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Carbon-13 Nuclear Magnetic Resonance Spectra of Thiols and Thiolacetates: Lipoic Acid and Derivatives

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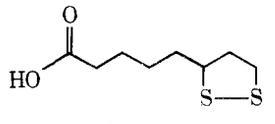
The ^{13}C NMR spectra of lipoic acid [5-(1,2-dithiolan-3-yl)pentanoic acid], lipoamide [5-(1,2-dithiolan-3-yl)pentanamide], dihydrolipoamide (6,8-dithioloctanamide), 6-*S*-acetyl-6,8-dithioloctanamide, 8-*S*-acetyl-6,8-dithioloctanamide, and methyl 6,8-*S*-diacetyl-6,8-dithioloctanoate are reported. Substituent effect parameters for primary and secondary –SH and –*S*-acetyl groups and primary $-CO_2H$, $-CONH_2$, –COCl, and $-CO_2Me$ groups have also been determined.

In connection with studies on the biochemistry of lipoic acid,[4,5] a complete analysis of the chemical shifts of lipoic acid and derivatives was needed. In this paper, we report the ^{13}C resonance assignments for lipoic acid (**1**), lipoamide (**2**), dihydrolipoamide (**3**), 6-*S*-acetyl-6,8-dithioloctanamide (**4**),



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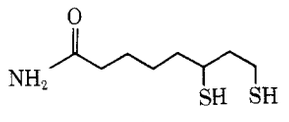

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8-*S*-acetyl-6,8-dithioloctanamide (**5**), and methyl 6,8-*S*-diacetyl-6,8-dithioloctanoate (**6**).

The ^1H-coupled ^{13}C spectra permitted unequivocal assignment of the 6 and 8 carbons of **1–6**. The remaining resonance assignments were made on the basis of T_1 measurements and were further verified by comparison with calculated chemical-shift values. To determine the calculated values, we measured the ^{13}C spectra of octanoic acid, octanamide, octanoyl chloride, methyl octanoate, 1-butanethiol, *S*-acetyl-1-butanethiol, 2-butanethiol, *S*-acetyl-2-butanethiol, propane-1,3-dithiol, and 1,3-*S*-diacetylpropane-1,3-dithiol and made chemical shift assignments. The α, β, γ, and δ substituent effects for primary and secondary –SH and –*S*-acetyl groups were determined, and were used in conjunction with the chemical shifts of the appropriate octanoic acid derivative to calculate expected chemical shifts for several lipoic acid derivatives.

Experimental Section

Lipoic acid and lipoamide were purchased from Sigma. Dihydrolipoamide was prepared by reducing lipoamide with $NaBH_4$.[6] 8-*S*-Acetyl-6,8-dithioloctanamide and 6-*S*-acetyl-6,8-dithioloctanamide were prepared by the enzymatic acetylation of dihydrolipoamide. This was accomplished by coupling the reverse of the physiological reaction catalyzed by the dihydrolipoyl transacetylase component of the pyruvate dehydrogenase complex to the phosphotransacetylase reaction.[6-8] Unreacted dihydrolipoamide [the enzyme reaction uses only the (–) isomer of dihydrolipoamide] was removed from the benzene extract by forming the dithioacetyl derivative of 2-pyridinecarboxaldehyde and then extracting with aqueous acid.

Methyl 6,8-*S*-diacetyl-6,8-dithioloctanoate was prepared from lipoic acid in several steps. First, we treated lipoic acid with methanol and concentrated H_2SO_4 to yield methyl lipoate, which we reduced with $NaBH_4$ to yield methyl 6,8-dithioloctanoate. Then acetylating with acetic anhydride and pyridine yielded methyl 6,8-diacetyl-6,8-dithioloctanoate in a fraction that distilled at 150 °C (0.2 mm).[6]

All other compounds were of either commercial origin or were prepared by standard procedures. The thiolacetates were generally prepared from the thiol and acetyl chloride.

Carbon-13 NMR spectra were obtained with a Varian XL-100-15 spectrometer operating at a frequency of 25.2 MHz and equipped 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 of commercially available materials were mea-

Table I. ^{13}C Chemical Shifts of Alkylthiols and Alkylthiolacetates[a]

Registry no.	Compound	δ_1	δ_2	δ_3	δ_4	$\delta_{C=O}$	δ_{CH_3}
109-79-5	1-Butanethiol	23.71	35.70	21.00	12.94		
928-47-2	*S*-Acetyl-1-butanethiol	27.98	31.08	21.27	12.81	193.50	29.54
513-53-1	2-Butanethiol	24.70	36.69	33.45	11.33		
2432-37-3	*S*-Acetyl-2-butanethiol	20.70	41.00	29.40	11.33	195.64	30.67
109-80-8	Propane-1,3-dithiol	22.21	36.51	22.21			
36648-08-5	1,3-*S*-Diacetylpropane-1,3-dithiol	27.56	29.19	27.56		194.64	30.25

[a] In ppm from Me_4Si, converted from $CDCl_3$ at 76.90 ppm.[9]

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

Registry no.	Compound	δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7	δ_8
124-07-2	Octanoic acid	180.33	33.83	24.53	28.86	28.86	31.57	22.54	13.79
111-64-8	Octanoyl chloride[b]	173.19	46.95	25.00	28.63	28.31	31.43	22.44	13.86
629-01-6	Octanamide	175.93	35.87	25.48	29.10	28.87	31.55	22.48	13.93
111-11-5	Methyl octanoate[b,c]	173.40	33.69	24.67	28.84	28.64	31.38	22.28	13.64

[a] In ppm from Me_4Si, 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 III. 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 IV. ^{13}C Chemical Shifts of Lipoic Acid Derivatives[a]

Registry no.	Compound		δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7	δ_8
62-46-4	Lipoic acid (**1**)		180.22	33.62	24.23	28.43	34.38	56.00	39.81	38.19
940-69-2	Lipoamide (**2**)		175.09	35.49	25.07	28.72	34.52	56.26	40.13	38.37
3884-47-7	Dihydrolipoamide (**3**)	(obsd)	175.26	35.55	24.95	26.47	38.58	39.19	42.61	22.15
		(calcd)		35.87	25.22	27.23	38.59	39.24	43.16	22.57
63640-91-5	8-Acetyl dihydrolipoamide (**5**)	(obsd)	175.41	35.35	24.81	26.57	38.39	39.61	38.18	26.27
		(calcd)		35.55	24.95	26.47	38.45	39.46	37.99	26.42
63640-92-6	6-Acetyl-dihydrolipoamide (**4**)	(obsd)		35.06	24.61	25.75	34.04	42.94	38.56	21.49
		(calcd)		35.55	24.95	26.47	34.58	43.50	38.61	22.15
63658-43-5	Methyl 6,8-diacetyldihydrolipoate (**6**)	(obsd)	173.49	33.71	24.52	26.10	34.61	43.39	34.32	26.41
		(calcd)		33.37	24.04	26.21	34.45	43.23	33.99	26.42

[a] In ppm from Me_4Si, 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.

sured in $CDCl_3$ (15–30%, w/v). The materials prepared by enzymatic reactions were examined at 1 to 10% concentrations w/v. The deuterium resonance of $CDCl_3$ was used as the lock signal. Carbon-13 chemical shifts were measured relative to $CDCl_3$ but are reported relative to external Me_4Si. The conversion to external Me_4Si was by the relationship:

$$\delta_{ext,\,Me_4Si} = \delta_{int,\,CDCl_3} + 76.90 \text{ ppm}^9$$

Spectral reproducibility was ±0.05 ppm. T_1 values were measured by the inversion–recovery or homogeneity-spoiling method. All spectra were measured at a temperature of 35 ± 2 °C.

Results

The ^{13}C chemical-shift data of thiols, thiolacetates, octanoic acid derivatives, and lipoic acid derivatives are presented in Tables I, II, and IV. The chemical-shift assignments are explained in subsequent discussion.

Substituent effect parameters for $-CO_2H$, $-CONH_2$, $-COCl$, and $-CO_2Me$ groups in Table III were calculated from the resonance assignments of the octanoic acid derivatives in Table II and were used rather than values previously reported.[10,12] The values in Table II gave a more accurate comparison to lipoic acid derivatives because the same chain lengths were considered and the spectra were taken under the same conditions.

Substituent effects for primary and secondary thiol and thiolacetate groups are listed in Table VI along with the primary and secondary hydroxyl and acetyl effects. The –SH effects agreed closely with those determined in other studies.[11,15] The –*S*-acetyl effects were not previously reported in the literature.

Table V contains the results of the T_1 measurements of several octanoic acid and lipoic acid derivatives. The nT_1

Table V. 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 (1)	11.00	1.98	2.44	2.68	2.92	2.85	5.76	8.18
Lipoamide (2)	20.43[b]	4.20	4.14	4.00	4.22	4.12	7.70	10.22
Dihydrolipoamide (3)		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 values measured by the inversion–recovery method are reported in seconds and are accurate to ±5%. (n = number of hydrogens attached to C). [b] Determined by the Homospoil Method.

Table VI. Substituent Effects[a] (ppm) of Primary and Secondary Thiol, Hydroxyl, Thiolacetyl, and Acetyl Groups

Carbon position	Primary				Secondary			
	SH[b]	OH[c]	SAc[d]	OAc[c]	SH[e]	OH[c]	SAc[h]	OAc[i]
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[f]	+9.7[f]	+7.5[f]	+6.6[f]
					+8.5[g]	+7.4[g]	+4.4[g]	+3.1[g]
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] Calculated from 1-butanethiol. [c] References 10 and 12. [d] Calculated from S-acetyl-1-butanethiol. [e] Calculated from 2-butanethiol. [f] C-1. [g] C-3. [h] Calculated from S-acetyl-2-butanethiol. [i] Calculated from *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.

values were generally assigned on the basis of segmented motion.[9]

Discussion

The ^{13}C spectra of lipoamide and dihydrolipoamide in Figure 1 each show seven resonances in a range of approximately 30 ppm. Only the C-6 resonance could be assigned unambiguously by examining the compounds with gated decoupling techniques. To assign the resonances of the six remaining methylene groups, thiols and thiolacetates served as models for C-5 through C-8, and octanamide for C-1 through C-4. Using the substituent effects of the thiol, thiolacetate, and amide groups, chemical-shift values were calculated for the dihydrolipoamide derivatives.

1-Butanethiol, S-acetyl-1-butanethiol, 2-butanethiol, and S-acetyl-2-butanethiol were used to determine the primary and secondary –SH and –S-acetyl substituent effect parameters. The resonances of 1-butanethiol and S-acetyl-1-butanethiol were assigned from the ^1H-coupled ^{13}C spectra. The C-1 resonances were assigned from the observed coupling constant $^1J_{CH}$ = 138 ± 2 Hz, typical of sulfur-substituted carbons.[10] For the C-2 through C-4 resonances, typical coupling constants of nonheteroatom-substituted carbons, $^1J_{CH}$ = 126 ± 2 Hz, were observed.[10] The C-4 resonances were identified as quartets. The C-2 and C-3 resonances, both triplets, were distinguished by comparing chemical shifts of 1-butanol and 1-butylacetate. Basis for the assignment was the assumption that the β, γ, and δ substituent effects of a primary –SH group and a primary –S-acetyl group would be similar to the effects of a primary –OH and primary –O-acetyl group, respectively. The α effects of the thiol and thiolacetate groups are in the same direction but substantially smaller than the α 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 calculated based on the relationship $\Delta\delta = (\delta_{\text{thiol or thiolacetate}} - \delta_{n\text{-butane}})$. To ensure that our substituent effect parameters were correct, we measured the ^1H noise-decoupled and ^1H-coupled ^{13}C spectra of propane-1,3-dithiol[13] and 1,3-S-diacetylpropane-1,3-dithiol. The chemical shifts obtained agreed within ±1.0 ppm of those estimated by adding the primary –SH or primary -S-acetyl substituent effects to the δ values of propane.[10] We also used the primary –SH substituent effect values to calculate δ values

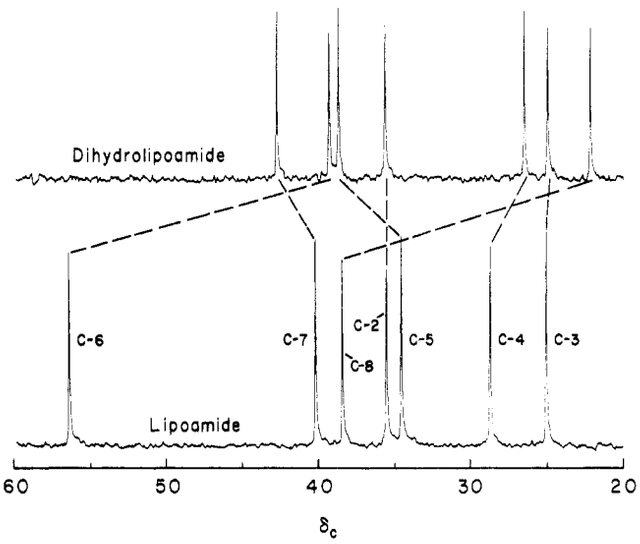

Figure 1. ^{13}C spectra of lipoamide and dihydrolipoamide showing the shifts observed on reduction of lipoamide.

expected for butane-1,2-dithiol[14] and 1-octanethiol.[14] The calculated values agreed within ±1.0 ppm with the observed values. In addition, our –SH parameters were similar to those determined by Nagata et al.[11] and Fava.[15]

The substituent effect parameters for secondary –SH and –S-acetyl groups were similarly determined. The C-2 resonances of 2-butanethiol and S-acetyl-2-butanethiol were observed as a doublet with $^1J_{CH}$ of 138 ± 2 Hz. The C-3 resonance was the only triplet. The C-1 and C-4 resonances, both quartets, were assigned by comparing with the chemical shifts of 2-butanol and 2-butylacetate. The α, β, and γ substituent effect parameters for a secondary –SH or –S-acetyl group were determined from the same $\Delta\delta$ relationship.

The C-2, C-3, C-7, and C-8 resonances of octanoic acid were assigned by comparing the observed δ values with δ values calculated from n-heptane δ values (Table III) and $-CO_2H$ substituent effects.[10] The remaining assignments of the C-4, C-5, and C-6 resonances could be made only from the T_1 measurements. The T_1 values of octanoic acid in Table V exhibit the segmented motion expected from a long,

straight-chain molecule with a hydrogen-bonded end. That required that nT_1 values uniformly decrease as one proceeds toward the hydrogen-bonded end.[9]

The octanamide resonances were assigned by comparisons with the octanoic acid assignments. The expected shifts for $-CONH_2$ vs. $-CO_2H$ and the T_1 measurements further verified the assignment. The T_1 values of octanamide also exhibited segmented motion. The resonance assignments of octanoyl chloride and methyl octanoate followed readily from the octanoic acid and octanamide assignments. The only significant changes in δ values of the octanoic acid derivatives were in the C-1, C-2, and C-3 resonances. The C-4 and C-5 resonances of octanoyl chloride and methyl octanoate are so close that they may require reverse assignment.

With the substituent effect parameters for –SH and the complete assignment of the octanamide resonances, the predicted ^{13}C spectrum of dihydrolipoamide (**3**) was calculated. The ^1H-coupled ^{13}C spectrum of dihydrolipoamide was used to unequivocally assign the C-6 and C-8 resonances. The C-6 resonance was identified as the only doublet, $^1J_{CH} = 138 \pm 2$ Hz. The C-8 resonance was identified as a triplet with $^1J_{CH} = 138 \pm 2$ Hz. All other resonances appeared as triplets with $^1J_{CH} = 126 \pm 2$ Hz. The remaining resonances were assigned by comparing observed and calculated δ values. The calculated and observed values shown in Table IV indicate clearly that all the carbons of dihydrolipoamide can be assigned unambiguously.

The C-6 and C-8 resonances of 6-*S*-acetyl-6,8-dithioloctanamide (**4**), 8-*S*-acetyl-6,8-dithioloctanamide (**5**), and methyl 6,8-*S*-diacetyl-6,8-dithioloctanoate (**6**) were also assigned from the $^1J_{CH}$ values of 138 ± 2 Hz. The remaining resonances of the *S*-acetyl derivatives were assigned by comparisons with calculated δ values. They were determined by adding the appropriate ($\delta_{-S\text{-}AC} - \delta_{-SH}$) factor to the observed δ values of dihydrolipoamide. The calculated values for the 6,8-*S*-diacetyl derivative were further corrected by a ($\delta_{\text{methyl octanoate}} - \delta_{\text{octanamide}}$) factor. Table IV shows the close agreement between the assigned and calculated shifts for all the *S*-acetyl derivatives.

The lipoic acid (**1**) 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 (Table V). The $^1J_{CH}$ values of 138 ± 2 Hz for C-6 and C-8 resonances obtained from the ^1H-coupled ^{13}C spectra also aided in the assignment.

Assignment of the lipoamide (**2**) resonances could not be based on T_1 values because segmented motion was not observed. The C-6 and C-8 resonances could be readily determined from the $^1J_{CH}$ of 138 ± 2 Hz. The remaining five resonances of lipoamide were assigned by comparisons with both the lipoic acid and dihydrolipoamide assignments. It was assumed that the chemical shifts of the C-2 and C-3 lipoamide resonances would not differ much from the dihydrolipoamide δ values. For that reason, $\delta_c = 35.49$ and 24.07 ppm were assigned to C-2 and C-3, respectively. Similarly, the change in chemical shifts of C-4, C-5, and C-7 on conversion of lipoic acid to lipoamide should be negligible. Therefore, chemical shifts of 28.72, 34.52, and 40.13 ppm were assigned to the C-4, C-5, and C-7 resonances of lipoamide, respectively. The close agreement (±0.3 ppm) of the δ values in Table IV supports the assignments.

Figure 1 shows upfield shifts of 17.07 and 16.22 ppm in the C-6 and C-8 chemical shifts, respectively, on conversion of lipoamide to dihydrolipoamide. These dramatic changes are attributed to the stereochemical change from a cyclic to an open-chain form and the chemical change from a disulfide to a dithiol.[11]

The effects of acetylation of the C-6 or C-8 thiol group of dihydrolipoamide on the C-6 or C-8 chemical shifts, though not so large, are still significant. Acetylation of the C-6 thiol group causes a downfield shift of 3.75 ppm in the C-6 resonance. Acetylation of the C-8 thiol group results in a downfield shift of 4.12 ppm in the C-8 resonance. Those changes are significant enough to distinguish between the acetyl derivatives (**4**, **5**, and **6**) of dihydrolipoamide and to encourage further studies on the enzymatic interconversion between the oxidized (**2**), reduced (**3**), and acetylated (**4–6**) forms of lipoamide.

Conclusion

The results of this study have two important and useful consequences. First, the substituent effect parameters of primary and secondary –SH and –*S*-acetyl groups should prove useful in studies of thiols and thiolacetates. Second, the ^{13}C resonance assignments of the lipoic acid derivatives (**1–6**) will be used in subsequent studies on the role of lipoic acid in biochemical systems.

Registry No.—*sec*-Butyl acetate, 105-46-4.

References and Notes

(1) Taken in part from the M.S. Thesis, Department of Chemistry, Kansas State University, 1977.
(2) This work was supported by United States Public Health Service Grant AM 18320.
(3) This work was also supported by the Kansas State Agricultural Experiment Station, Contribution No. 677-j (Chem) and 190-j (Biochem).
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