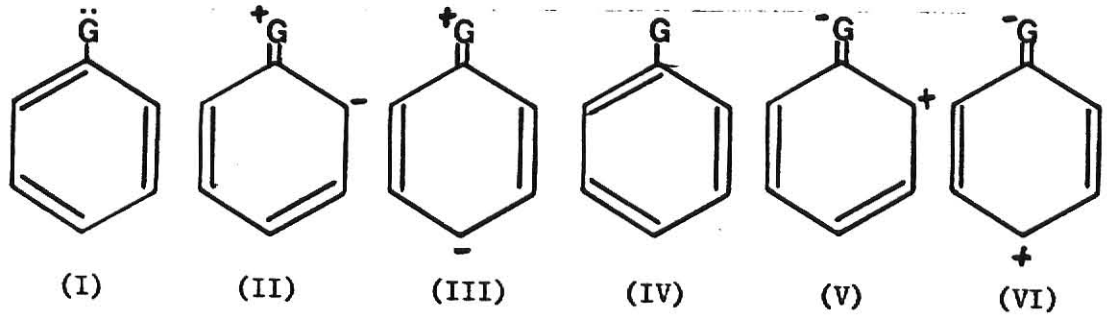
The results of this study have two important and useful consequences. First, the substituent effect parameters of 1° and 2° -SH and -S-acetyl groups, not previously found in the literature, should prove useful in other studies of thiols and thiolacetates. Second, the ^{13}C resonance assignment of the lipoic acid derivatives (I-VI) will be used in subsequent studies on the role of lipoic acid in biochemical systems.

PART II
BIPHENYLS

INTRODUCTION

The ^{13}C resonance assignment of a series of biphenyls was undertaken. The approach to assigning these resonances was to treat the biphenyls as substituted benzenes. Many ^{13}C studies have involved examinations of the spectra of substituted benzenes.[10-15] These studies indicated that the shielding effects of substituents tend to follow an additive relationship in polysubstituted systems, provided the groups are not ortho. The ^{13}C substituent effects for a number of substituted benzenes have been collected from available data and are listed in Table 7. From these results it is apparent that the carbon bonded to the substituent is most affected, +39.6 to -32.0 ppm relative to benzene. The carbons meta to substituents are hardly affected, +2.9 to -1.5 ppm, while the ortho and para carbons exhibit appreciable shifts, +10.2 to -15.6 ppm.

Resonance theory accounts for both the magnitude and direction of the para and meta substituent effects quite reasonably. The resonance structures I-III show the electronic effects of electron donating groups on the ortho, meta, and para positions. The electronic effects of electron withdrawing groups are illustrated in resonance structures IV-VI.



(I) (II) (III) (IV) (V) (VI)

TABLE 7

^{13}C Substituent Effects[a] of Substituted Benzenes

substituent	C-1	o	m	p	reference
$-NH_2$	+18.0	-13.3	+0.9	-9.8	b
$-NH_3^+$	+2.0	-4.8	+2.4	+1.5	d
$-NMe_2$	+22.6	-15.6	+1.0	-11.5	c
-NHAc	+9.3	-8.4	-0.1	-4.7	d
$-NO_2$	+20.0	-4.8	+0.9	+5.8	b
-OH	+26.9	-12.7	+1.4	-7.3	b
$-O^-$	+39.6	-8.2	+1.9	-13.6	c
$-OCH_3$	+31.4	-14.4	+1.0	-7.7	b
-OPh	+29.2	-9.4	+1.6	-5.1	c
-OAc	+23.0	-6.4	+1.3	-2.3	c
$-CO_2H$	+2.1	+1.5	+0.0	+5.1	b
-COCl	+4.6	+2.4	+0.0	+6.2	b
$-CO_2Me$	+1.3	-0.5	-0.5	+3.5	c
-CHO	+8.6	+1.3	+0.6	+5.5	b
$-COCH_3$	+9.1	+0.1	+0.0	+4.2	b
-COPh	+9.4	+1.7	-0.2	+3.6	b
$-CH_2OH$	+11.7	-2.3	-1.0	-2.0	d
$-C\equiv N$	-15.4	+3.6	+0.6	+3.9	b
$-C\equiv CH$	-6.1	+3.8	+0.4	-0.2	b
$-CH=CH_2$	+9.5	-2.0	+0.2	-0.5	c
$-CH_3$	+8.9	+0.7	-0.1	-2.9	b
-Ph	+13.1	-1.1	+0.4	-1.2	b

TABLE 7 (continued)

^{13}C Substituent Effects[a] of Substituted Benzenes

substituent	C-1	o	m	p	reference
-F	+34.8	-12.9	+1.4	-4.5	b
-Cl	+6.2	+0.4	+1.3	-1.9	b
-Br	-5.5	+3.4	+1.7	-1.6	b
-I	-32.0	+10.2	+2.9	+1.0	c
$-SO_3H$	+14.7	-2.3	+1.3	+3.8	d
$-SO_2Cl$	+15.0	-2.2	+0.8	+6.5	d
-SH	+1.9	+0.5	+0.1	-3.4	d

[a] Defined as $(\delta_C - \delta_{benzene})$, $\delta_{benzene}$ = 128.5 ppm.

[b] (reference 3)

[c] (reference 8)

[d] Determined by this study.

Electron donating groups such as $-NH_2$, -OH, $-NHCOCH_3$, $-OCH_3$, $-CH_3$, and -Ph tend to increase electron density at the para carbon (structure III). More electron density at a carbon results in increased shielding of that carbon relative to benzene and an upfield shift in δ. For this reason, the para substituent effects of electron donating groups are negative.

For electron withdrawing groups as $-NO_2$, -CN, $-CO_2H$, and $-SO_3H$, the resonance form VI becomes important. This resonance form shows a decrease of electron density at the carbon para to the substituent. This results in a net deshielding of that carbon relative to benzene and a downfield shift. Thus, a positive para substituent effect is observed for electron withdrawing groups.

The resonance structures I-VI also predict that neither electron withdrawing nor electron donating groups will have any significant electronic effect on the carbon meta to the substituent. This prediction is verified by the very small meta substituent effects observed for all substituted benzenes.

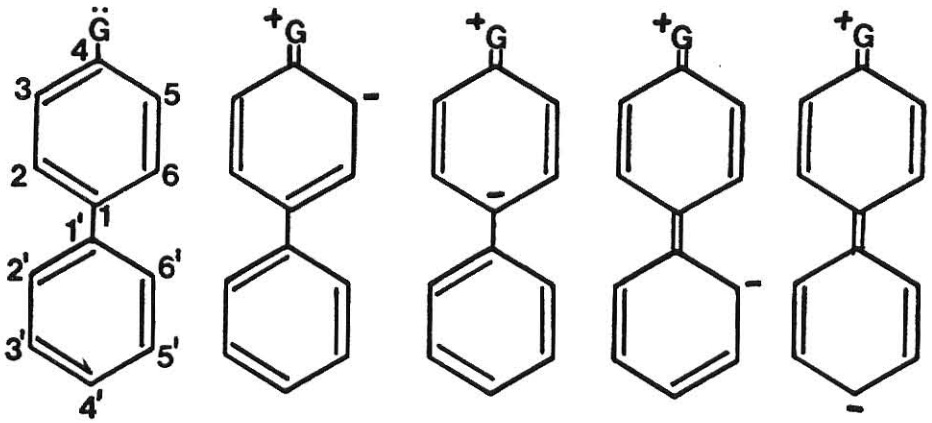
According to resonance theory, the substituent effects on the ortho carbons are expected to be quite similar to the para effects (structures II and V). Ortho carbons, however, absorb over a wider range than the para nuclei. This implies that additional perturbations are operating for ortho nuclei. In fact, for the $-NO_2$, -Br, and -Cl substituents, the shifts relative to benzene are opposite for the ortho and para carbons. In general, the ortho carbons are shielded relative to their para counterparts. Although a detailed interpretation of the trends in the ortho position has not been made, the additivity of ortho substituent effects still remains valid and useful.

Predicting chemical shifts for substituted benzenes becomes a problem in ortho-substituted systems because additivity is not generally observed. This suggests that steric interference between neighboring groups upsets their normal interactions with the ring. The deviatiions from additivity may even provide a measure of the steric hindrance.

Several cases have been found in which the usual additivity of substituent effects failed to predict the aryl shieldings in o-disubstituted aromatic systems. In o-substituted nitrobenzenes and N,N-dimethylanilines,[10,13] the additivity relation breaks down for both the substituted and para carbons. This indicates that resonance structures such as III and VI are less important than in the meta and para substituted isomers for which additivity is observed. It was also found that the carbonyl carbon shieldings in several substituted acetophenones are sensitive to the nature of ortho substituents, but are essentially independent of a wide variety of polar groups in either the meta

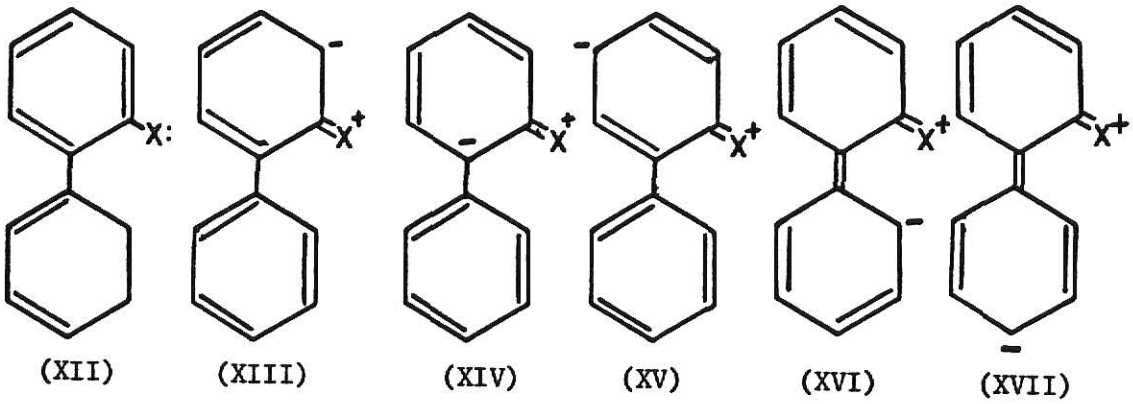
or para positions.[16,17] The simplest interpretation of these observations attributes the trend to steric inhibition of resonance. Substituents in the ortho positions prevent π systems from becoming coplanar and thus prevent full conjugation.

All this information about substituted benzenes can be immediately applied to substituted biphenyls because they are, of course, substituted benzenes. Resonance theory, however, suggests an extra interesting feature that may be observed in the ^{13}C shieldings. If the biphenyl is substituted at the 4-position of one ring, the normal substituent effects are expected at positions 1 through 6 (structures VIII and IX). In addition, secondary ring effects are expected for the 2', 4', and 6' positions (structures X and XI).



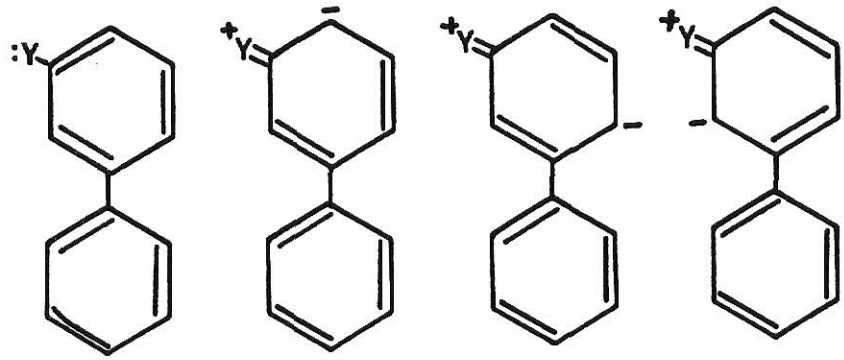
(VII) (VIII) (IX) (X) (XI)

According to resonance theory, substitution at the 2 position should result in the same electronic effects in both rings as 4-substitution (structures XII-XVII). Observation of the secondary ring effects in 2-substituted biphenyls, however, would be complicated by steric effects.



(XII) (XIII) (XIV) (XV) (XVI) (XVII)

Substitution at the 3-position of biphenyl should not result in any significant secondary ring effects according to resonance theory (structures XVIII-XXI).



(XVIII) (XIX) (XX) (XXI)

It would be interesting to note if any secondary ring effects are actually observed in a series of substituted biphenyls. If the secondary ring effects are observed it may be useful to determine whether they are consistent or unpredictable.

OBJECTIVE

To assign the ^{13}C resonances of a series of substituted biphenyls and to determine if any secondary ring effects are observed.

APPROACH

To accomplish these objectives:

1. The expected ^{13}C spectra of several substituted biphenyls were calculated using the biphenyl δ's and the substituent effects from Table 7.

2. The ^{1}H-decoupled ^{13}C spectra of the biphenyls were measured. The ^{1}H-coupled ^{13}C spectra of several of these were also measured.

3. Resonance assignments were made from calculated values, peak intensities, and multiplicities.

RESULTS

The ^{1}H-decoupled ^{13}C spectra of several monosubstituted benzenes and several substituted biphenyls were measured. The resonance assignments of the monosubstituted benzenes are listed in Table 8. Chemical shift assignments for several 4,4'-substituted biphenyls are reported in Table 9. The incomplete assignments of various 2,2'-substituted biphenyls are listed in Table 10. And the ^{13}C resonance assignments of several other biphenyls are found in Tables 11 and 12.

TABLE 8

^{13}C Chemical Shifts[a] of Monosubstituted Benzenes

compound	δ_1	δ_2	δ_3	δ_4
benzenesulfonic acid	143.15	126.14	129.78	132.33
benzenesulfonyl chloride	143.55	126.30	129.34	135.00
thiophenol	130.40	128.93	128.59	125.08
acetanilide[b]	137.80	120.06	128.35	123.83
benzyl alcohol[c]	140.19	126.16	127.50	126.45
anilinium hydrochloride	130.55	123.74	130.90	130.01

[a] In ppm from TMS.

[b] $\delta_{C=O}$ 169.11, δ_{CH_3} 23.89.

[c] δ_{CH_2} 63.59.

TABLE 9

^{13}C Chemical Shifts[a] of 4,4'-Substituted Biphenyls

compound		1,1'	2,2'	3,3'	4,4'
biphenyl	(obs)	140.90	126.83	128.47	126.95
	(calc)[c]	141.6	127.4	128.9	127.3
4,4'-dinitrobiphenyl	(obs)	144.79	128.15	124.20	147.94
	(calc)[d]	146.7	127.7	123.7	147.0
4,4'-diaminobiphenyl	(obs)	131.46	126.97	115.21	144.71
	(calc)[d]	131.1	127.7	115.2	145.0
benzidine hydrochloride	(obs)	138.43	127.89	123.79	131.63
	(calc)[d]	142.4	129.2	123.7	129.0
4,4'-diacetamidobiphenyl[b]	(obs)	138.23	126.21	119.23	134.15
	(calc)[d]	136.2	126.7	120.1	136.3

[a] In ppm from TMS.

[b] $\delta_{C=O}$ 168.00, δ_{CH_3} 24.05.

[c] Determined as ($\delta_{benzene}$ + phenyl substituent effects).

[d] Determined as ($\delta_{biphenyl(obs)}$ + substituent effects).

TABLE 10

^{13}C Chemical Shifts[a] of 2,2'-Substituted Biphenyls

compounds		1,1'	2,2'	3,3'	4,4'	5,5'	6,6'
2,2'-biphenyldicarboxylic acid[b]	(obs)[d]	143.82	129.96	130.48	126.98	131.27	129.96
	(calc)[e]	142.4	128.9	130.0	127.0	133.6	126.8
2,2'-biphenyldisulfonic acid	(obs)[d]	138.39	141.11	127.80	128.65	132.43	130.78
	(calc)[e]	138.6	141.5	126.2	128.3	132.3	128.1
2,2'-biphenyldisulfonyl chloride	(obs)[d]	135.66	142.61	128.88	131.91	134.25	129.78
	(calc)[e]	138.7	141.9	126.3	127.8	135.0	127.6
2,2'-biphenyldithiol	(obs)[d]	138.83	131.56	130.05	129.21	125.58	128.46
	(calc)[e]	141.0	128.7	128.6	127.5	125.1	127.3
2,2'-dihydroxymethylbiphenyl[c]	(obs)[d]	139.73	138.42	129.41	129.26	127.81	127.35
	(calc)[e]	138.6	138.5	126.2	126.0	126.5	125.8

[a] In ppm from TMS.

[b] $\delta_{C=O}$ 167.46.

[c] δ_{CH_2} 62.54.

[d] Assignments are tentative.

[e] Determined as ($\delta_{biphenyl(obs)}$ + substituent effects).

TABLE 11

^{13}C Chemical Shifts[a] of Substituted Biphenyls

compound		1,1'	2,2'	3,3'	4,4'	5,5'	6,6'
o-tolidine[b]	(obs)	131.63	128.17	122.18	142.81	115.00	124.58
	(calc)[c]	131.0	128.4	124.1	145.7	115.1	124.8
4,4'-diamino-3,3'-dichloro-biphenyl	(obs)	131.14	126.91	119.50	141.47	115.94	125.40
	(calc)[c]	132.4	128.1	121.4	145.4	116.5	125.8
4,4'-diamino-2,2'-biphenyl-disulfonic acid	(obs)	129.43	142.18	115.31	145.98	118.54	134.07
	(calc)[c]	128.8	142.4	112.9	146.3	119.0	129.0

[a] In ppm from TMS.

[b] δ_{CH_3} 17.31.

[c] Determined as ($\delta_{biphenyl(obs)}$ + substituent effects).

TABLE 12

^{13}C Chemical Shifts[a] of Substituted Biphenyls

compound		1	2	3	4	5	6	1'	2'	3'	4'
3-chloro-4-hydroxybiphenyl	(obs)	134.63	127.18	120.01	150.33	116.27	126.75	139.12	126.34	128.52	126.92
	(calc)[d]	134.9	128.6	122.0	152.3	117.1	126.3	140.9	126.8	128.5	127.0
4-amino-3-nitrobiphenyl	(obs)	130.22	123.71	132.37	143.52	119.10	134.24	138.59	126.12	128.75	127.15
	(calc)[d]	132.0	122.9	135.2	140.2	116.1	133.5	140.9	126.8	128.5	127.0
4-amino-3-chlorobiphenyl	(obs)	129.86	135.09	120.82	146.12	120.82	123.88	139.88	126.94	129.86	127.99
	(calc)[d]	132.4	128.1	121.4	145.4	116.5	125.8	140.9	126.8	128.5	127.0
4-bromobiphenyl	(obs)	139.58	128.61	131.55	121.28	-----	-----	139.73	126.59	128.38	127.35
	(calc)[d]	139.3	128.5	131.9	121.5	-----	-----	140.9	126.8	128.5	127.0
3-bromobiphenyl[b]	(obs)	143.7	130.4	123.1	130.4	130.4	125.6	138.8	127.1	129.0	127.9
	(calc)[d]	142.6	130.2	123.0	130.4	130.2	125.2	140.9	126.8	128.5	127.0
3-nitrobiphenyl[b]	(obs)	142.8	121.2	149.3	121.9	130.0	133.0	138.6	127.0	129.2	128.6
	(calc)[d]	141.8	122.0	148.5	122.2	129.4	132.6	140.9	126.8	128.5	127.0
4-amino-4'-nitrobiphenyl	(obs)[c]	128.34	128.25	115.19	145.98	-----	-----	147.28	126.26	123.94	147.33
	(calc)[d]	131.1	127.7	115.2	145.0	-----	-----	146.7	127.7	123.7	147.0

[a] In ppm from TMS.

[b] Reference 1.

[c] Tentative assignments.

[d] Determined as ($\delta_{biphenyl(obs)}$ + substituent effects).

DISCUSSION

Monosubstituted Benzenes (Table 8)

The ^{1}H-decoupled ^{13}C spectra of several monosubstituted benzenes were measured to determine the substituent effects for the $-SO_3H$, $-SO_2Cl$, $-SH$, $-NHAc$, $-NH_3^+$, and $-CH_2OH$ groups. Resonance assignments were made primarily from peak intensities. The C-1 resonances of all the monosubstituted benzenes were the smallest peaks due to their relative lack of NOE. The intensity of the para resonance of each was greater than the C-1 because of NOE, but approximately half that of the ortho and meta resonances as expected from symmetry. The only problem encountered was distinguishing the ortho and meta resonances. These were differentiated by analogy to substituent effect parameters for similar groups. In all cases, the meta effect was considered to be very small, while the ortho effect was expected to be in a direction consistent with the electronic effect of the group. From the resonance assignments of the monosubstituted benzenes in Table 8, substituent effects were determined and listed in Table 7. These substituent effect parameters were subsequently used to calculate the expected chemical shifts for several substituted biphenyls.

4,4'-Substituted Biphenyls (Table 9)

The ^{1}H-decoupled ^{13}C spectra of the 4,4'-substituted biphenyls showed only four carbon resonances for each as expected from the symmetry of the molecules. The peak intensities immediately differentiated the C-1 and C-4 from the C-2 and C-3 resonances. The C-2 and C-3 resonances were considerably more intense than the C-1 and C-4 resonances because the 2 and 3 carbons are twice as abundant and also experience NOE from a directly attached hydrogen. So the problem in assigning the resonances for the 4,4'-substituted biphenyls was to distinguish the C-1 resonance from the C-4 and the C-2 from the C-3. The assignments of the C-2 and C-3 resonances were facilitated by the rather large

chemical shift differences of at least 4ppm and by the small deviations from the calculated values. The C-1 and C-4 resonances of 4,4'-diaminobiphenyl and benzidine hydrochloride were also quite different in chemical shift and were easily distinguished. The C-1 and C-4 resonances of 4,4'-diacetamidobiphenyl and 4,4'-dinitrobiphenyl, however, were too close and varied considerably from the calculated values so that their assignments are questionable.

TABLE 13

Secondary Ring Effects[a] of 4,4'-Substituted Biphenyls

compound	1,1'	2,2'	3,3'	4,4'
4,4'-diaminobiphenyl	+0.4	-0.7	+0.0	-0.3
4,4'-diacetamidobiphenyl	+2.0	-1.5	-0.9	-2.1
4,4'-dinitrobiphenyl	-1.9	+0.5	+0.5	+0.9
benzidine hydrochloride	-4.0	-1.3	+0.0	+2.6

[a] Defined in ppm as $(\delta_{obs} - \delta_{calc})$.

The secondary ring effects of the 4,4'-substituted biphenyls are summarized in Table 13. The $(\delta_{obs} - \delta_{calc})$ values are listed to represent these effects. A positive value indicates that the observed chemical shift is more downfield than expected. A negative value indicates a more upfield shift.

For the electron donating substituents, $-NH_2$ and -NHAc, the C-2 and C-4 resonances are more upfield than expected. This is consistent with resonance theory which predicts increased shielding, relative to a nonsubstituted phenyl ring, at the 2 and 4 carbons. The opposite effect is expected for the electron withdrawing substituents, $-NO_2$ and $-NH_3^+$, that is, a more downfield shift. The $(\delta_{obs} - \delta_{calc})$ values in Table 13 indicate that this general trend is observed

except in the C-2 position of benzidine hydrochloride where a more upfield shift is observed.

The large secondary effects observed at the 1,1' position imply that the electronic effect of a substituted ring considerably differs from the nonsubstituted. Certainly, many more substituents must be investigated to validly generalize the secondary ring effects. A series of electron donating and withdrawing groups should be considered. This small sampling of 4,4'-substituted biphenyls does seem encouraging, since the expected trends are observed in all but one resonance.

2,2'-Substituted Biphenyls (Table 10)

Steric interactions complicated the treatment of the ^{13}C spectra of the 2,2'-substituted biphenyls. For this reason, resonance assignments are tentative and no attempt was made to interpret secondary ring effects.

Other Biphenyls (Tables 11 and 12)

The ^{13}C resonance assignments of ten more substituted biphenyls are summarized in Tables 11 and 12. The secondary ring effects of several of these are not considered because of ortho substitution.

Since no steric effects are expected to complicate the ^{13}C spectra of 4-amino-4'-nitrobiphenyl, the secondary ring effects were investigated. The $(\delta_{obs} - \delta_{calc})$ values for positions 1 through 4 of 4-amino-4'-nitrobiphenyl are -2.7, +0.6, +0.0, and +0.9 ppm, respectively. These values are similar to those observed in Table 13 for 4,4'-dinitrobiphenyl. The $(\delta_{obs} - \delta_{calc})$ values for the 1' through 4' positions of 4-amino-4'-nitrobiphenyl, +0.6, -1.4, +02, and +0.3 ppm, respectively, are somewhat analogous to the values for 4,4'-diaminobiphenyl in Table 13. These similarities give further evidence to the additivity of the secondary ring effects.

For 3-nitrobiphenyl, ($\delta_{obs} - \delta_{calc}$) values of -2.3, +0.2, +0.7, and +1.6 ppm are observed for the 1' through 4' resonances, respectively. These values are also quite similar to the values in Table 13 for 4,4'-dinitrobiphenyl. This seems to indicate that a 3-substituted phenyl group may have the same effect on a secondary ring as a 4-substituted phenyl. The similar ($\delta_{obs} - \delta_{calc}$) values of 3-bromobiphenyl and 4-bromobiphenyl also point to this conclusion. For 4-bromobiphenyl, the ($\delta_{obs} - \delta_{calc}$) values of -0.8, -0.2, -0.1 and +0.4 ppm are observed for the 1' through 4' resonances, respectively. For 3-bromobiphenyl, values of -0.9, +0.3, +0.5, and +0.9 are observed.

SUMMARY

This study provides the resonance assignment of several monosubstituted benzenes and several substituted biphenyls. It also seems to indicate that secondary ring effects are observed for the biphenyls. Any definite conclusions concerning these effects, however, would be premature. The additivity of the secondary ring effects must be verified by the investigation of a complete series of 4-substituted, 4,4'-substituted, 3-substituted, and 3,3'-substituted biphenyls.

EXPERIMENTAL

Carbon-13 NMR spectra were obtained with a Varian XL-100-15 spectrometer operating at a frequency of 25.2 MHz and equpped with Nicolet TT-100 Data System with quadrature phase detection and 20K of memory, allowing 16K data points, 8K points in the frequency domain. All spectra were measured at 35±2°C in deuterated solvents at 15-30% concentrations by weight/volume. The deuterium resonance of the solvent was used as the lock signal. ^{13}C chemical shifts were measured relative to several different standards but are reported relative to external TMS. The conversions to external TMS were done by the values in Table 14. Spectral reproducibility was ±0.05 ppm. T_1's were measured by the inversion-recovery or homogeneity spoiling method.

TABLE 14[18]

^{13}C Shieldings[a,b] of Some Solvents[c] and Reference Materials

solvent	$\delta_C(^1H)$		$\delta_C(^2H)$	
	ppm	Hz	ppm	Hz
acetone	30.43	765.77	29.22	735.32
dimethyl sulfoxide	40.48	1018.68	39.56	995.53
dichloromethane	54.02	1359.41	53.61	1349.10
dioxane	67.40	1696.12	-----	-------
chloroform	77.17	1941.98	76.91	1935.44
carbon tetrachloride	95.99	2415.59	-----	--------
benzene	128.53	3234.46	127.96	3220.11

[a] Relative to TMS at 0.00 ppm, 0.00 Hz.

[b] H = 25.165 MHz.

[c] For CS_2(int) δ_C = 192.8 ppm, 4851.81 Hz; CS_2 (ext) δ_C = 193.7 ppm, 4876 Hz.

Synthesis

1-Thiolacetylbutane. 1-Thiobutane[19], 5 ml., was added dropwise to 25 ml. of acetyl chloride. The solution stood at room temperature for 1 hour. The acetyl chloride was then removed by rotary evaporator and the product distilled b.p. 155°. The ^{13}C δ's are reported in Table 1.

2-Thiolacetylbutane. 2-Thiobutane[19], 5 ml., was added dropwise to 25 ml. of acetyl chloride. The solution stood at room temperature for 1 hour and the acetyl chloride was then removed by a rotary evaporator. The product was distilled, b.p. 145°. The ^{13}C δ's are reported in Table 1.

1,3-Dithiolacetylpropane. 1,3-Dithiopropane[19], 5 ml., was added dropwise to 25 ml. of acetyl chloride. The solution stood at room temperature for 1 hour. The acetyl chloride was removed by rotary eavporator and the product analyzed without further purification. The ^{13}C δ's are reported in Table. No impurities greater than 2% were detected in the ^{13}C spectrum.

Octanamide. Five ml. of octanoyl chloride[20] were added dropwise to 25 ml. of concentrated NH_4OH and stirred for 30 minutes. The solution was poured into ice water. The precipitate was filtered and washed several times with cold water. The dry product was a white solid, m.p. 110°C. The ^{13}C δ's are reported in Table 2.

Methyl Octanoate. Five ml. of octanoyl chloride[20] were added to 25 ml. of MeOH and allowed to stand for 1 hour. The MeOH was removed by a rotary evaporator. The sweet smelling liquid that remained was analyzed without further purification. The ^{13}C δ's are reported in Table 2.

Dihydrolipoamide.[21,22] Lipoamide[23], 200 mg., in 4 ml. of MeOH and 1 ml. of H_2O was stirred on ice and a cool solution of 200 mg. $NaBH_4$ in 1 ml. H_2O was added. The resulting solution was then acidified with dilute HCl and extracted with $CHCl_3$. The $CHCl_3$ extract was dried and the $CHCl_3$ evaporated

in vacuo. The resulting residue was recrystallized from benzene-Skelly B (5:2). The yield of white plates, m.p. 66-67°, was 75%. The ^{13}C δ's are reported in Table 4.

Methyl 6,8-S-diacetyldihydrolipoate.[21,22]

A. Methyl lipoate. 30 ml. of MeOH, 3 g. of lipoic acid[23], and 3 ml. of conc. H_2SO_4 were refluxed for 1 hr. The solution was then extracted with ether. The ether layer was washed once with H_2O, twice with $NaHCO_3$, and once with saturated NaCl. The ether layer was then dried with anhydrous Na_2SO_4 and evaporated in vacuo. This yielded 1.1 g. of a viscous liquid, b.p. 129-31°, (.7mm).

B. Methyl dihydrolipoate. To a cooled solution of 1.1 g. methyl lipoate in 30 ml. of MeOH, a cooled solution of 1 g. $NaBH_4$ in 5 ml. H_2O was slowly added. The solution was then stirred on ice for 1 hour and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried with anhydrous Na_2SO_4, and evaporated in vacuo. The product was a colorless liquid, b.p. 125-28° (.5mm). The yield was approximately 100%.

C. Methyl 6,8-S-diacetyldihydrolipoate. A solution of 1.1 g. methyl dihydrolipoate, 0.71 ml. of pyridine, and 0.71 ml. of acetic anhydride was allowed to stand at room temperature for 48 hours. The solution was then diluted with 10.7 ml. of benzene, washed with water, cold 1% HCl, dilute $NaHCO_3$, and water. The solution was dried and distilled in a Hickman-Still. The ^{13}C δ's are reported in Table 4.

6-S-Acetyldihydrolipoamide and 8-S-acetyldihydrolipoamide.[21,22,24,25] These compounds were synthesized by enzymatic reactions. The ^{13}C δ's are reported in Table 4.

4,4'-Diacetamidobiphenyl.[26] Benzidine dihydrochloride[20], 0.5 g., was dissolved in 25 ml. 5% HCl. A solution of 5% NaOH was added until the solution just turned

cloudy and then 2 ml. of 5% HCl were added to clear the solution. Acetic anhydride, 5 ml., was added and the solution stirred vigorously. White crystals precipitated immediately, m.p. over 300°. The ^{13}C δ's are reported in Table 9.

Disodium salt of 2,2'-biphenyldisulfonic acid.[27] 4,4'-Diamino-2,2'-biphenyldisulfonic acid[20], 40 gm., was dissolved in 350 ml. of 5% NaOH solution. To this solution was added 20 gm. of $NaNO_2$. H_3PO_2 (50%), 250 ml., was placed in a 1 liter beaker equipped with a stirring bar and cooled in a salt-ice bath. The diamine-$NaNO_2$-NaOH solution was then added dropwise from a seperatory funnel. The temperature was maintained at 10-20° throughout the addition. After addition was complete, the solution was stirred for 1 hour and allowed to stand overnight to release gases. The next day the solution was neutralized with Na_2CO_3 to pH 8. The solution was then evaporated to a volume of 300 ml. and cooled in an ice bath without agitation. Yellow needles result. (Scratching the beaker may be necessary to induce crystallization.) Net yields were approximately 40 gm. There is always a small amount of NaH_2PO_2 impurity as observed by ^1H-NMR, 2 peaks at δ = 2.3 and 11.1 ppm. (^{13}C δ's in Table 11)

2,2'-Biphenyldisulfonyl chloride.[28] The disodium salt of 2,2'-biphenyldisulfonic acid, 40 g., was dissolved in 150 ml. of DMF and the solution was filtered to remove NaH_2PO_2. $SOCl_2$, 50 ml., was added dropwise over a period of 15 minutes and the solution was allowed to cool. The solution was then poured into ice water, the resulting white precipitate was collected and washed several times with cold water. The yields were about 30 grams of white product that were recrystallized from $CHCl_3$ as white needles, m.p. 135-7°. The ^{13}C δ's are reported in Table 10.

2,2'-Biphenyldithiol.[29] 2,2'-Biphenyldisulfonyl chloride, 10 g., was suspended in 300 ml. of 33% H_2SO_4 in a 1000 ml. bulb equipped with heating mantel, reflux condenser, drying tube, and magnetic stirrer. Zn dust, 50 g., was added slowly

in small portions to prevent foaming. When addition was complete the solution was refluxed for 6 hours and left to stand overnight. The following day, the solution was filtered through a sintered glass filter. The filtrate was discarded and the solid was extracted several times with 50 ml. portions of diethyl ether. The extracts were combined, dried with anhydrous $NaSO_4$, and filtered. The ether was evaporated and the yellow product was recrystallized from EtOH to yield white needles, 5-6 gm., m.p. 75°. The ^{13}C δ's are reported in Table 10.

4-Amino-4'-nitrobiphenyl.[30] 4,4'-Dinitrobiphenyl[19], 1 g., was dissolved in 100 ml. of 95% EtOH. To this solution was added a solution of 0.5 gm. NaHS, 50 ml. 95% EtOH, and 2 ml. of conc. NH_4OH. The resulting solution was refluxed for 1 hour when the yellow solution turned red-brown. The solution was then cooled. The resulting orange prdouct was collected and recrystallized from 95% EtOH to give orange plates, m.p. 200-203°. The ^{13}C δ's are reported in Table 12.

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ILLEGIBLE DOCUMENT

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THIS IS THE BEST COPY AVAILABLE

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b 23.71t
c 21.00t
d 12.94q

d c a b
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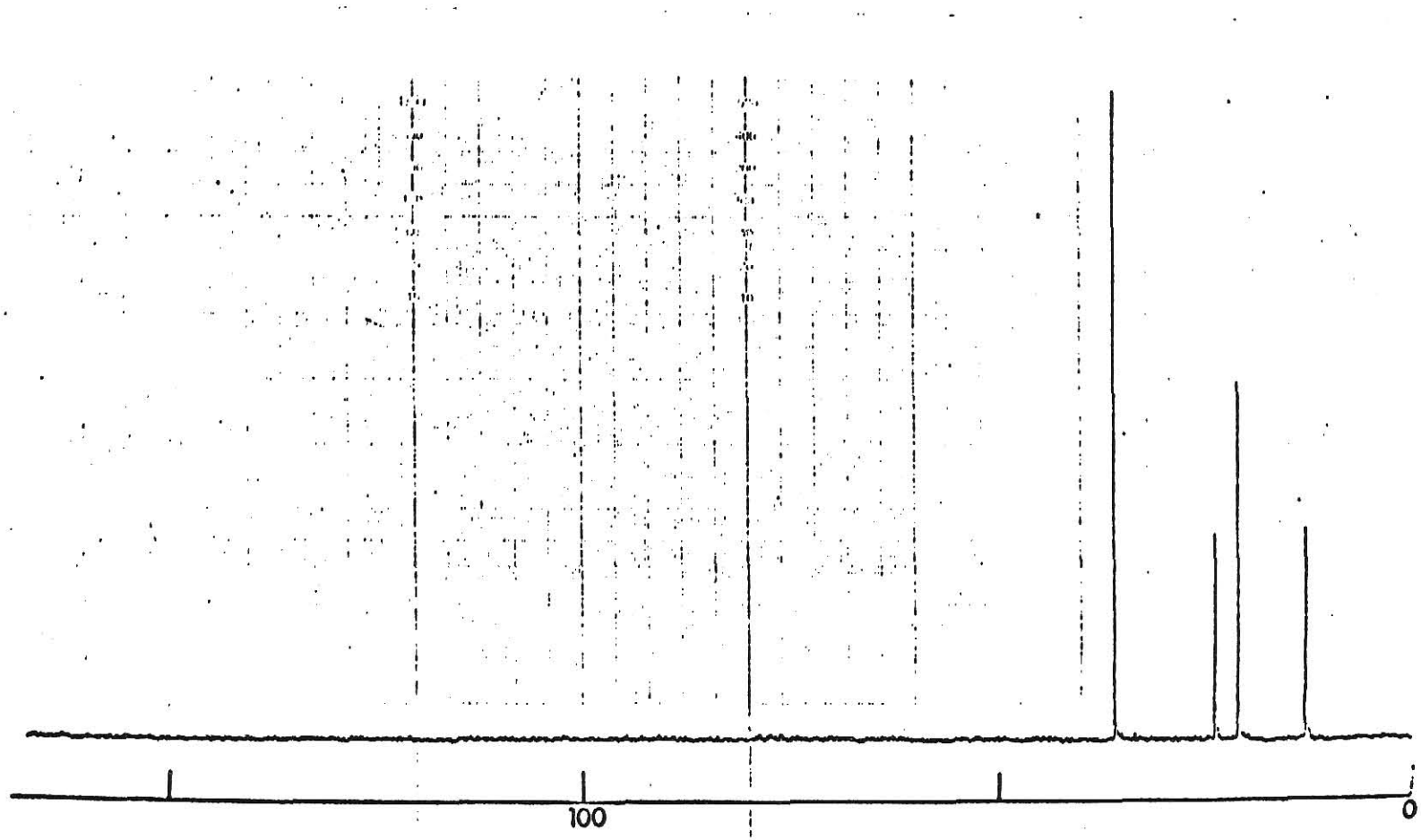
1-BUTANETHIOL

$C_4H_{10}S$

in $CDCl_3$



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e 21.27 t
f 12.81 q

f e b d a c
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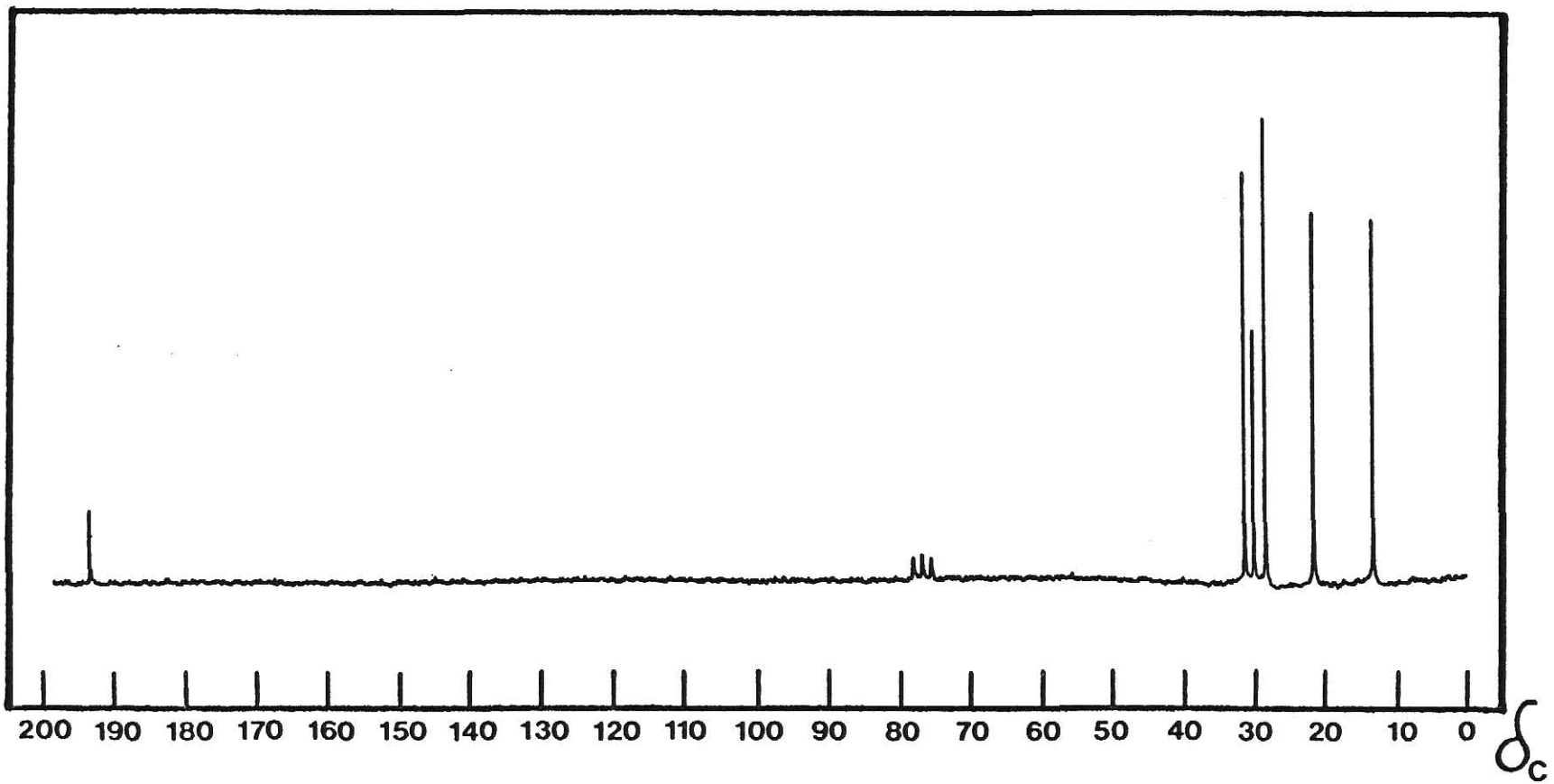
1-THIOLACETYLBUTANE

$C_6H_{12}OS$

in $CDCl_3$



a 36.69d
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c 24.70q
d 11.33q

d b a c
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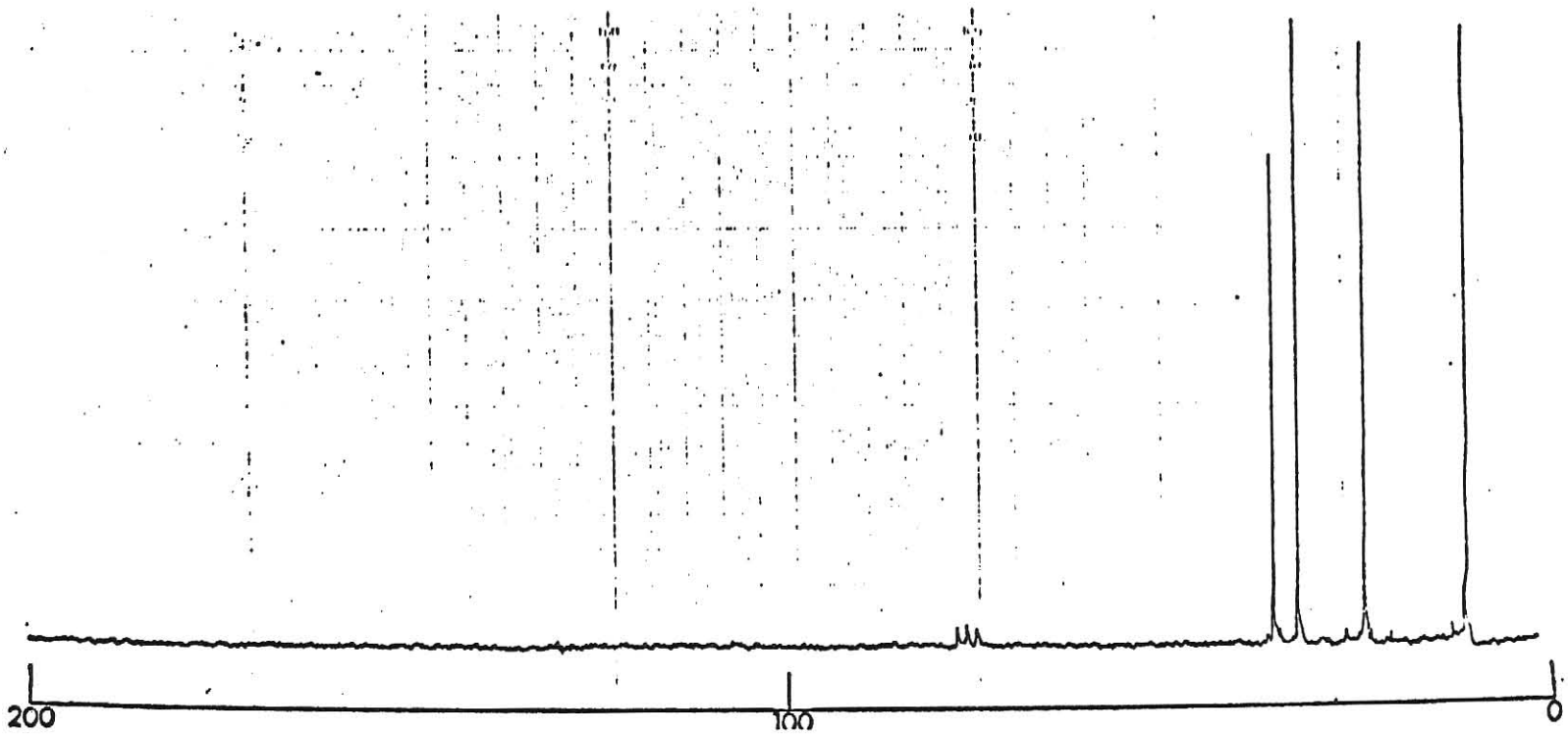
2-BUTANETHIOL

$C_4H_{10}S$

in $CDCl_3$



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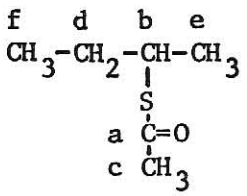
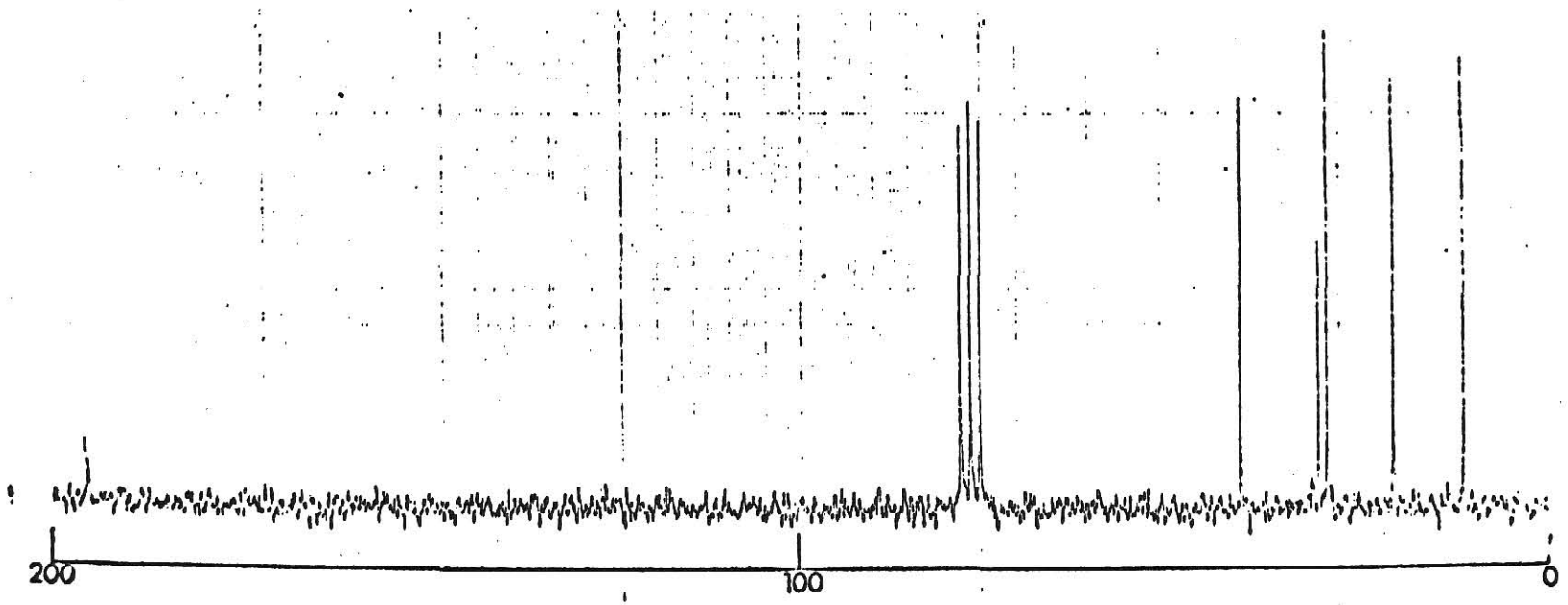


2-THIOACETYLBUTANE

$C_6H_{12}OS$

in $CDCl_3$



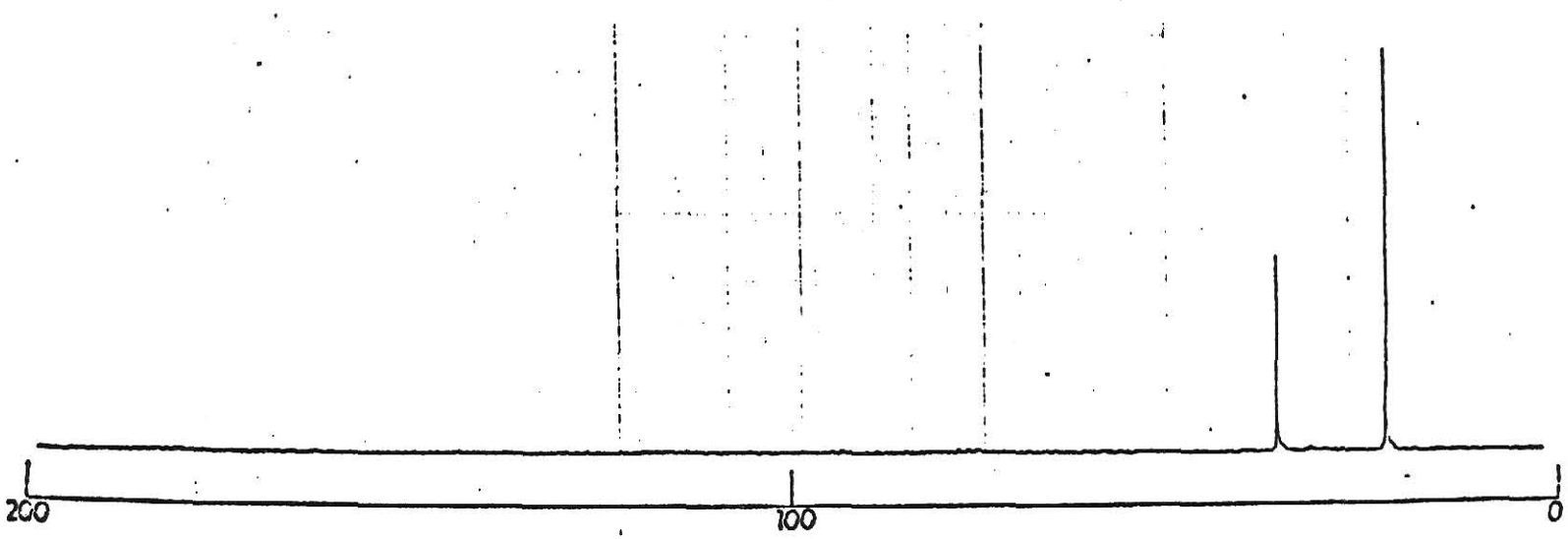
a 36.51t
b 22.21t

b a
HS-CH_2-CH_2-CH_2-SH

1,3-DITHIOPROPANE

$C_3H_8S_2$

in $CDCl_3$



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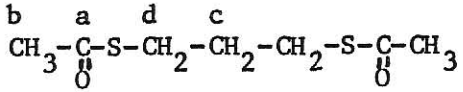
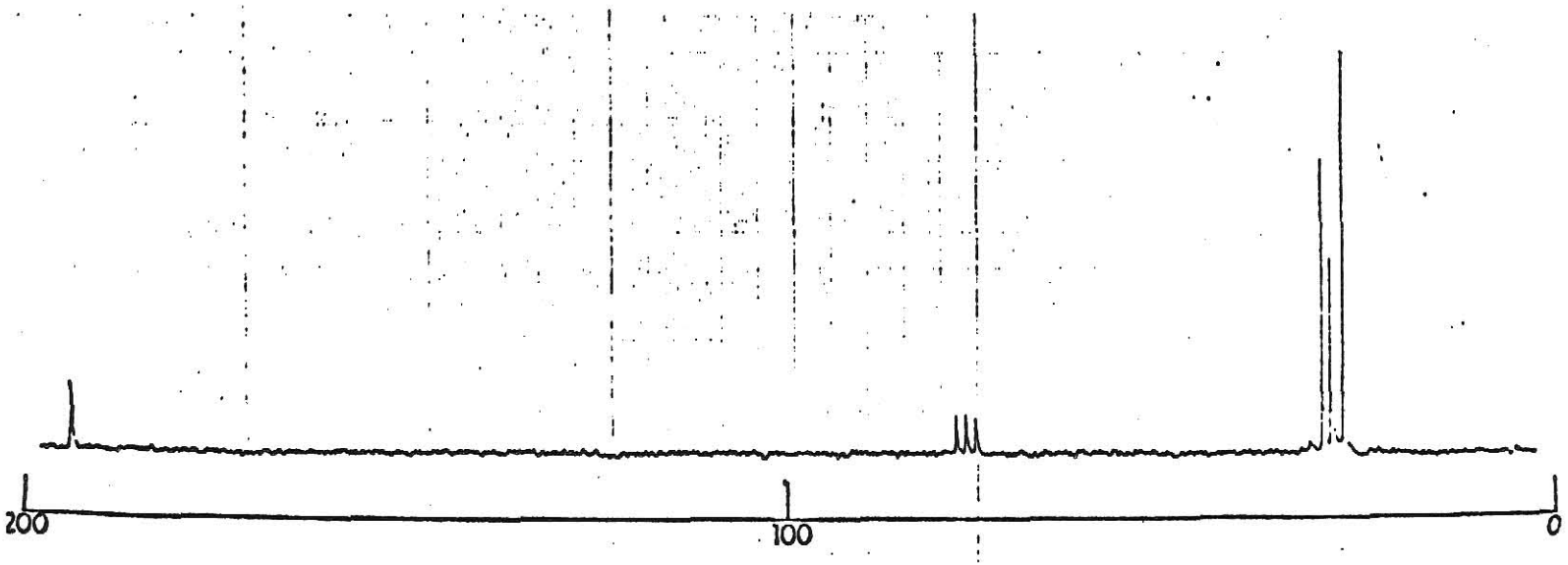


1,3-DITHIOACETYLPROPANE

$C_7H_{12}O_2S_2$

in $CDCl_3$



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c 22.95t
d 14.08q

d c a b
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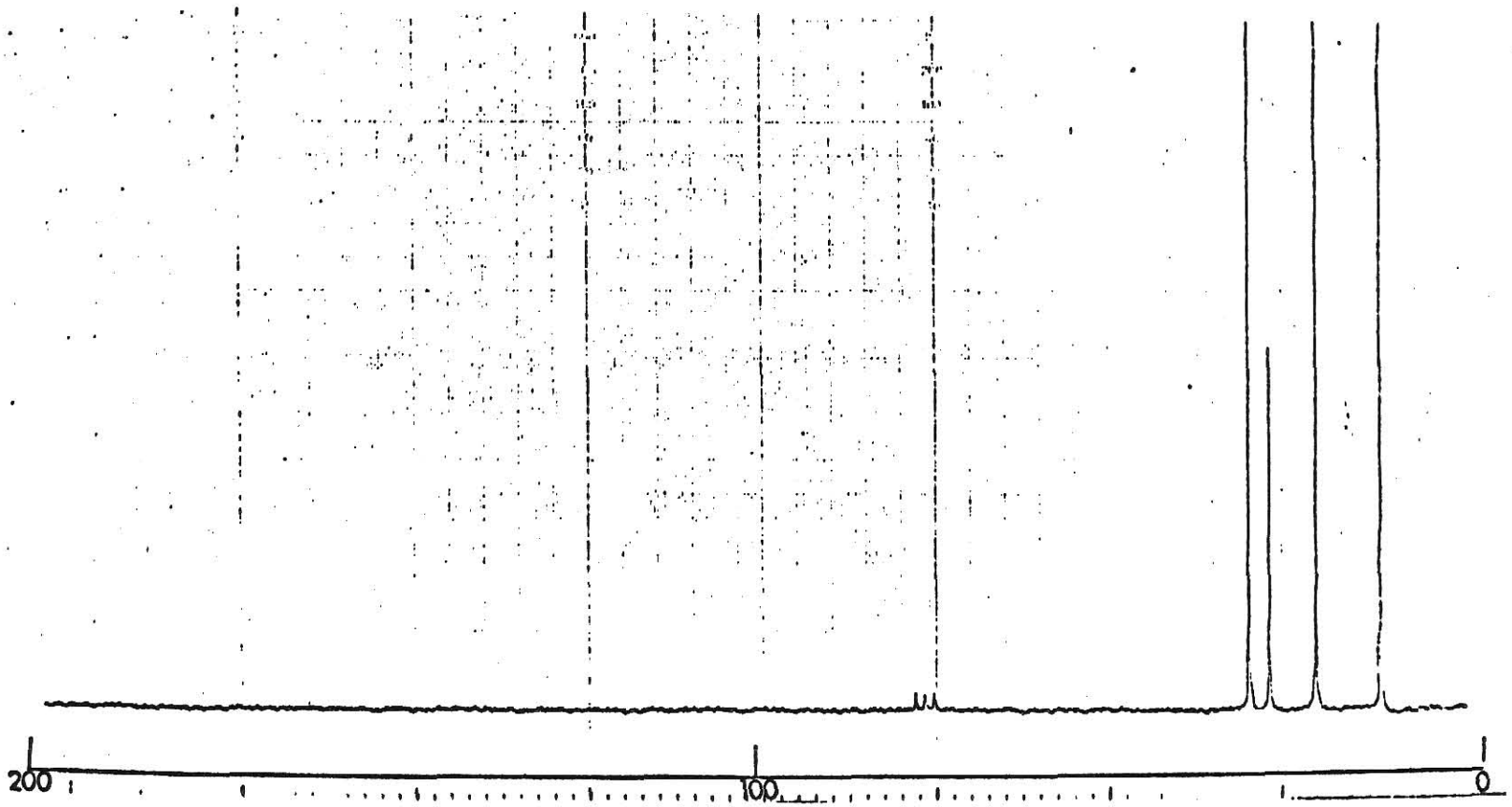
HEPTANE

C_7H_{16}

in $CDCl_3$



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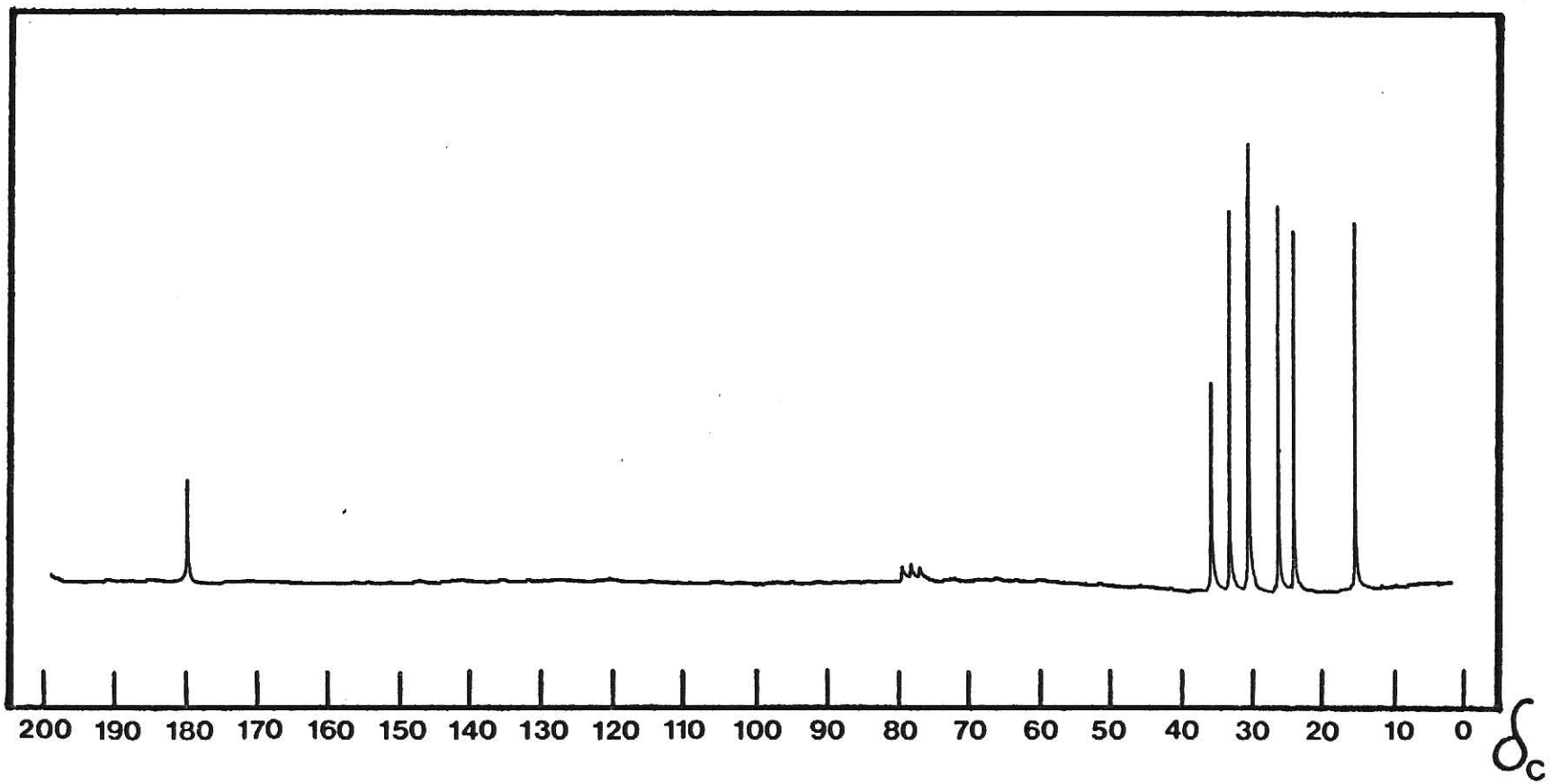
f 24.53t
g 22.54t
h 13.79q

h g c e d f b a
$CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-COOH$

OCTANOIC ACID

$C_8H_{16}O_2$

in $CDCl_3$



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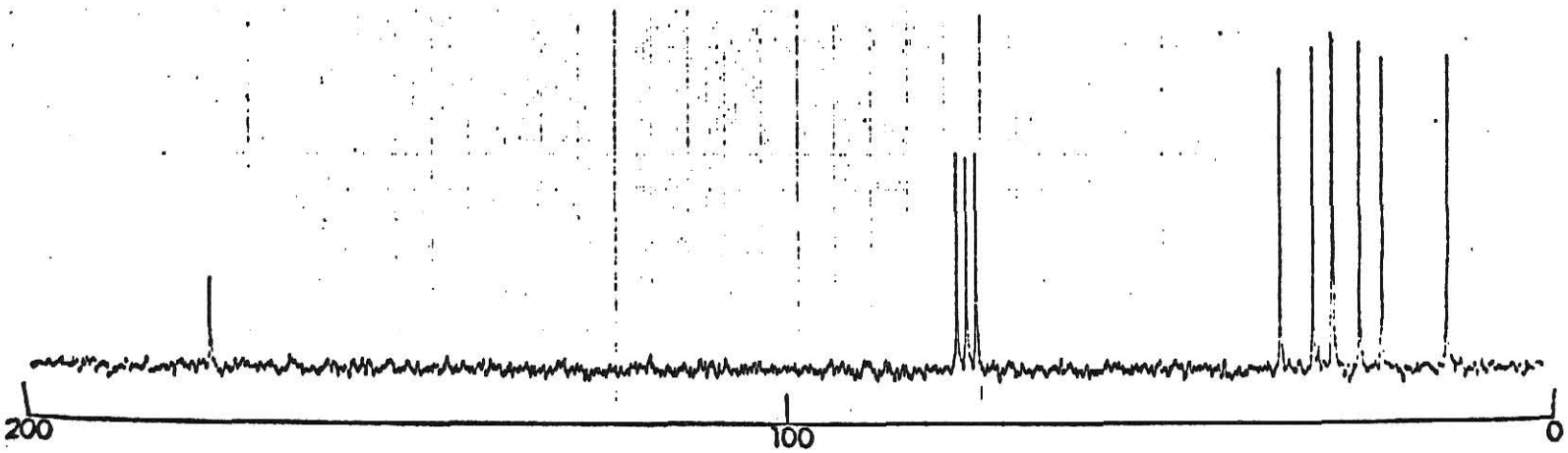
g 22.48t
h 13.93q

h g c e d f b a
$CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-NH_2$

OCTANAMIDE

$C_8H_{17}NO$

in $CDCl_3$



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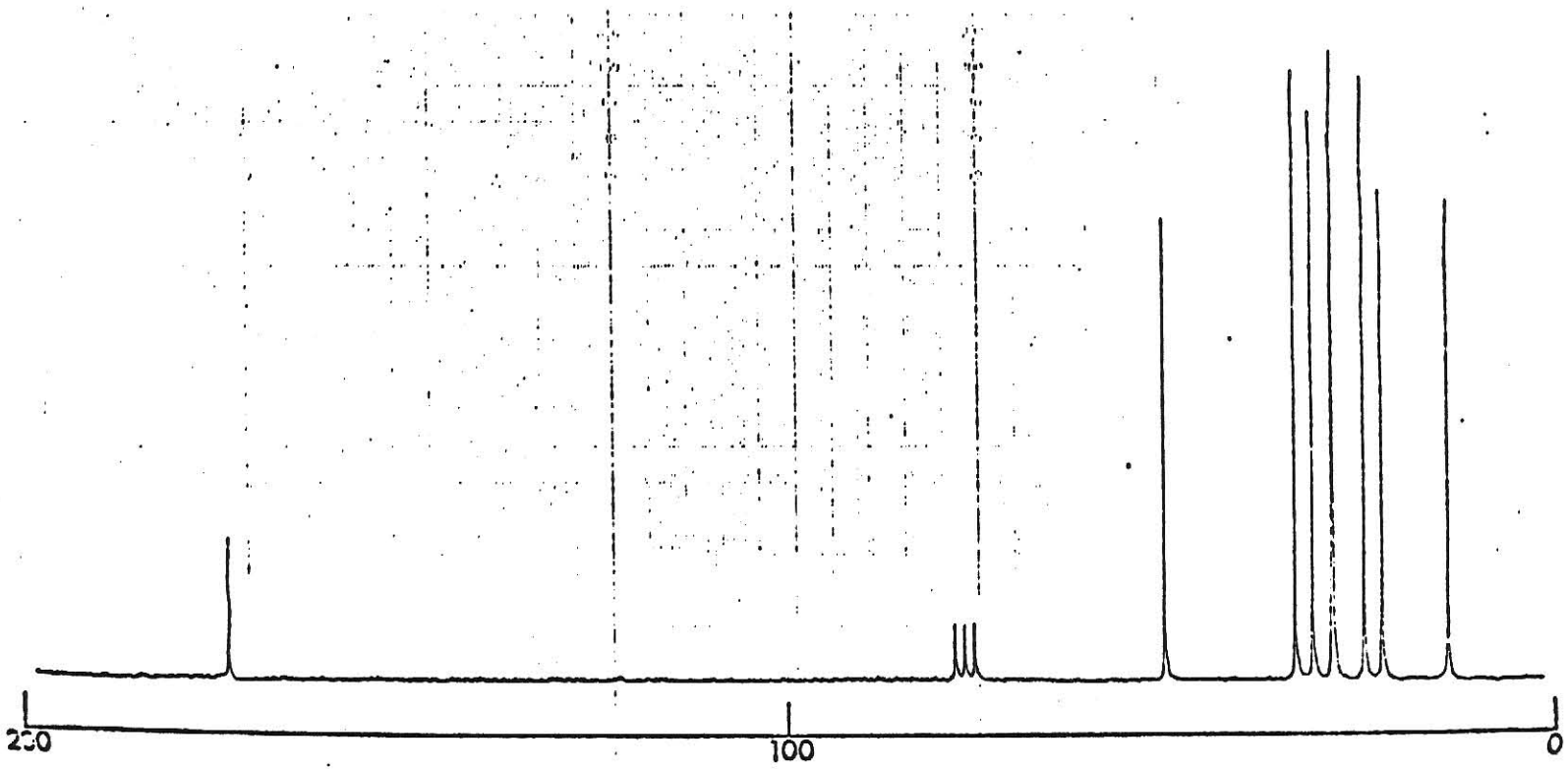
g 24.67t
h 22.28t
i 13.64q

i h d f e g c a b
$CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-OCH_3$

METHYL OCTANOATE

$C_9H_{18}O_2$

in $CDCl_3$



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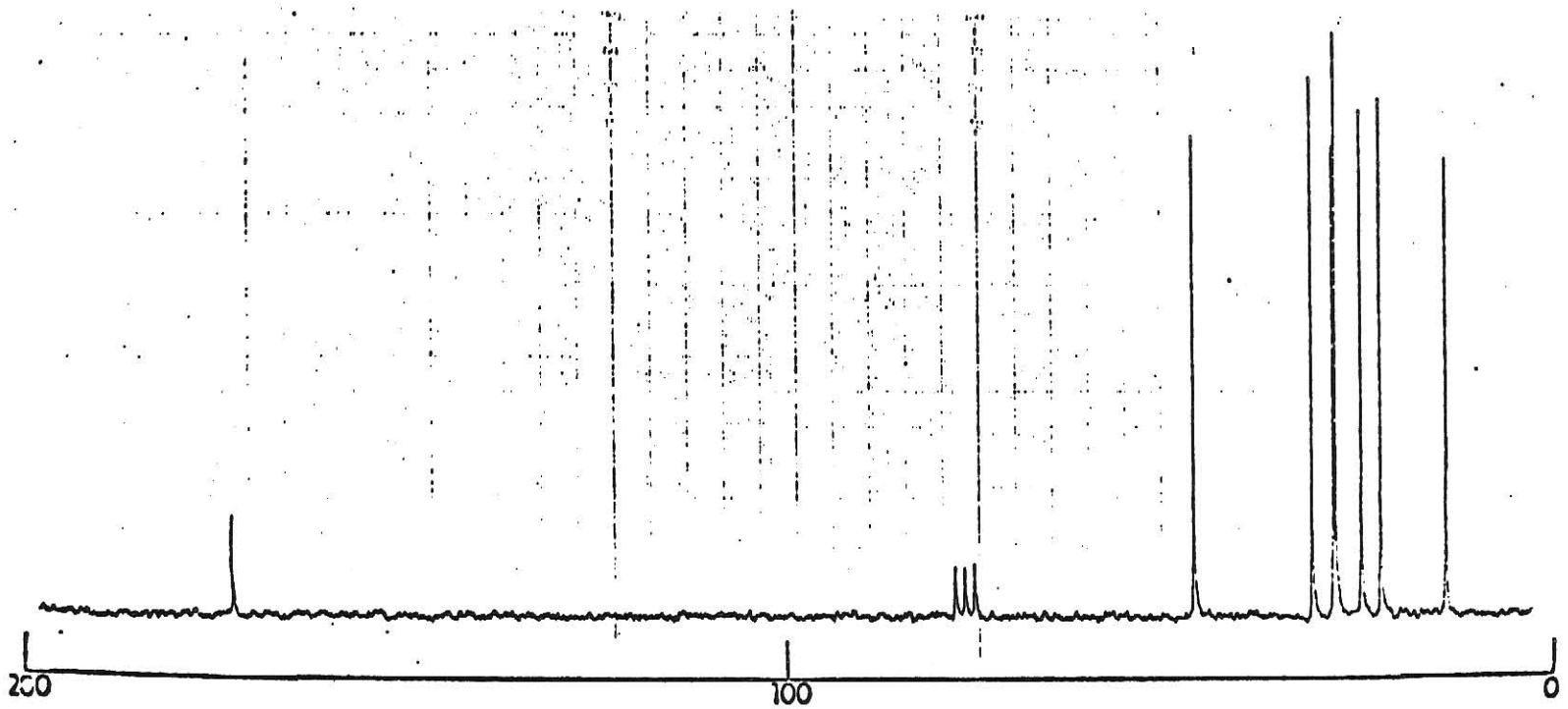
h g c e d f b a
$CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-Cl$

OCTANOYL CHLORIDE

$C_8H_{15}OCl$

in $CDCl_3$



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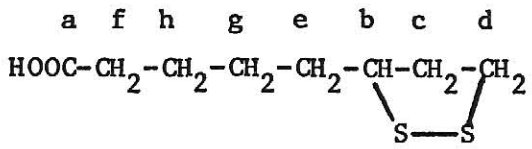

LIPOIC ACID

$C_8H_{14}O_2S_2$

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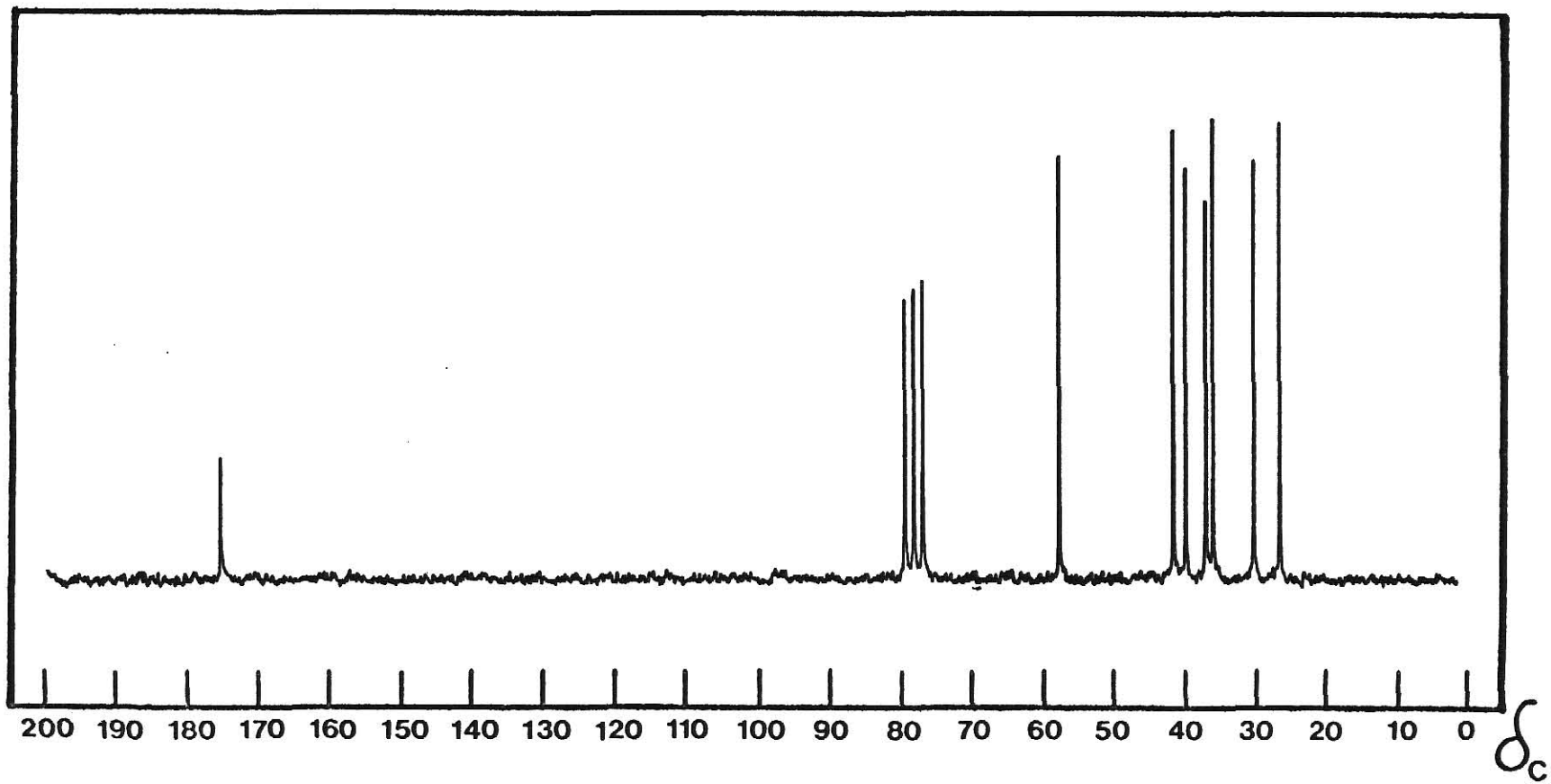

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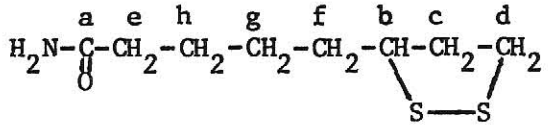
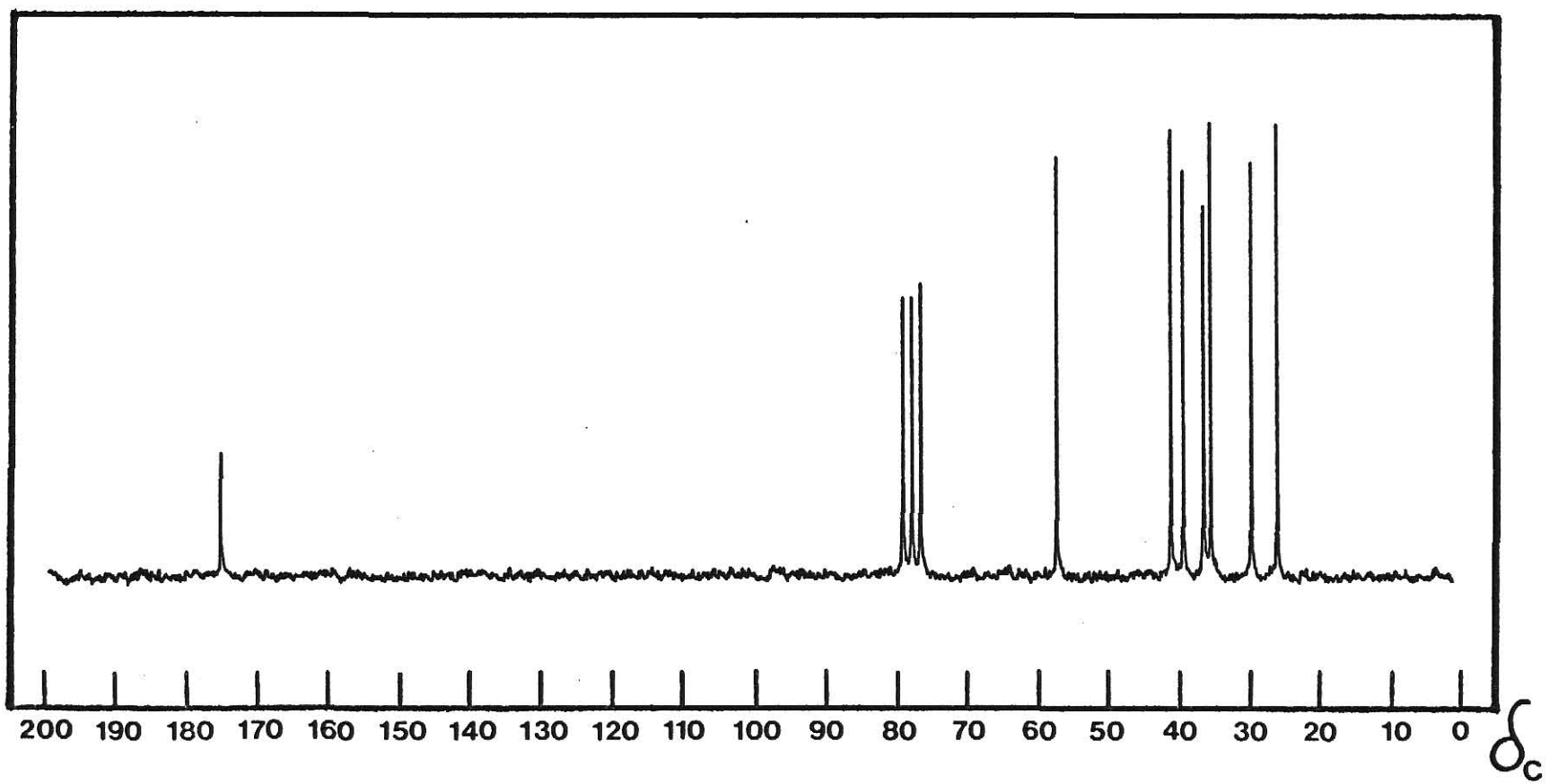
h 25.07 t



LIPOAMIDE

$C_8H_{15}NOS_2$

in $CDCl_3$



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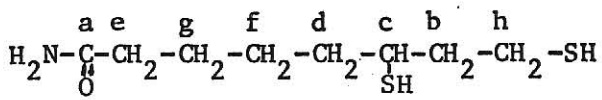
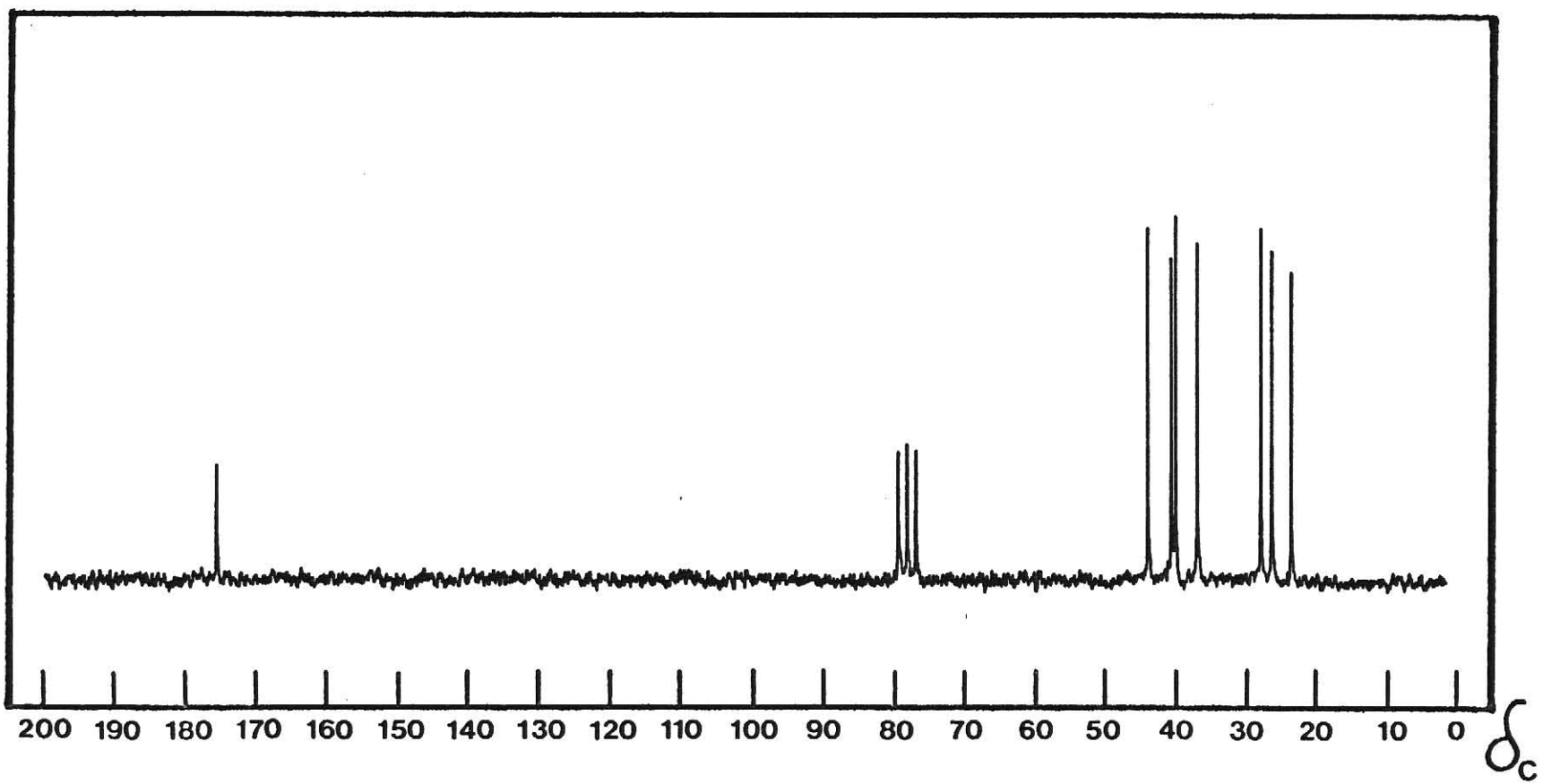


DIHYDROLIPOAMIDE

$C_8H_{17}NOS_2$

in $CDCl_3$



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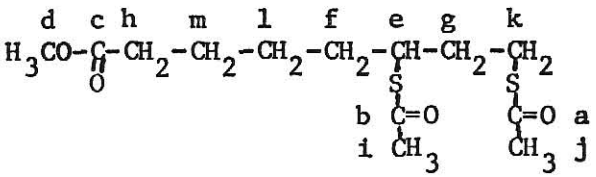
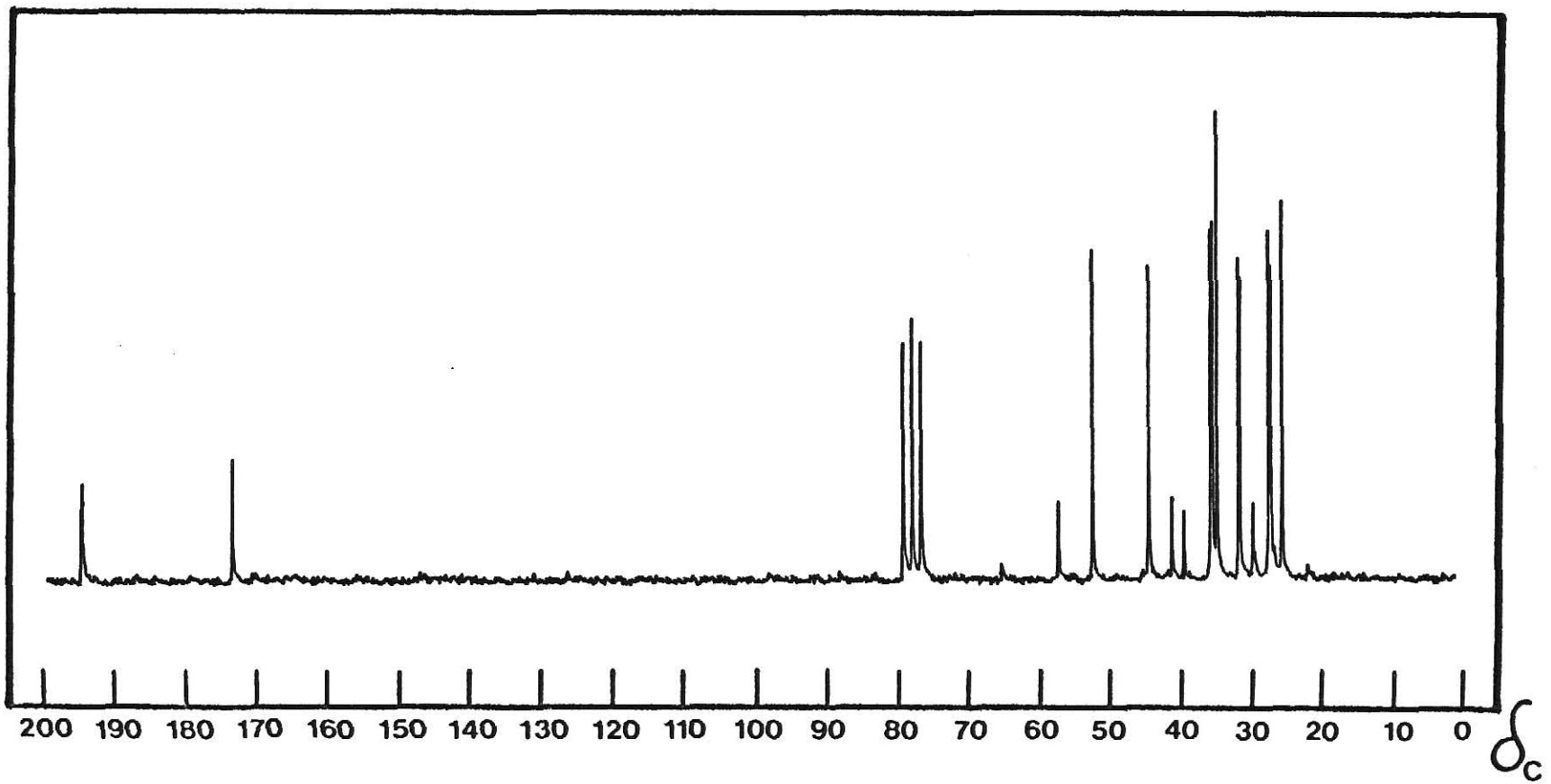


METHYL 6,8-DIACETYLLIPOATE

$C_{13}H_{22}O_4S_2$

in $CDCl_3$



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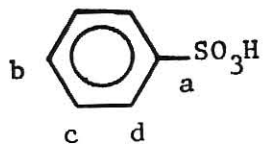
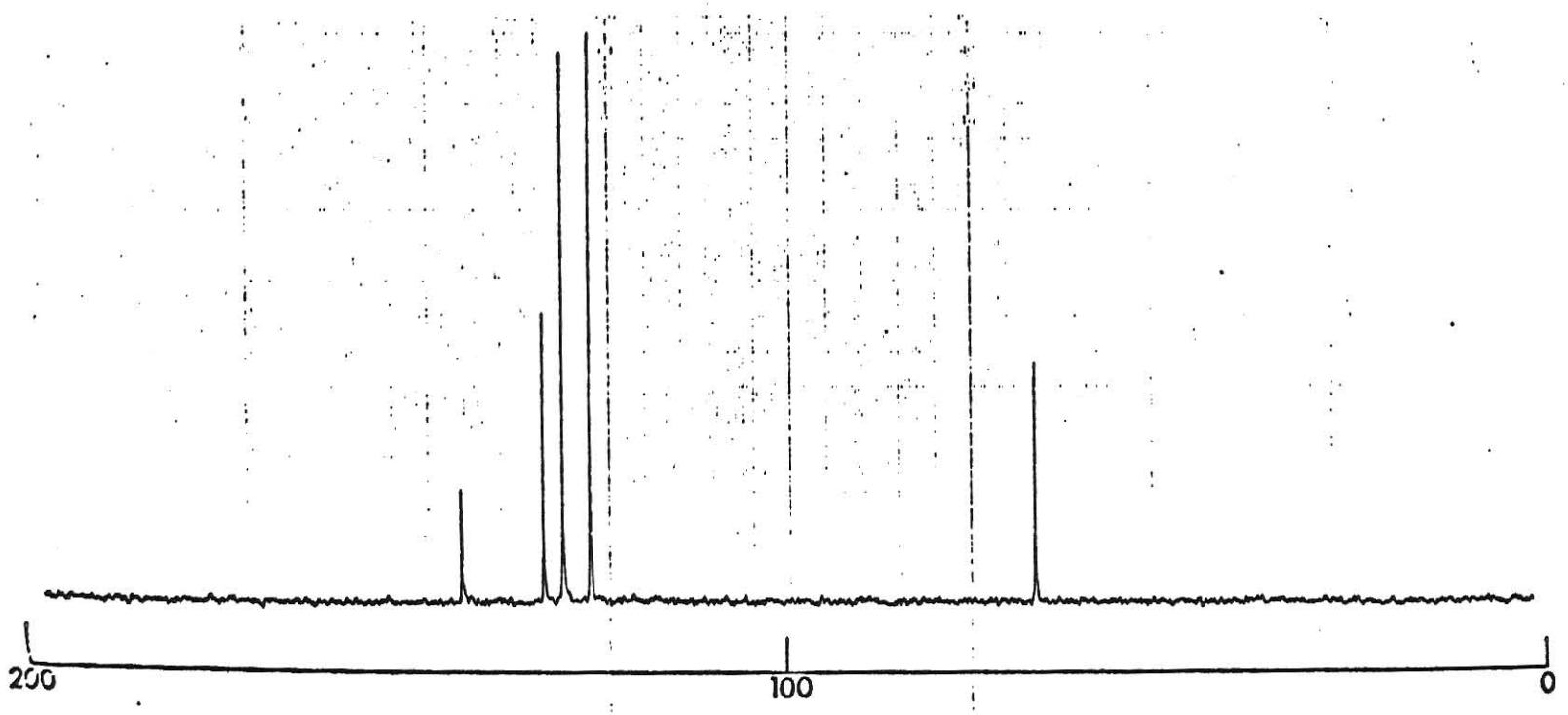
d 126.14



BENZENESULFONIC ACID

$C_6H_6O_3S$

in D_2O with dioxane



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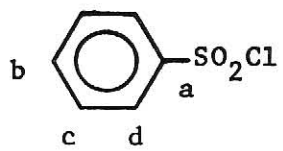
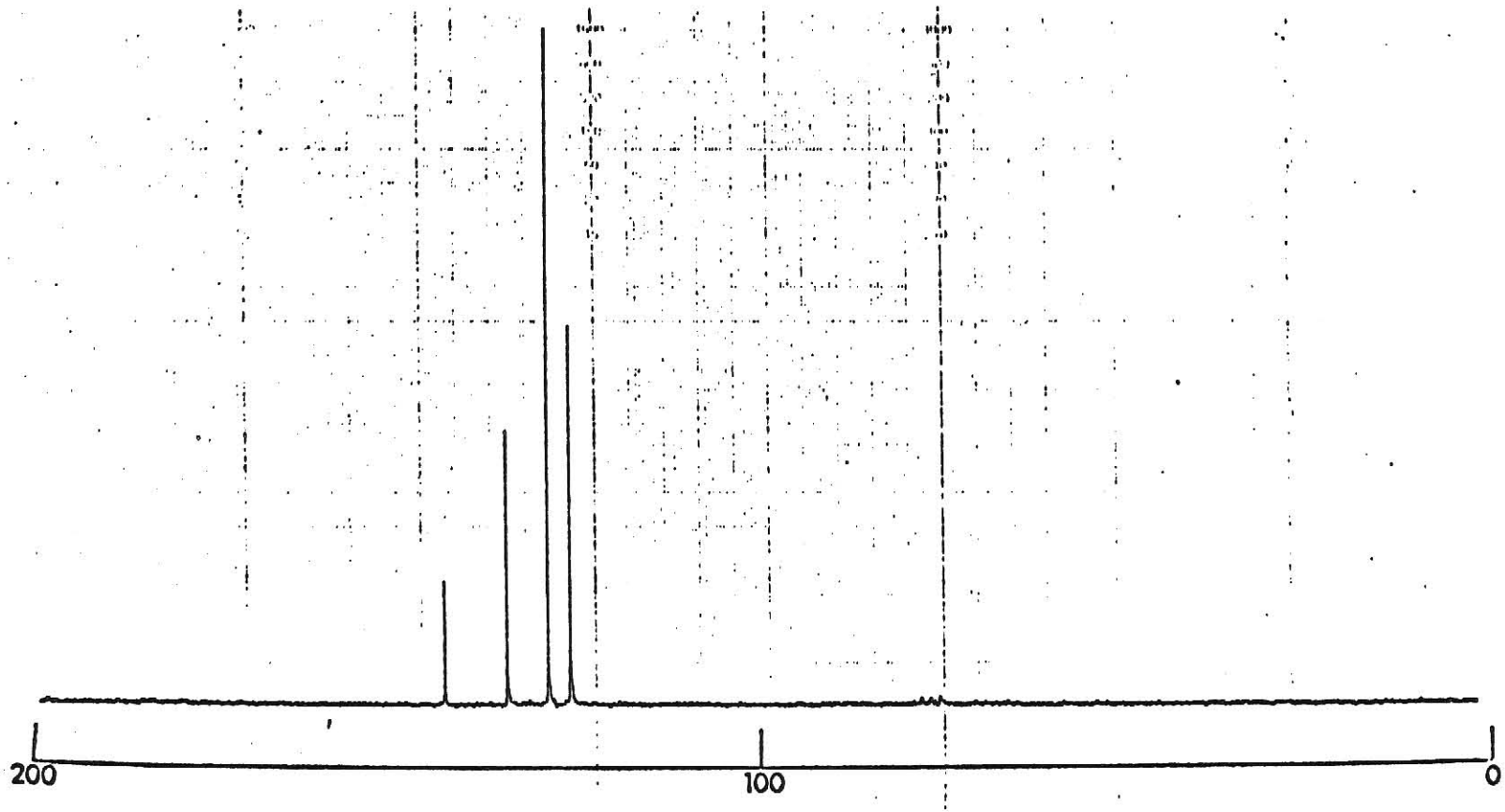


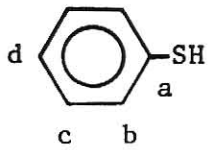
BENZENESULFONYL CHLORIDE

$C_6H_5O_2SCl$

in $CDCl_3$



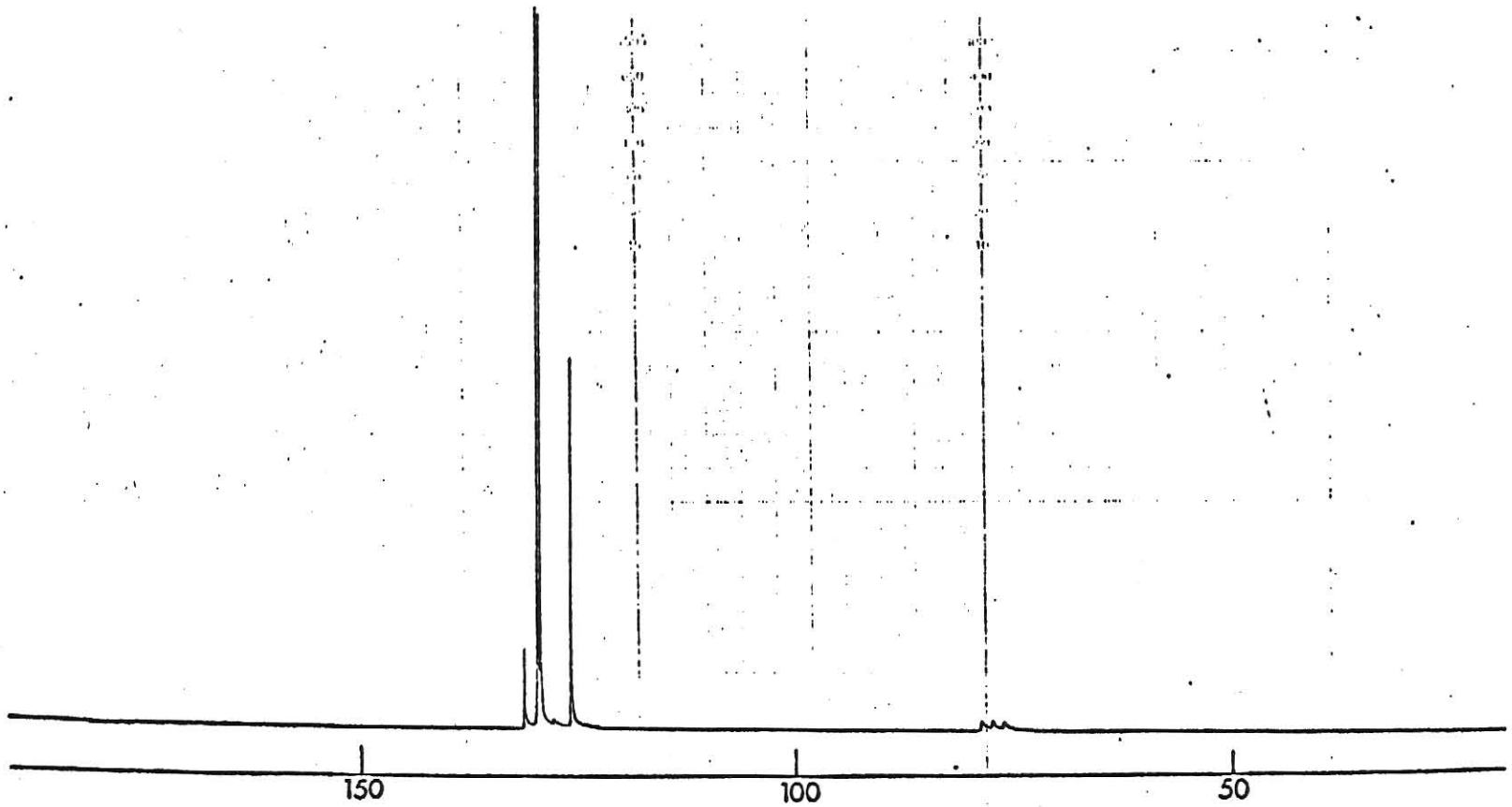
a 130.40
b 128.93
c 128.59
d 125.08



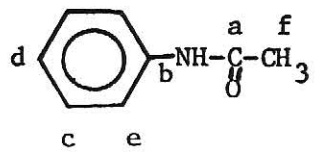
THIOPHENOL

C_6H_6S

in $CDCl_3$



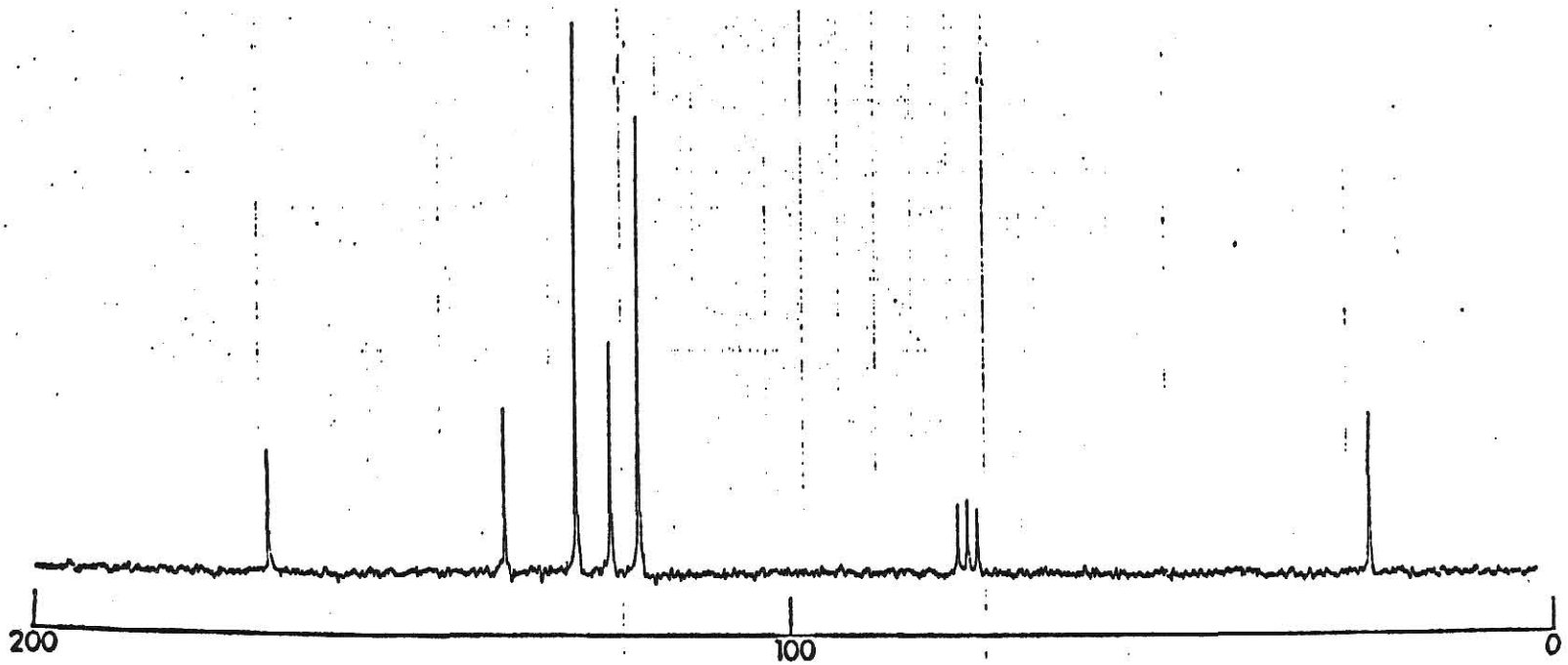
a	169.11
b	137.80
c	128.35
d	123.83
e	120.06
f	23.89



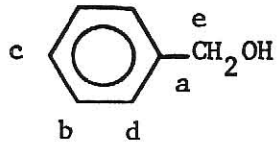
ACETANILIDE

C_8H_9NO

in $CDCl_3$



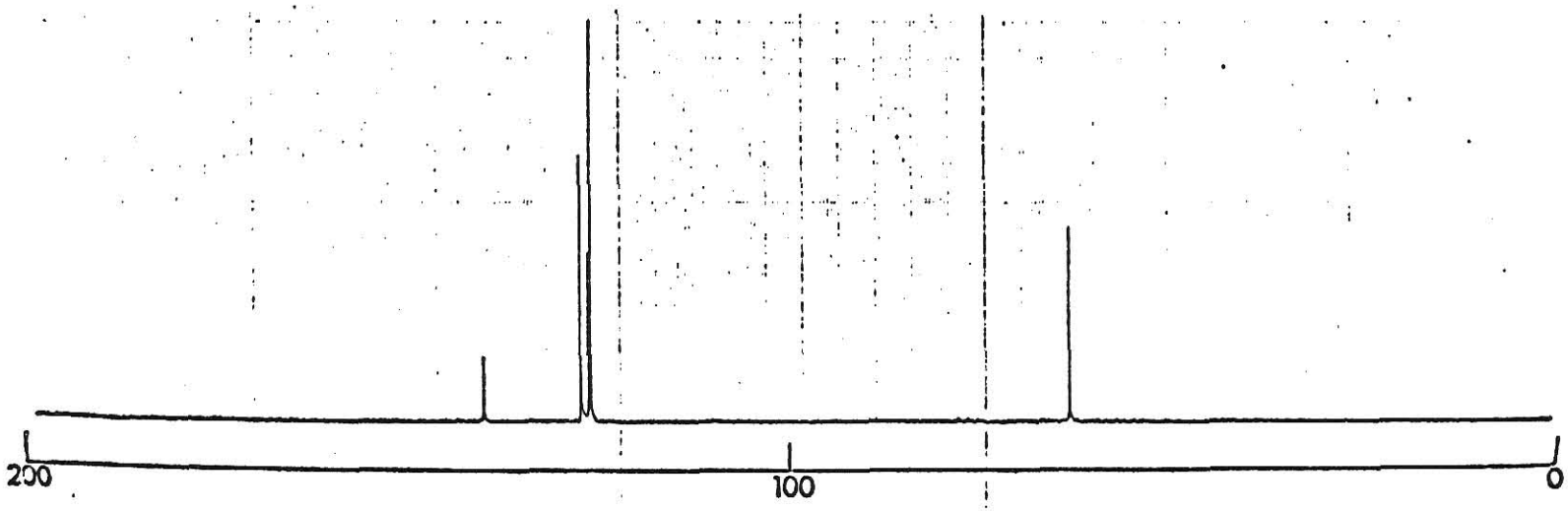
a 140.19
b 127.50
c 126.45
d 126.16
e 63.59



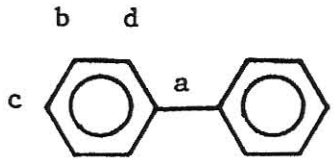
BENZYL ALCOHOL

C_7H_8O

in $CDCl_3$



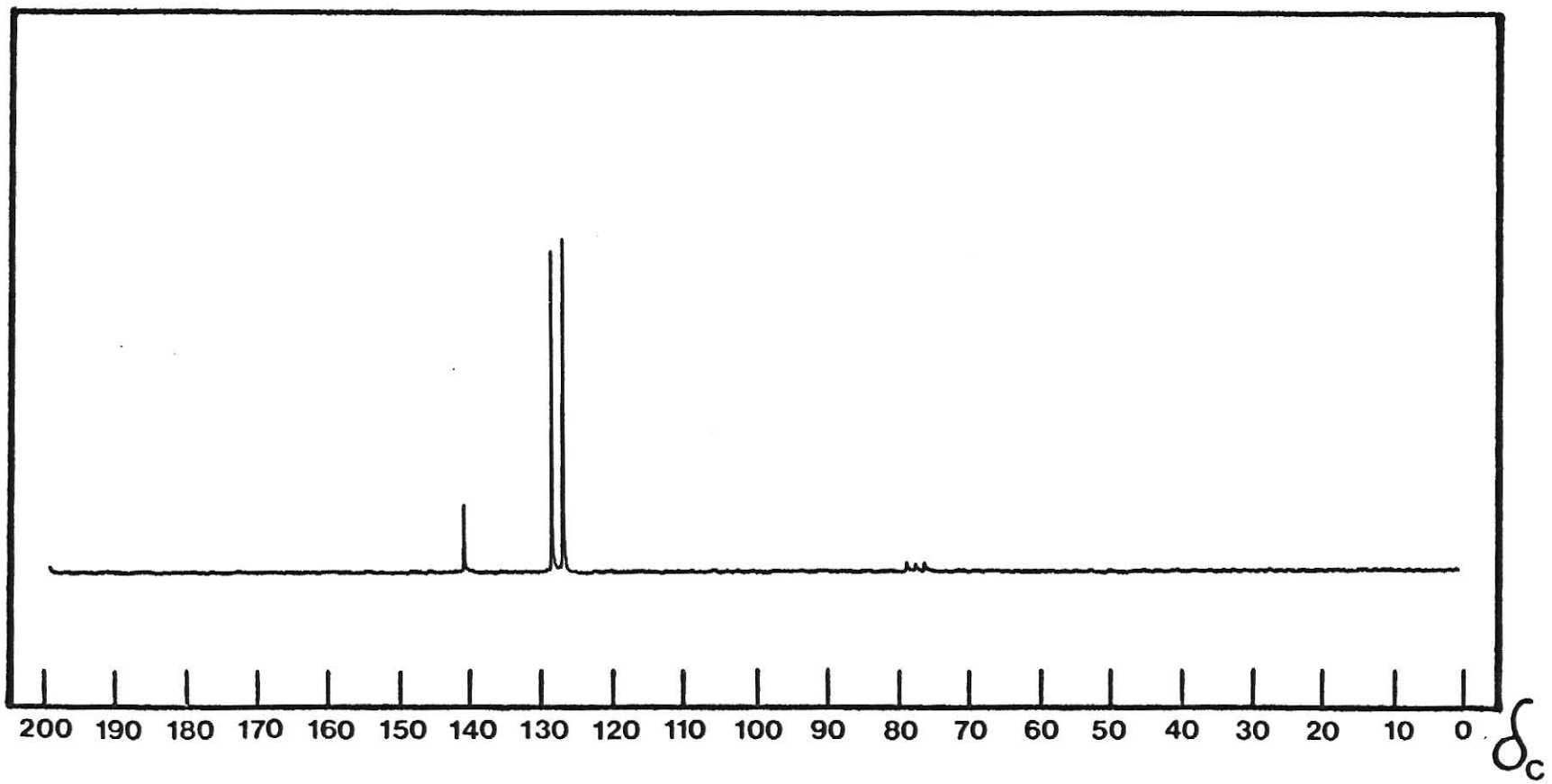
a 140.90
b 128.47
c 126.95
d 126.83



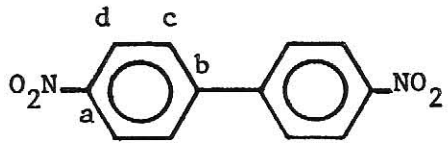
BIPHENYL

$C_{12}H_{10}$

in $CDCl_3$



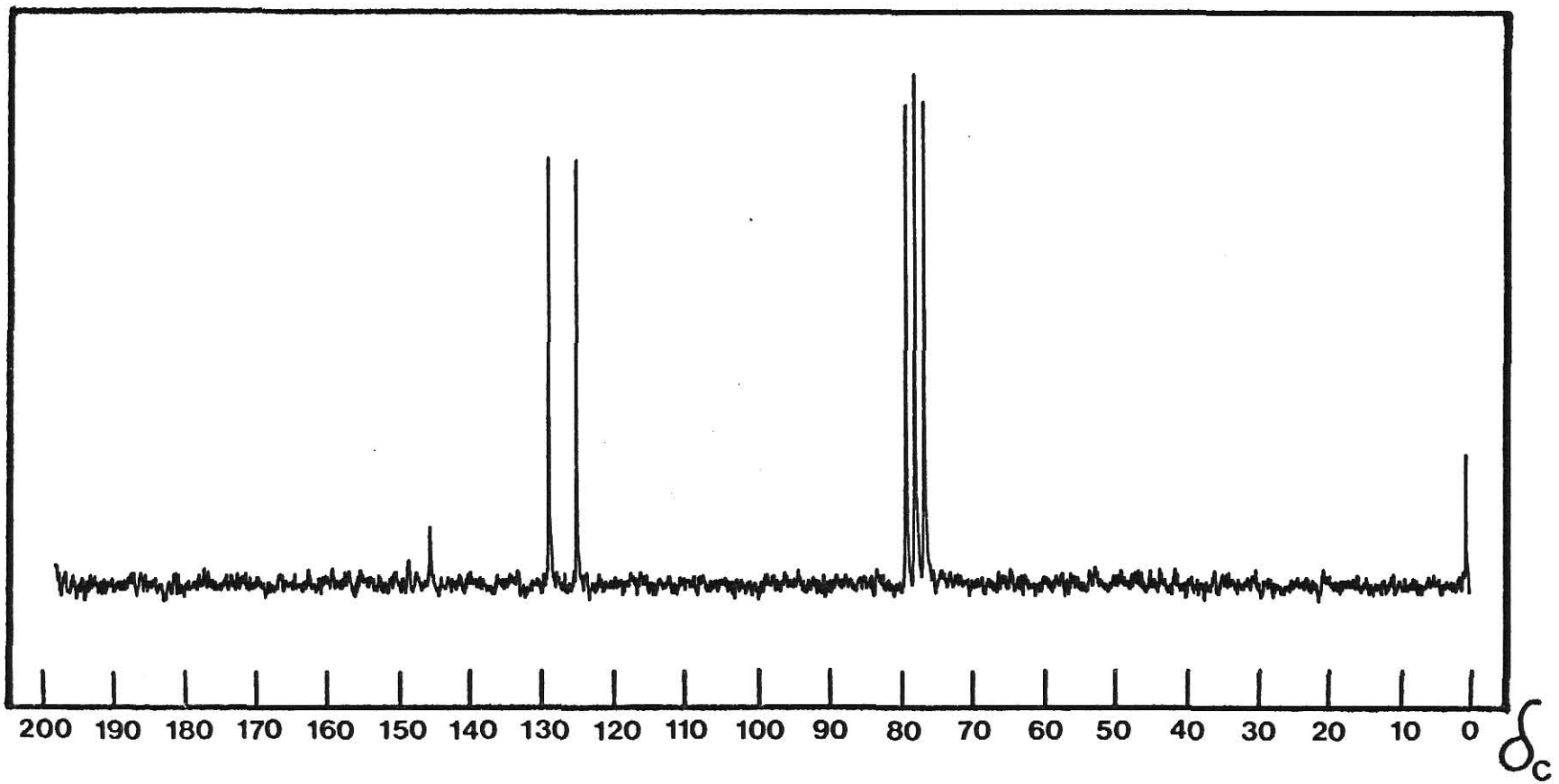
a 147.93 s
b 144.79 s
c 128.15 d
d 124.20 d



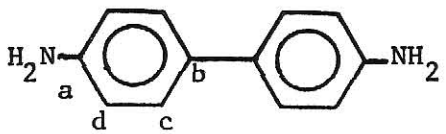
4,4'-DINITROBIPHENYL

$C_{12}H_8N_2O_4$

in $CDCl_3$



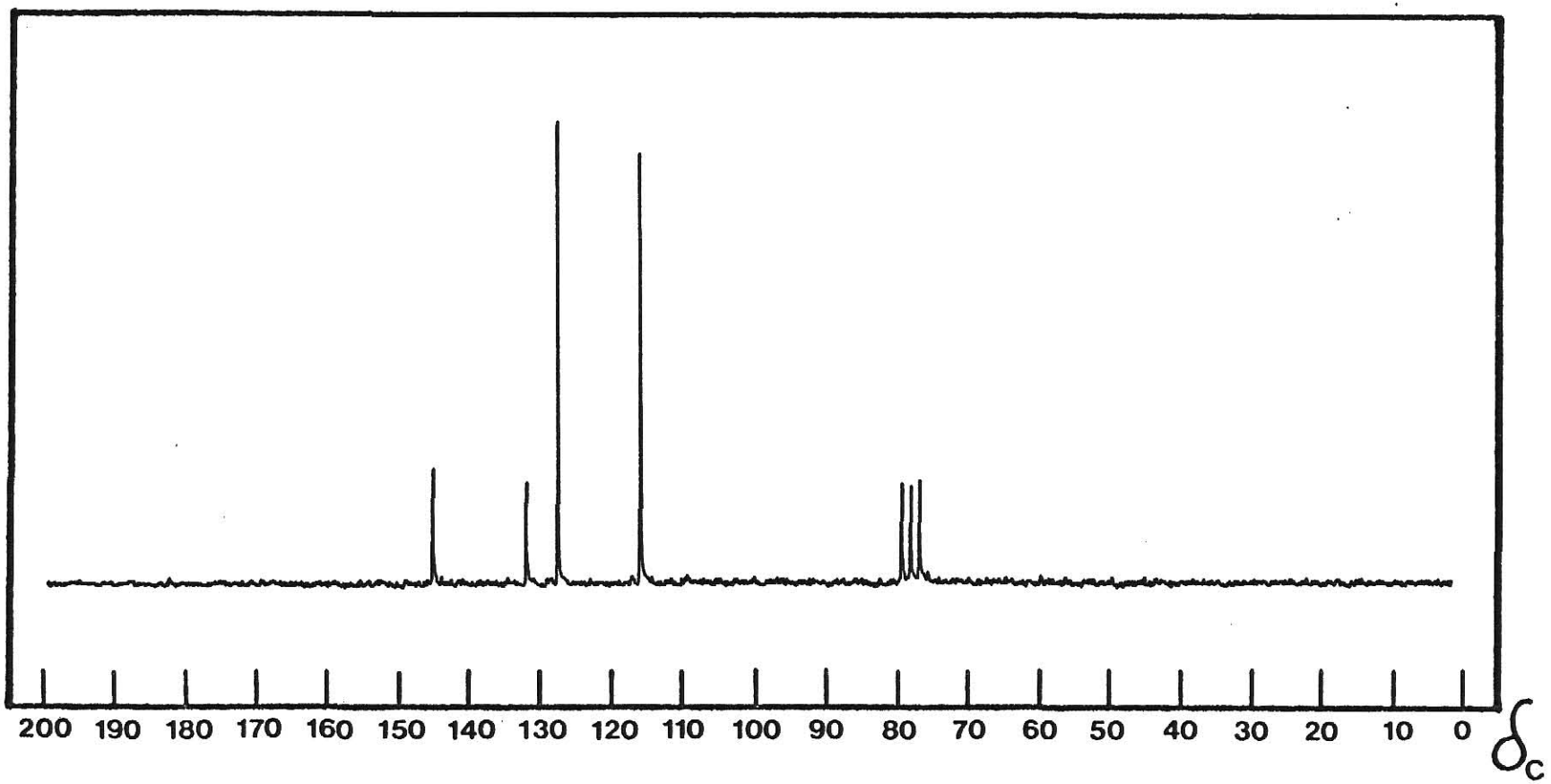
a 144.71s
b 131.46s
c 126.97d
d 115.21d



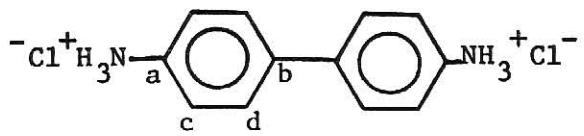
4,4'-DIAMINOBIPHENYL

$C_{12}H_{12}N_2$

in $CDCl_3$



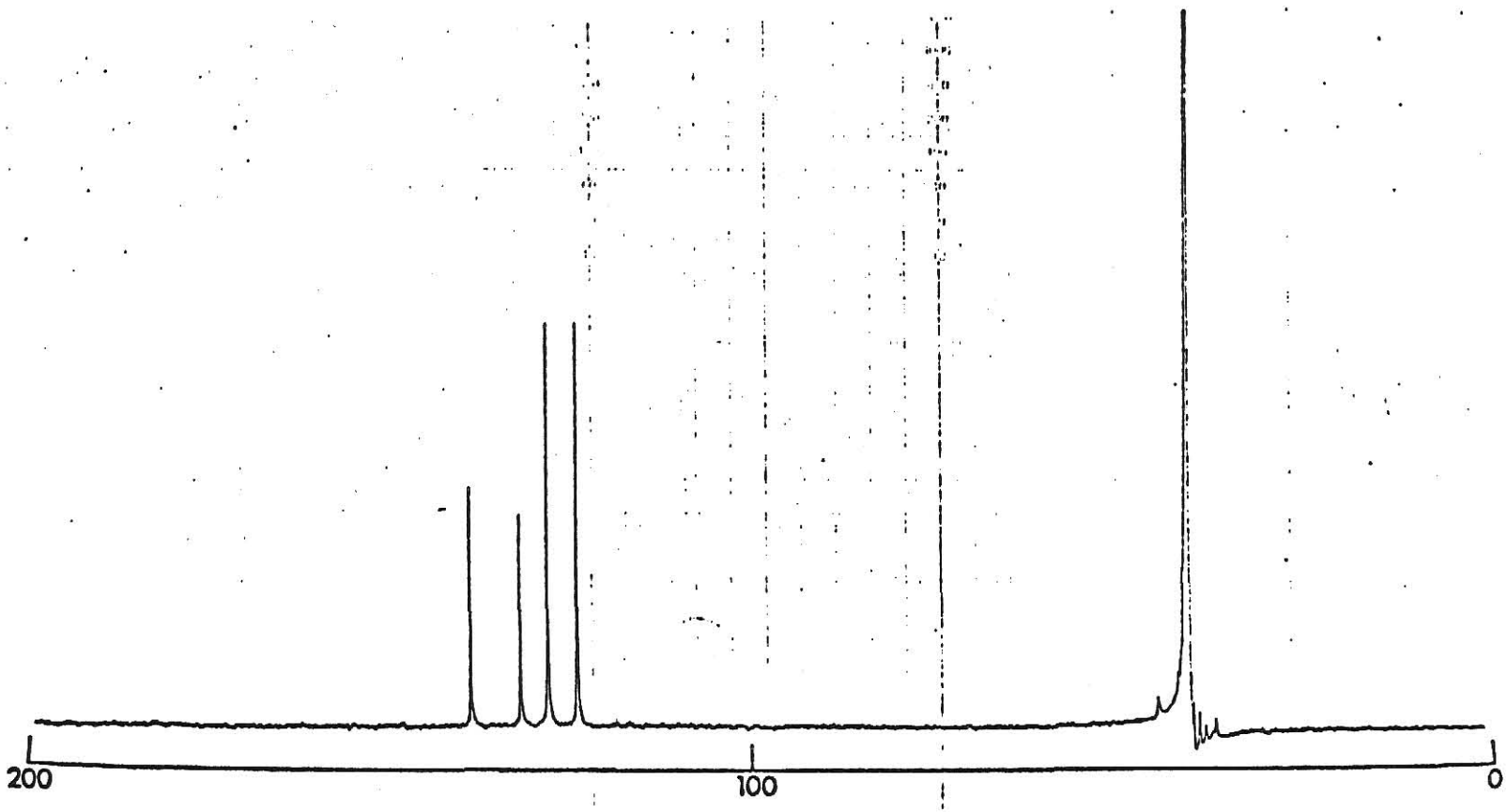
a 138.43
b 131.63
c 127.89
d 123.79



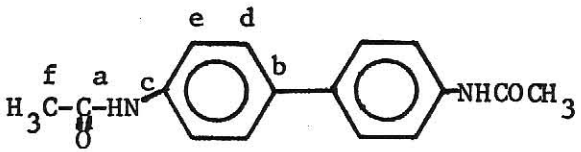
BENZIDINE HYDROCHLORIDE

$C_{12}H_{14}N_2Cl_2$

in d_6-DMSO



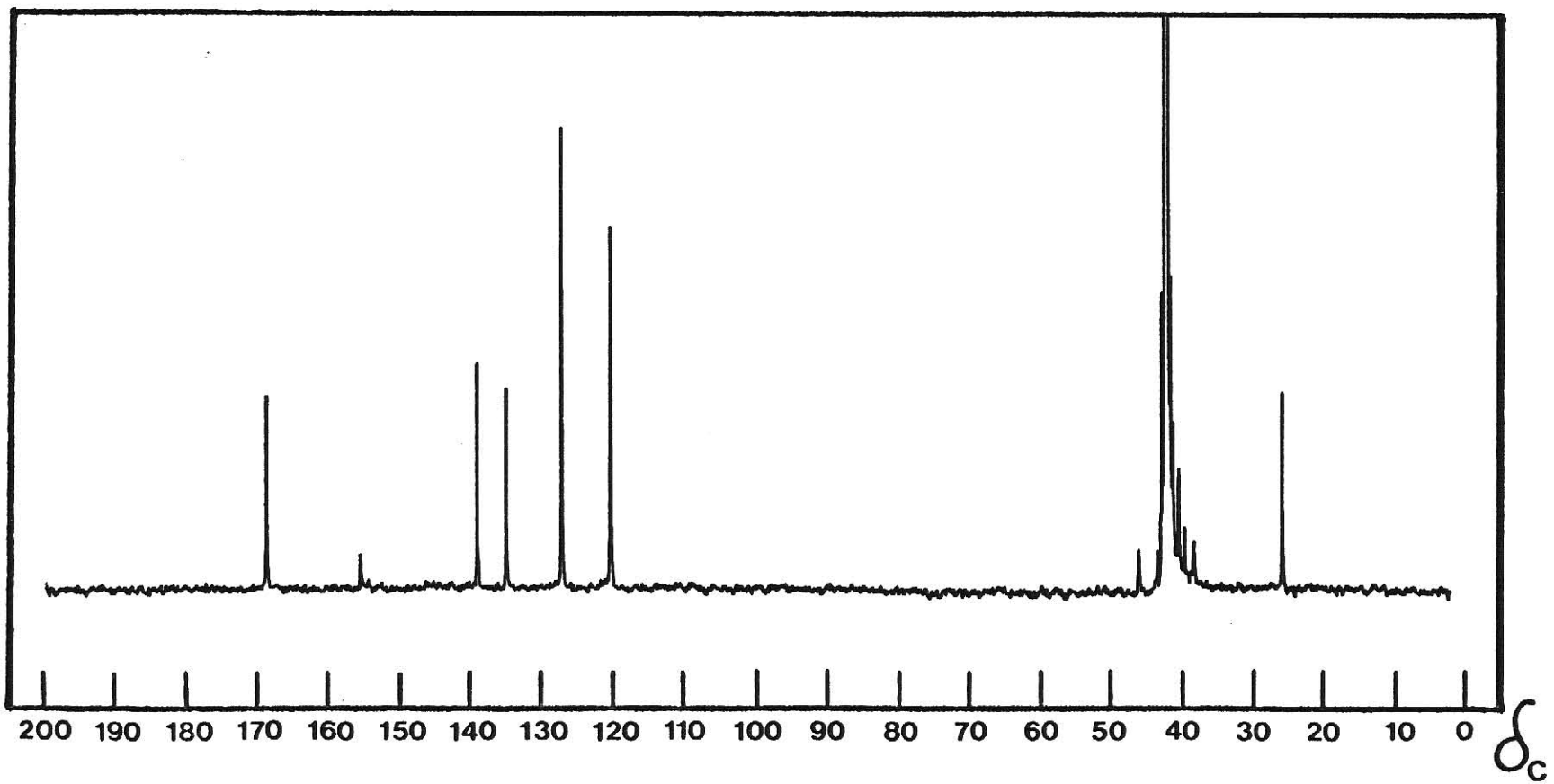
a 168.00
b 138.23
c 134.15
d 126.21
e 119.23
f 24.05



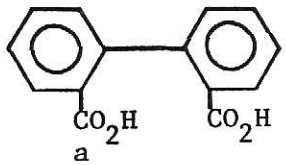
4,4'-DIACETAMIDOBIPHENYL

$C_{16}H_{16}N_2O_2$

in d_6-DMSO



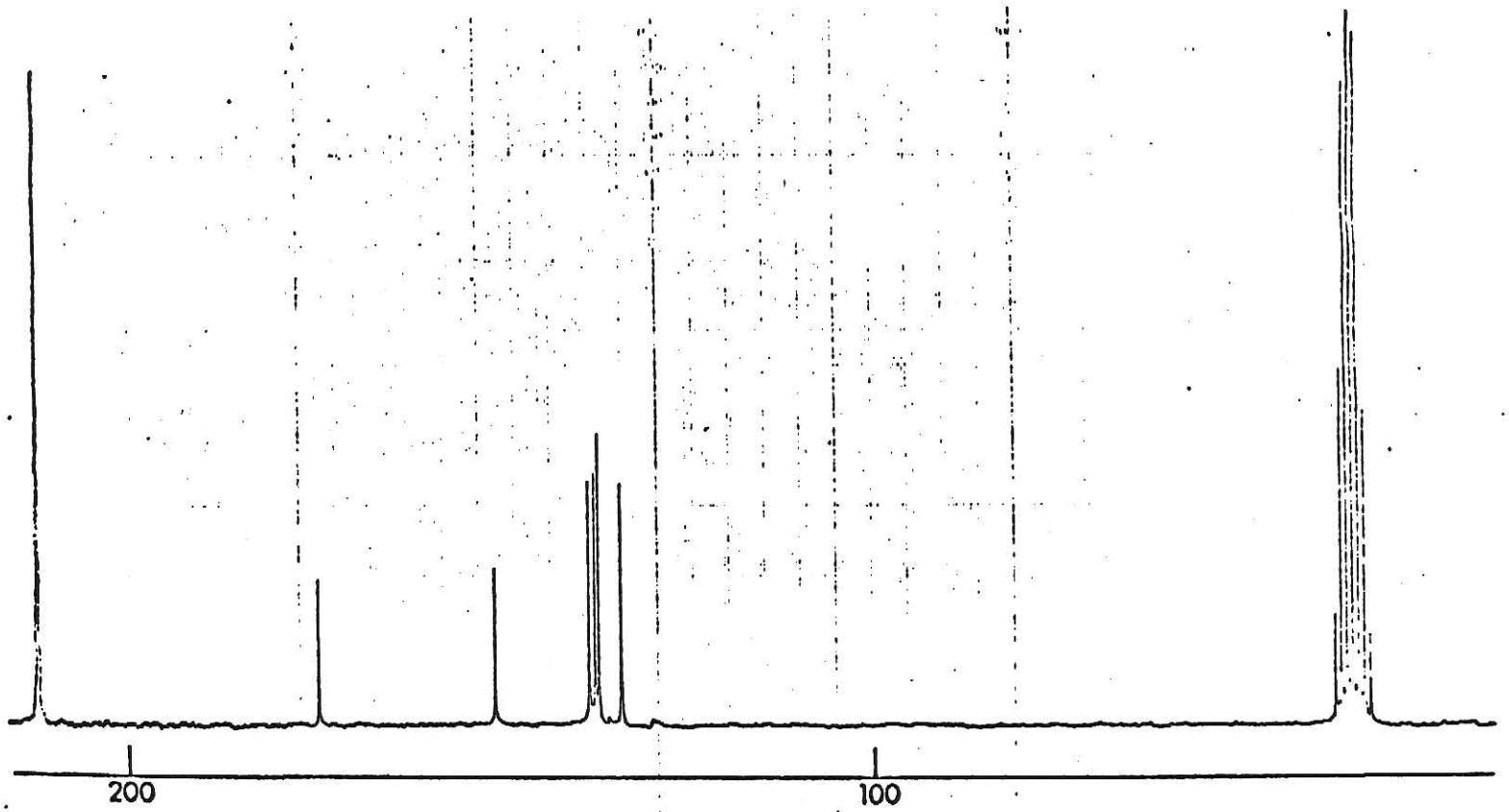
a 167.46
b 143.82
c 131.27
d 130.48
e 129.96
f 126.98
g 126.81



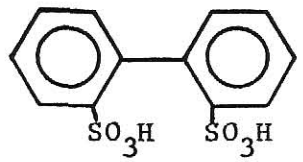
DIPHENIC ACID

$C_{14}H_{10}O_4$

in d_6-acetone



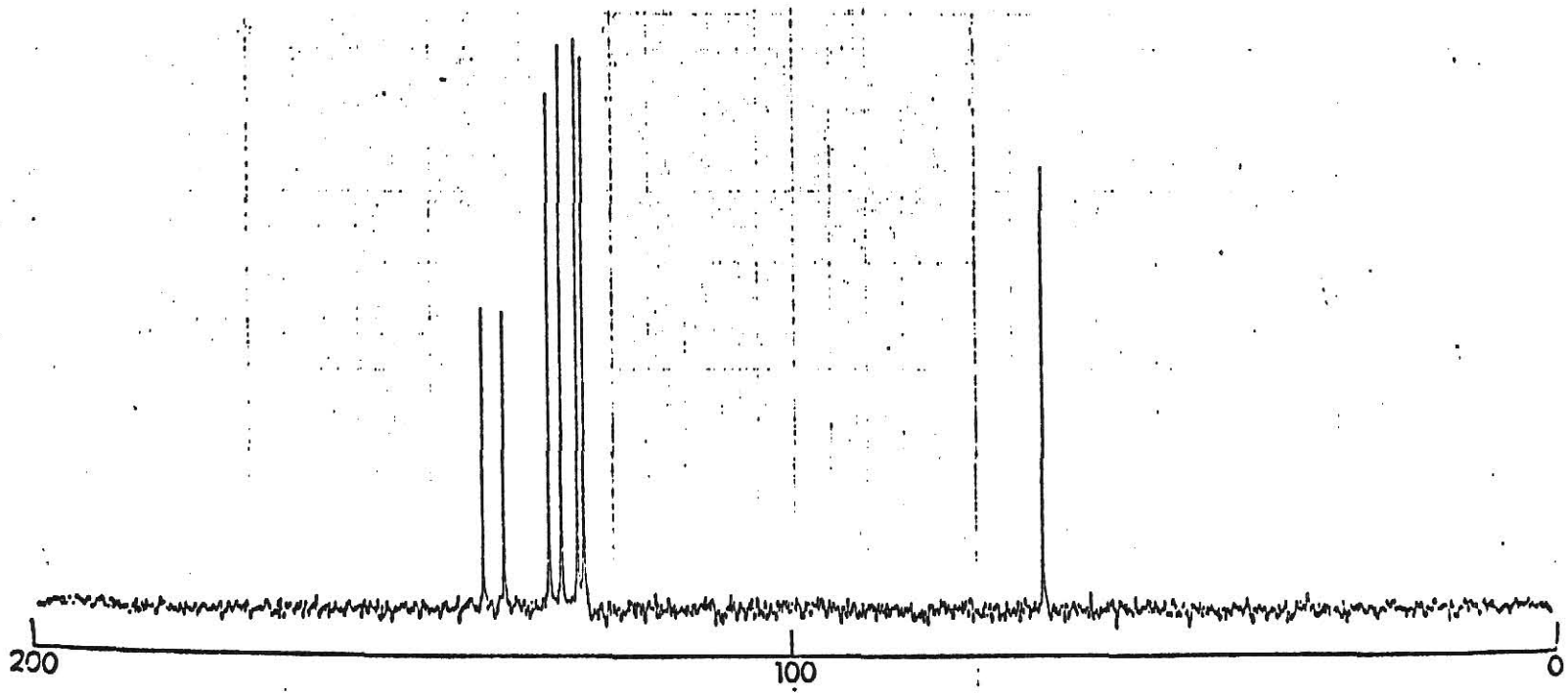
a	141.11
b	138.39
c	132.43
d	130.78
e	128.65
f	127.80



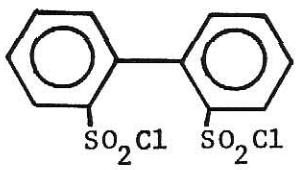
2,2'-BIPHENYLDISULFONIC ACID

$C_{12}H_{10}O_6S_2$

in D_2O and dioxane



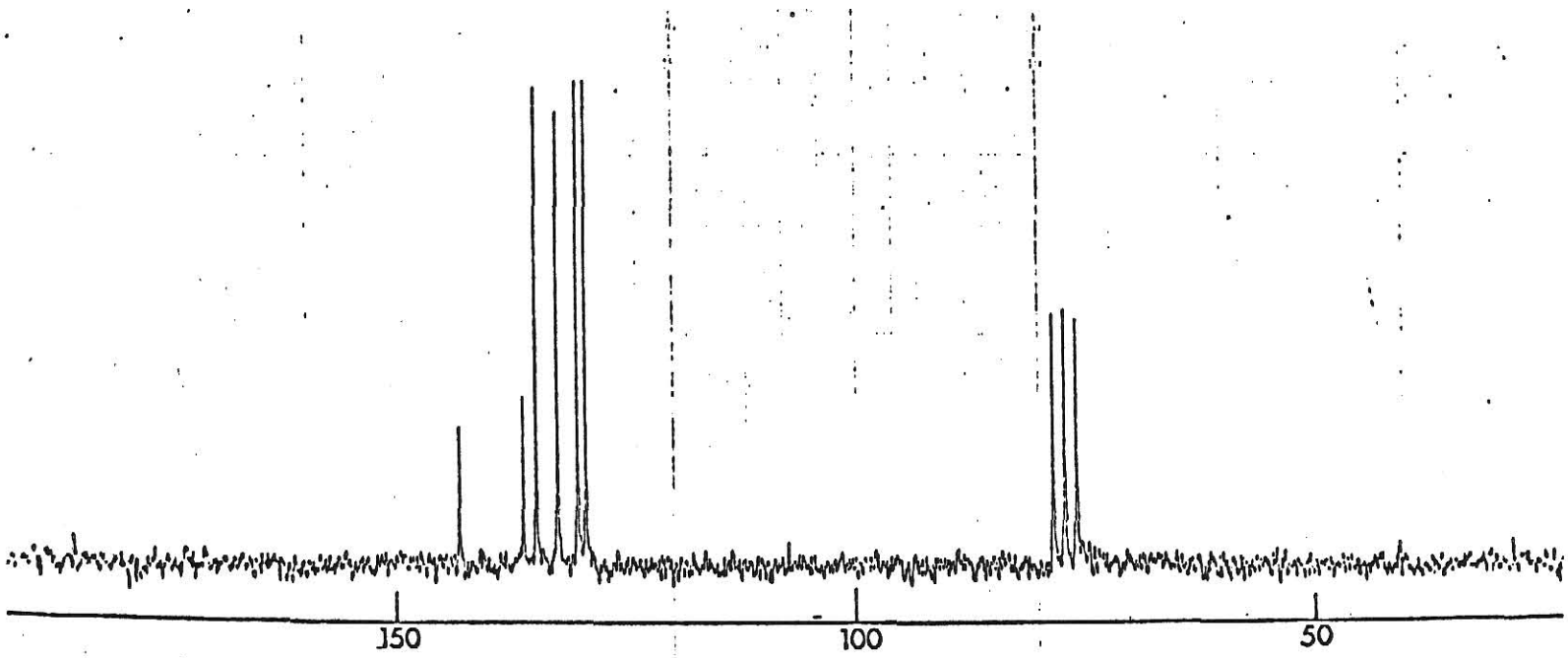
a	142.61
b	135.66
c	134.25
d	131.91
e	129.78
f	128.88



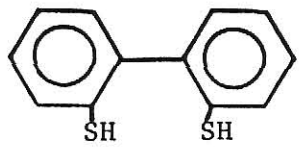
2,2'-BIPHENYLDISULFONYL CHLORIDE

$C_{12}H_8O_4S_2Cl_2$

in $CDCl_3$



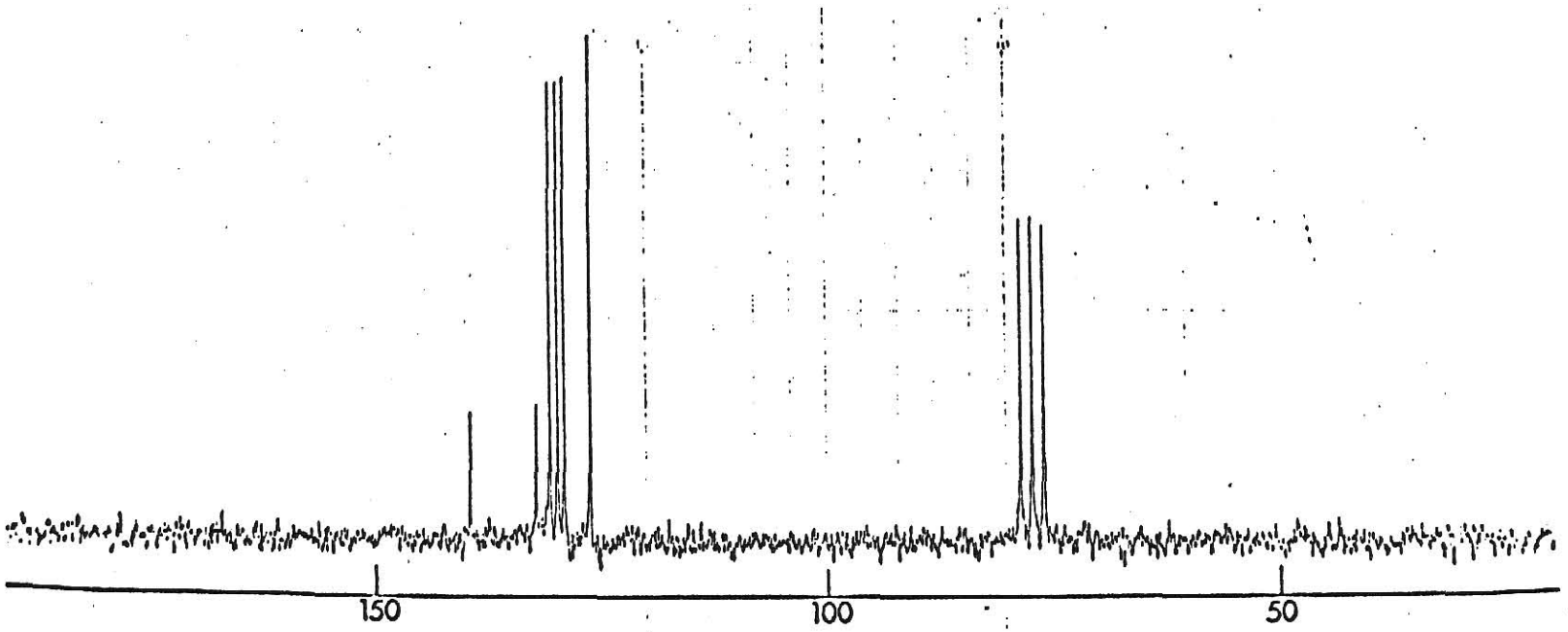
a	138.83
b	131.57
c	130.05
d	129.21
e	128.46
f	125.58



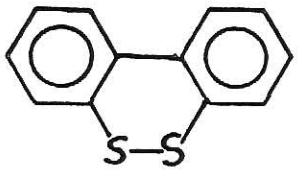
2,2'-DITHIOBIPHENYL

$C_{12}H_{10}S_2$

in $CDCl_3$



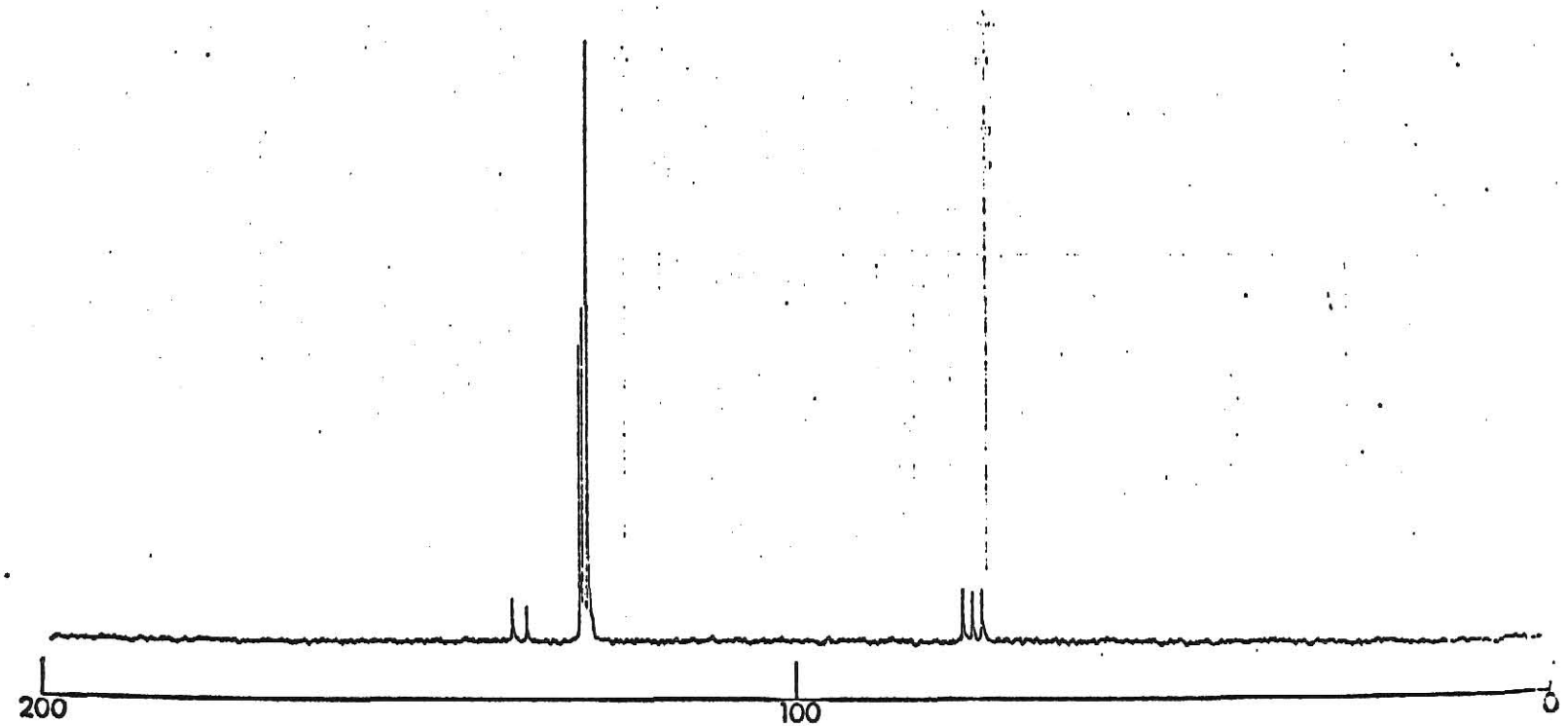
a 137.71
b 135.82
c 128.69
d 128.28
e 127.62



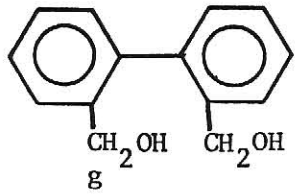
9,10-DITHIA-9,10-DIHYDROPHENANTHRENE

$C_{12}H_8S_2$

in $CDCl_3$



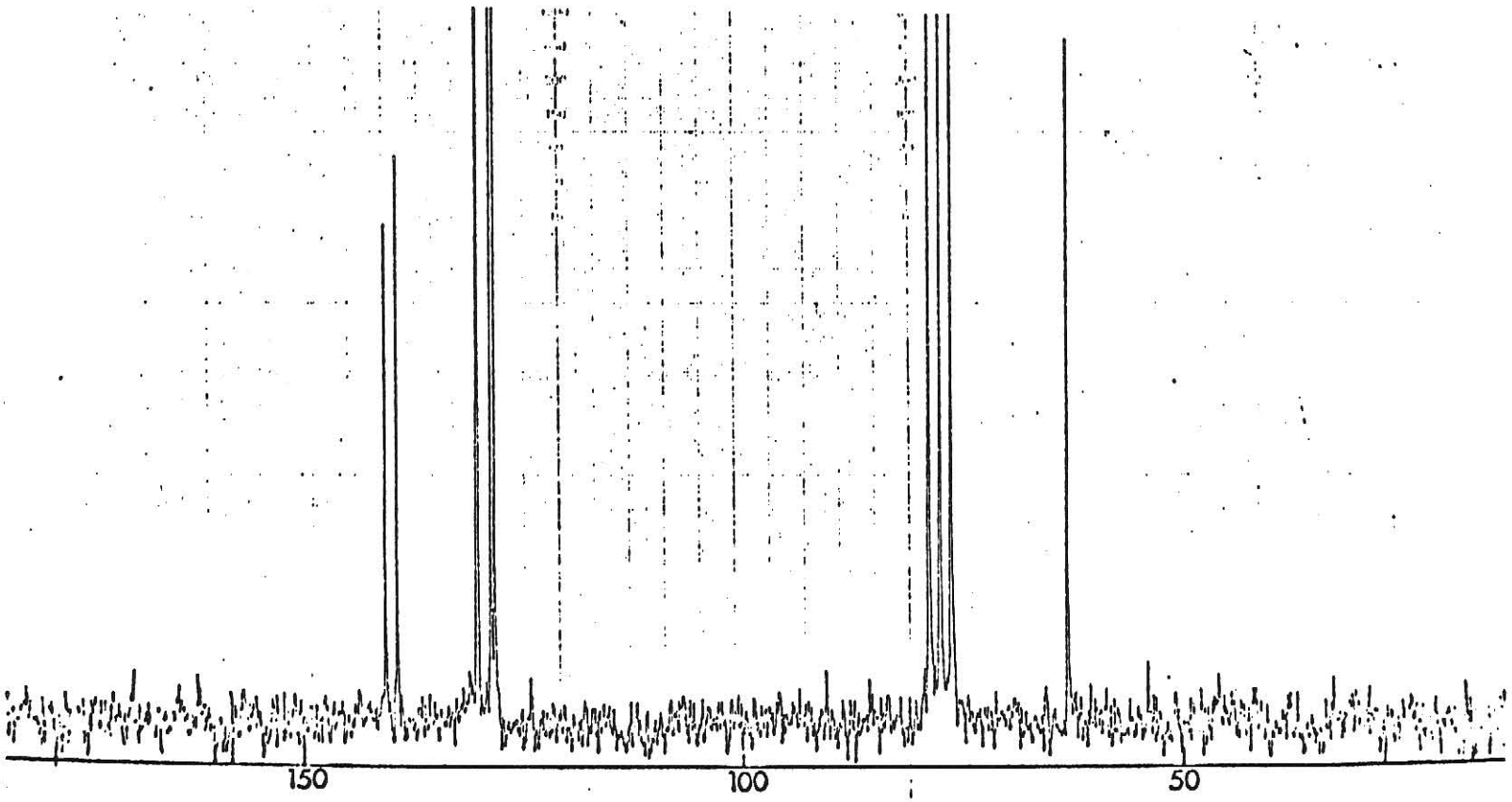
a	139.73
b	138.42
c	129.41
d	129.26
e	127.81
f	127.35
g	62.54



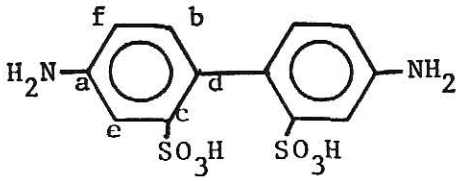
2,2'-HYDROXYMETHYLBIPHENYL

$C_{14}H_{14}O_2$

in $CDCl_3$



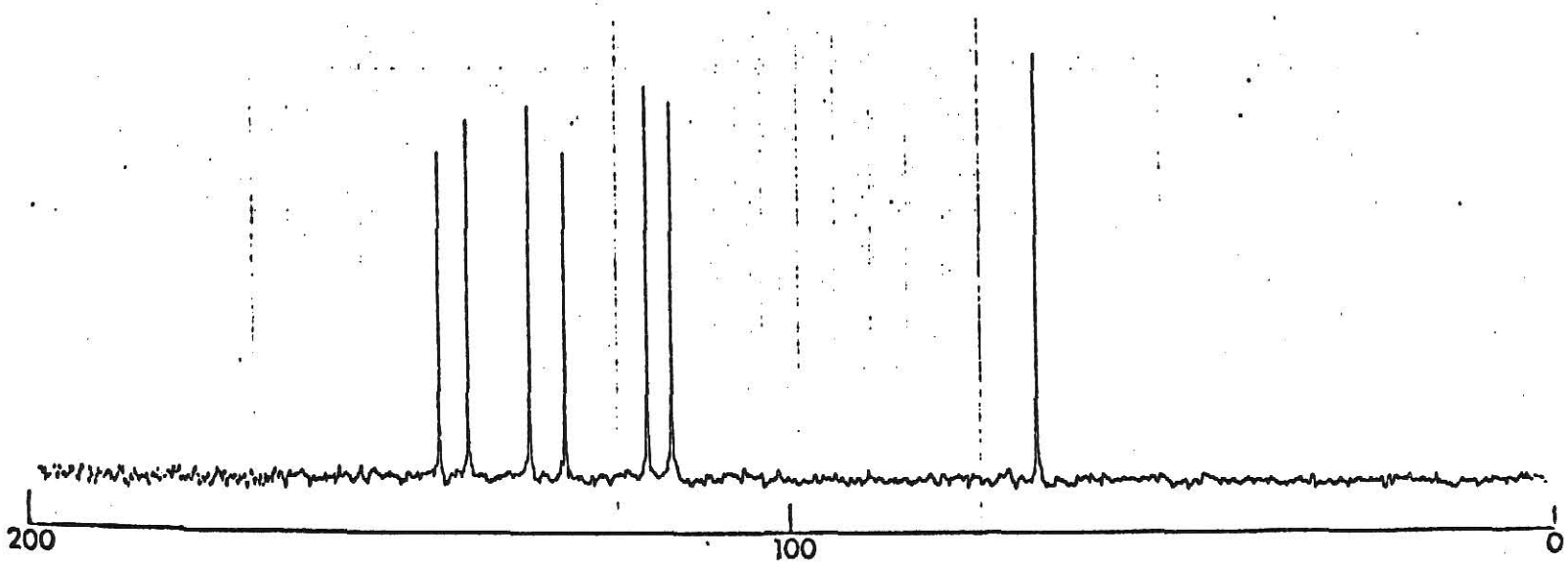
a	145.98
b	142.18
c	134.07
d	129.43
e	118.54
f	115.31



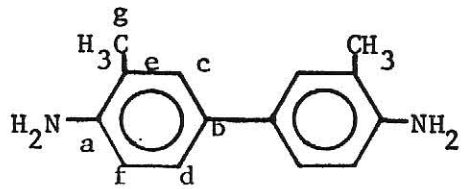
4,4'-DIAMINO-2,2'-BIPHENYLDISULFONIC ACID

$C_{12}H_{12}N_2O_6S_2$

in D_2O with dioxane



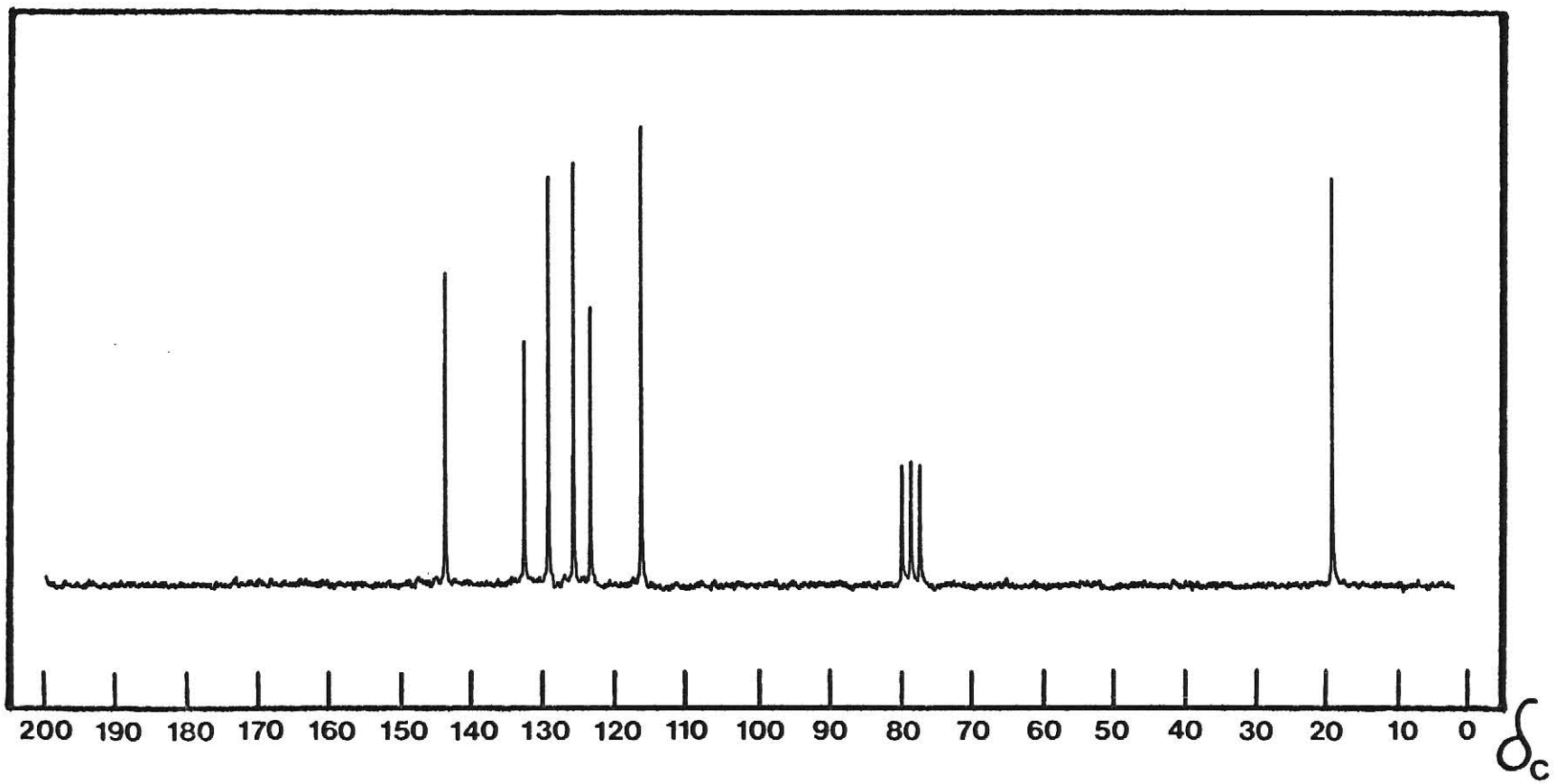
a 142.81
b 131.63
c 128.17
d 124.58
e 122.18
f 115.00
g 17.31



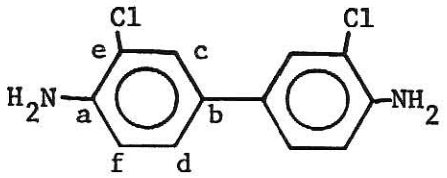
o-TOLIDINE

$C_{14}H_{16}N_2$

in $CDCl_3$



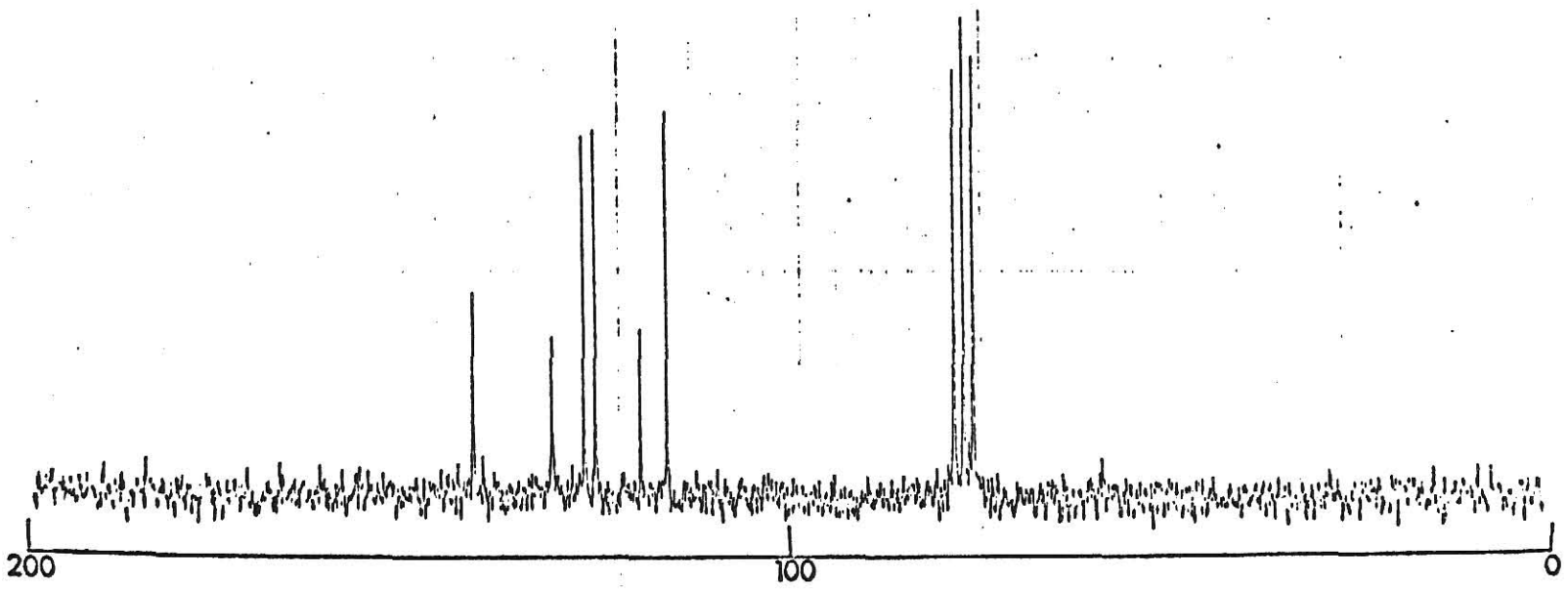
a	141.47
b	131.14
c	126.91
d	125.40
e	119.50
f	115.94



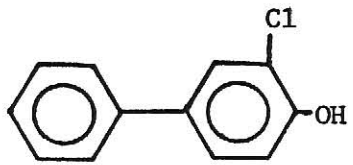
4,4'-DIAMINO-3,3'-DICHLOROBIPHENYL

$C_{12}H_{10}N_2Cl_2$

in $CDCl_3$



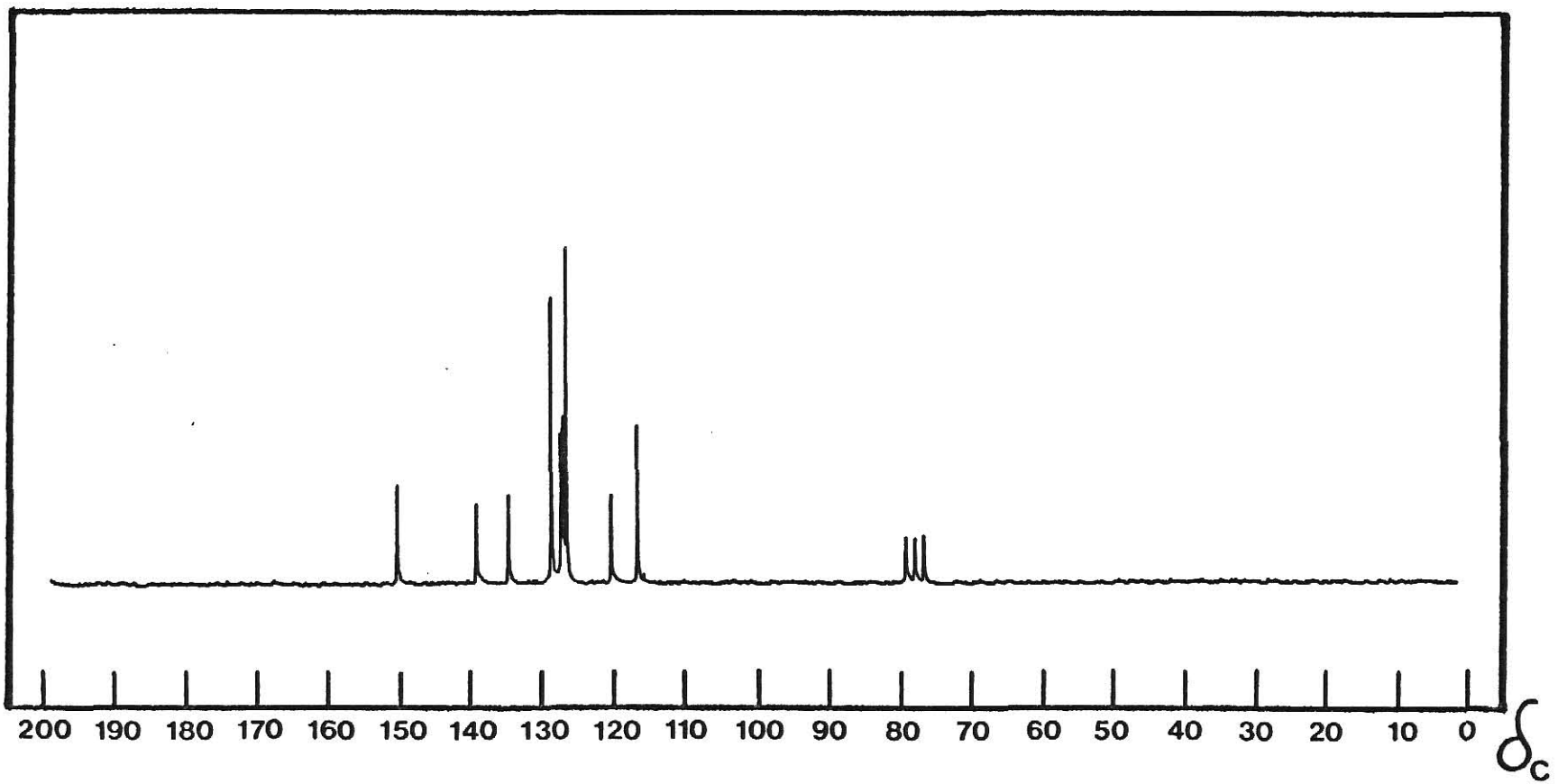
a	150.33	f	126.92
b	139.12	g	126.75
c	134.63	h	126.34
d	128.52	i	120.01
e	127.18	j	116.27



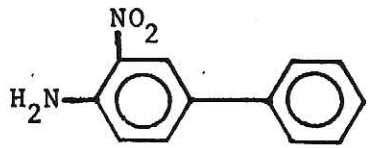
3-CHLORO-4-HYDROXYBIPHENYL

$C_{12}H_9OCl$

in $CDCl_3$



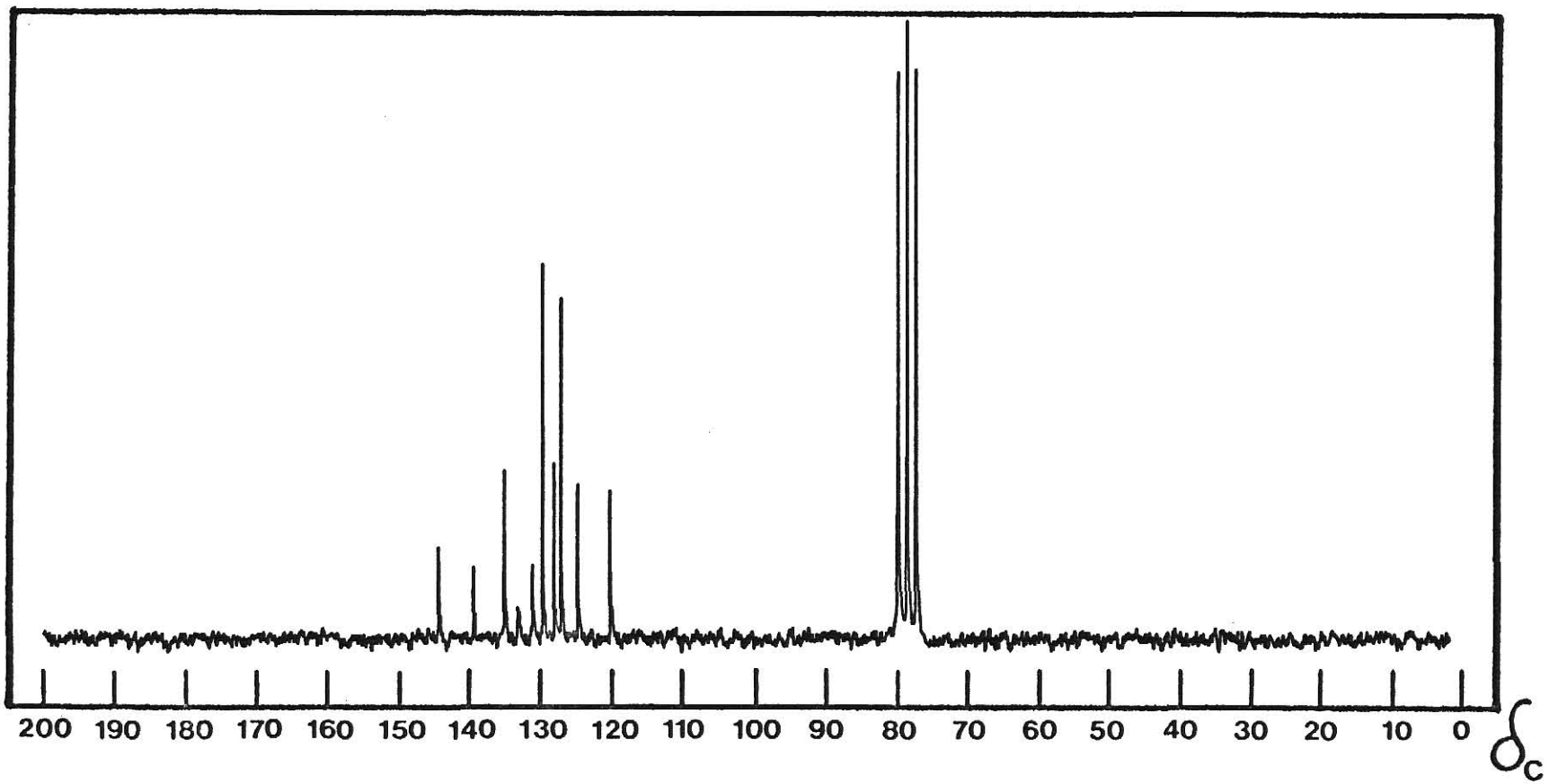
a 143.52
b 138.59
c 134.24
d 132.37
e 130.22
f 128.75
g 127.15
h 126.12
i 123.71
j 119.10



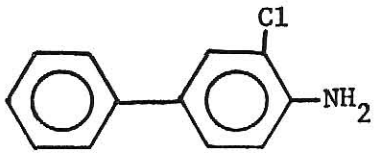
4-AMINO-3-NITROBIPHENYL

$C_{12}H_{10}N_2O_2$

in $CDCl_3$



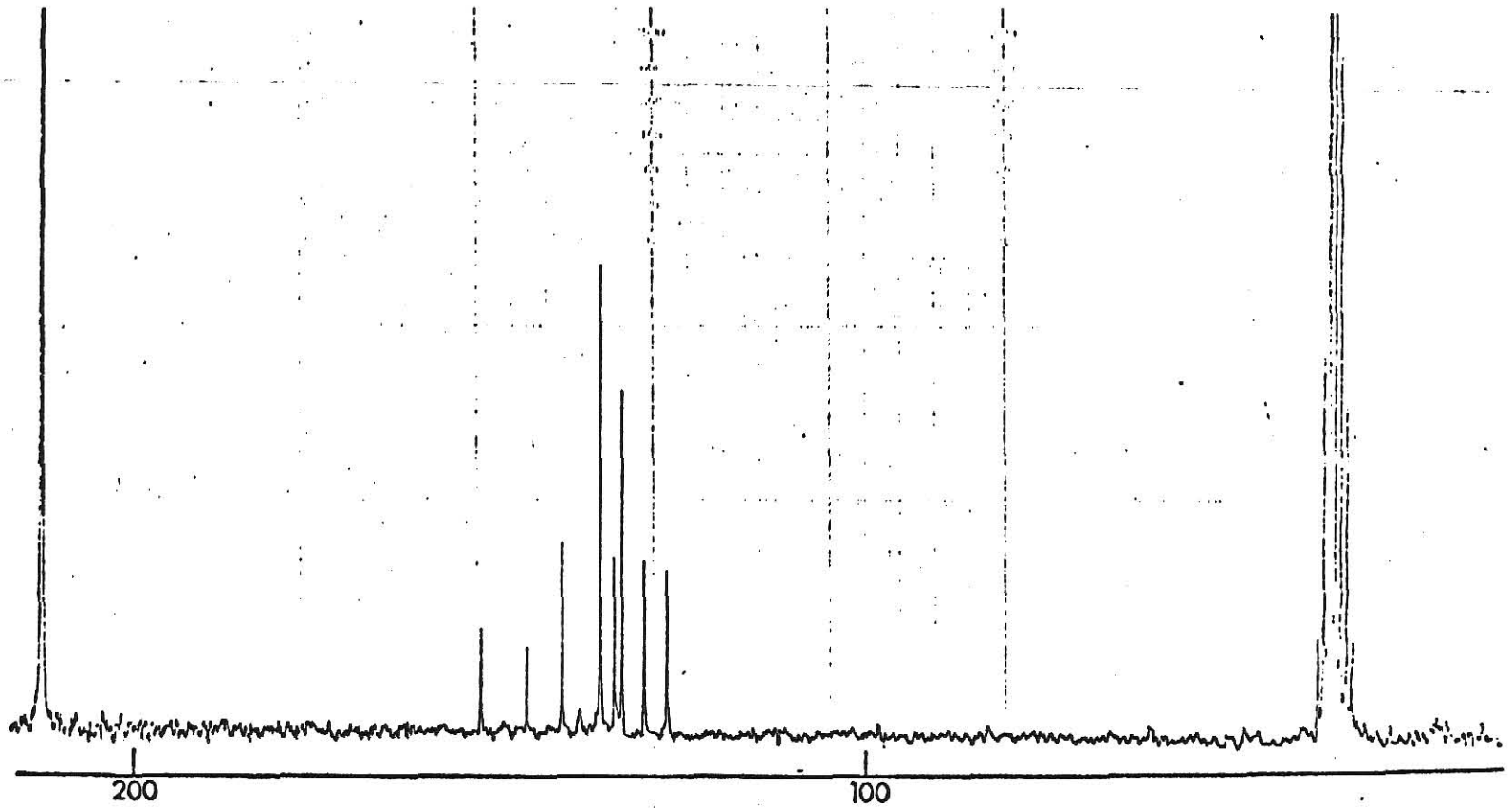
a 146.12
b 139.88
c 135.09
d 129.86
e 127.99
f 126.94
g 123.88
h 120.82



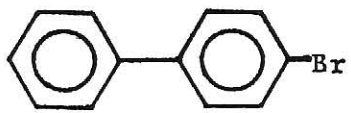
4-AMINO-3-CHLOROBIPHENYL

$C_{12}H_{10}NCl$

in d_6-acetone



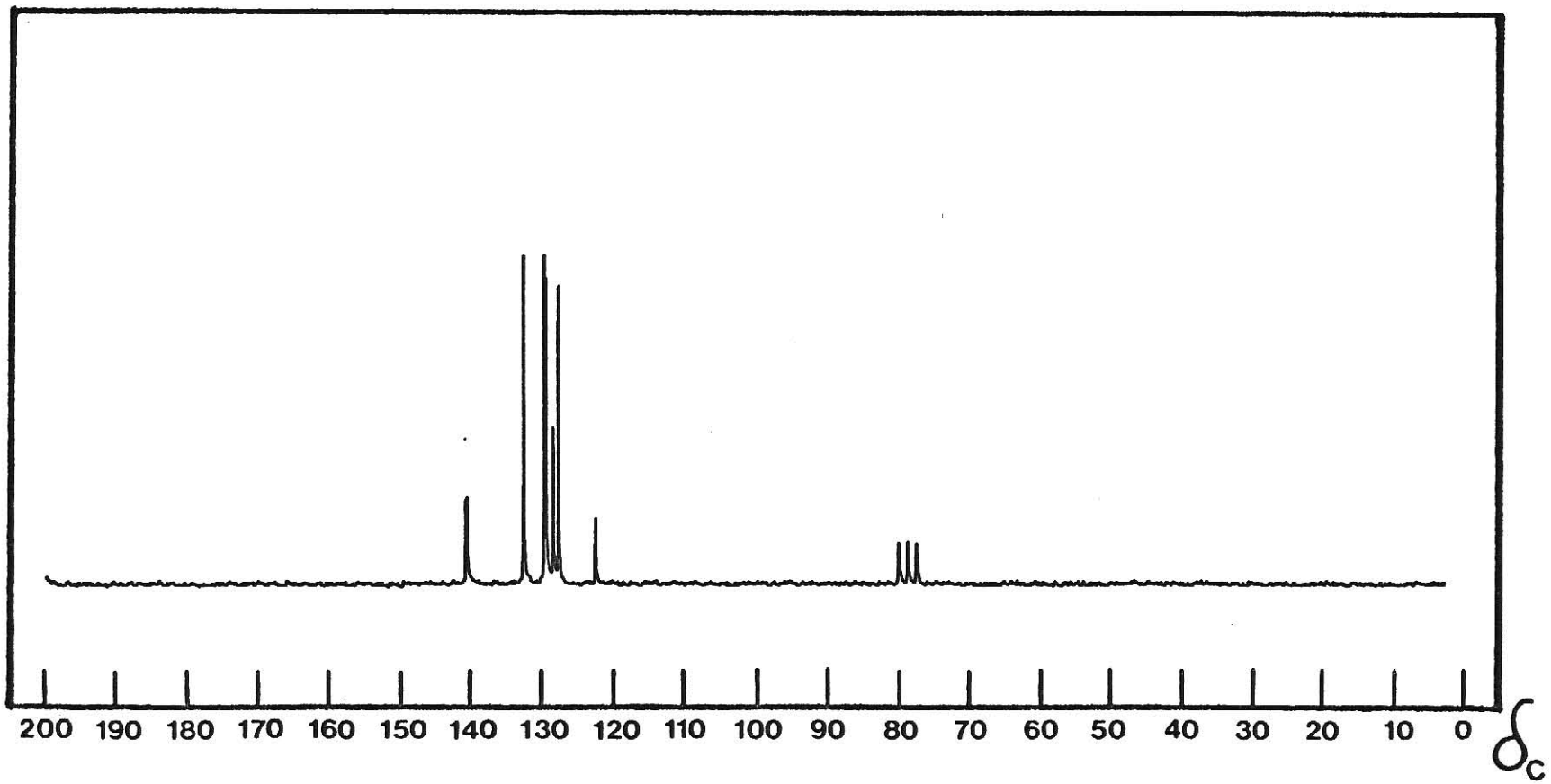
a 139.73
b 139.58
c 131.55
d 128.61
e 128.38
f 127.35
g 126.59
h 121.28



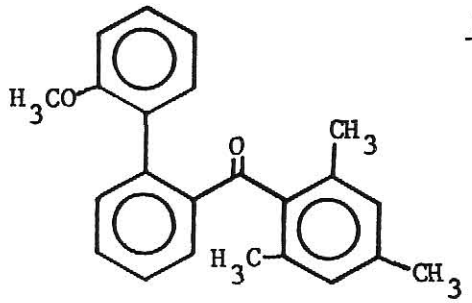
4-BROMOBIPHENYL

$C_{12}H_9Br$

in $CDCl_3$



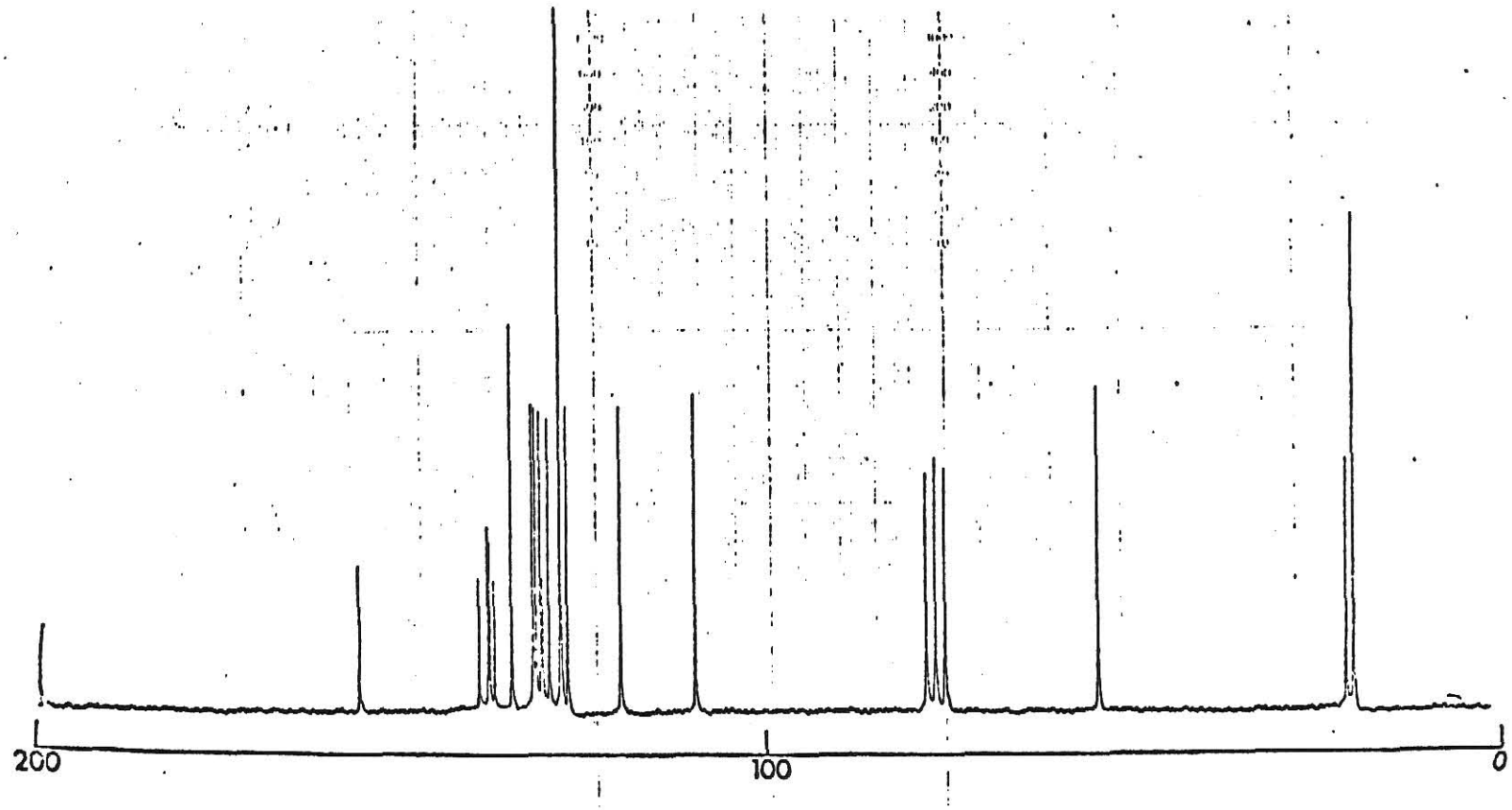
a	199.81	j	130.86
b	155.75	k	130.59
c	139.30	l	129.75
d	138.06	m	128.14
e	137.80	n	127.21
f	137.19	o	119.91
g	134.85	p	109.67
h	131.94	q	54.78
i	131.53	r	20.93
		s	19.89



1,3,5-TRIMETHYL-2'-(2-METHOXYPHENYL) BENZOPHENONE

$C_{23}H_{22}O_2$

in $CDCl_3$



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CARBON-13 NMR SPECTROSCOPY
PART I: THIOLS, THIOLACETATES, AND LIPOIC ACID DERIVATIVES
PART II: SUBSTITUTED BIPHENYLS

by

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ABSTRACT

High resolution ^{13}C Nuclear Magnetic Resonance Spectroscopy is applied to two problems. Part I provides the ^{13}C resonance assignments for several lipoic acid derivatives. Part II contains the ^{13}C resonance assignments of some substituted biphenyls. The assignments are based on peak intensities, multiplicities, coupling constants, and chemical shifts obtained from the ^{1}H-coupled or ^{1}H-decoupled ^{13}C-NMR spectra. T_1 relaxation times, measured by the inversion-recovery or homogeneity spoiling method, also led to several assignments.

Part I

Lipoic acid is an essential cofactor for multienzyme complexes that help oxidize several α-keto acids. It functions in acyl transfer to Coenzyme A and electron transfer to flavin adenine dinucleotide. The acetyl group that is transferred may be attached to lipoic acid at either of two positions. It may be possible to determine the exact position of attachment by using ^{13}C-NMR techniques to study the role of lipoic acid in the reactions catalyzed by multienzyme complexes.

This work furnishes a complete analysis of the ^{13}C chemical shifts of the lipoic acid derivatives; lipoic acid [5-(1,2-dithiolan-3-yl)pentanoic acid] (I), lipoamide [5-(1,2-dithiolan-3-yl)pentanamide] (II), dihydrolipoamide [6,8-dithioloctanamide] (III), 6-S-acetyl-6,8-dithioloctanamide (IV), 8-S-acetyl-6,8-dithioloctanamide (V), and methyl 6,8-S-diacetyl-6,8-dithioloctanoate (VI).

Calculation of the expected ^{13}C shieldings of several lipoic acid derivatives was an integral part of their analysis. This required the knowledge of the chemical shifts of several octanoic acid derivatives in addition to the substituent effect parameters for primary and secondary thiol and thiolacetyl groups. The ^{1}H-coupled and ^{1}H-decoupled ^{13}C-NMR spectra of octanoic acid, octanamide, octanoyl chloride, and methyl octanoate were used in conjunction with the T_1

relaxation measurements of octanoic acid and octanamide to determine the chemical shifts of the octanoic acid derivatives. The -SH and -S-acetyl substituent effects, not available in the literature, were determined by analyzing the ^{1}H-coupled and ^{1}H-decoupled ^{13}C-NMR spectra of 1-thiobutane, 2-thiobutane, 1,3-dithiopropane, 1-thiolacetylbutane, 2-thiolacetylbutane, and 1,3-dithiolacetylpropane.

This study concludes that the lipoic acid derivatives (I-VI) can be readily distinguished by ^{13}C-NMR spectroscopy. Therefore, it encourages further ^{13}C-NMR studies on the enzymatic interconversion of the oxidized (II), reduced (III), and acetylated (IV-VI) forms of lipoamide.

Part II

The ^{13}C resonance assignment of a series of substituted biphenyls is undertaken. The assignments are modelled after substituted benzenes, for which many ^{13}C-NMR studies have been made. These studies indicated that the shielding effects of substituents tend to follow an additive relationship in polysubstituted systems, provided the groups are not ortho. The ^{13}C substituent effects for a number of groups were available and were used to calculate expected ^{13}C shieldings for several biphenyls. The substituent effects for $-SO_3H$, $-SO_2Cl$, -SH, $-CH_2OH$, -NHAc, and $-NH_3^+Cl^-$ groups are also determined in this study for use in calculations.

Resonance theory suggests an interesting feature that may be observed in the ^{13}C shieldings of the biphenyls, referred to as "secondary ring effects". The resonance forms of biphenyls show charges at the ortho and para carbons of the unsubstituted ring. This implies that the effect of a substituent on biphenyl ^{13}C shieldings should be observed in the unsubstituted as well as the substituted ring.

The ^{13}C resonance assignments of several substituted biphenyls are reported in this study. The secondary ring effects are determined as $(\delta_{obs} - \delta_{calc})$

values. For several biphenyls, the secondary ring effects could not be investigated because their treatment was complicated by steric effects or ortho substitution. The 4,4'-disubstituted and 3 or 4-monosubstituted biphenyls, not complicated by steric effects or ortho substitution, show secondary ring effects that are consistent with resonance theory. Any definite conclusions, however, would be premature. The additivity of the secondary ring effects must be verified by the investigation of a complete series of substituted biphenyls.