Properties of Lipoic Acid Analogs (1)

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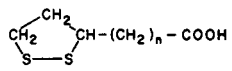
Two chain-shortened analogs of lipoic (1,2-dithiolane-3-pentanoic) acid, *viz.* bisnor-lipoic (1,2-dithiolane-3-propanoic) and tetranorlipoic (1,2-dithiolane-3-carboxylic) acids, have been chemically synthesized. The ir, uv, pmr, and mass spectroscopic properties and paper, thin-layer, and column chromatographic mobilities of lipoic acid and its analogs were investigated and are given.

Lipoic (thioctic) acid (Figure 1) was first isolated in 1951 (5), and its structure was soon established as 1,2-dithiolane-3-pentanoic acid (6). Lipoic acid is an essential



Lipoic (1,2-dithiolane-3-pentanoic) acid, n=4
Bisnor-lipoic (1,2-dithiolane-3-propanoic) acid, n=2
Tetranor-lipoic (1,2-dithiolane-3-carboxylic) acid, n=0

Figure 1. Chemical structure of lipoic acid and its analogs.

coenzyme for all systems of α-keto acid dehydrogenase complexes that have been investigated. Although considerable information is available concerning the mechanism of lipoic acid action (7,8), little is known concerning the metabolism of this compound. A bacterium identified as *Pseudomonas putida* LP was isolated and adapted to grow in a synthetic medium, which contained 0.4% lipoic acid as the sole source of carbon, sulfur, and energy. One major catabolite was identified as bisnor-lipoic (1,2-dithiolane-3-propanoic) acid, and β-oxidation of the side chain of lipoate was thus indicated (9).

In order to identify and characterize the catabolites of the degradation of lipoate, chain-shortened analogs of lipoic acid, *viz.* bisnor-lipoic and tetranor-lipoic (1,2-dithiolane-3-carboxylic) acids were chemically synthesized. Their spectroscopic and chromatographic properties will be presented here, and their biological properties and catabolic utilization will be reported elsewhere (10).

The ir spectra of lipoic acid and analogs are shown in Figure 2. Strong C=O stretchings at 5.8-5.9 μ and characteristic C-S stretchings at 14.5-15.0 μ were observed in all three spectra. The expected S-S stretching, falling between 20-25 μ, is outside the range shown. A weak absorption at 13.6 μ, indicating $-(CH_2)_n-$ where $n > 3$, is significant only in the spectrum of lipoic acid.

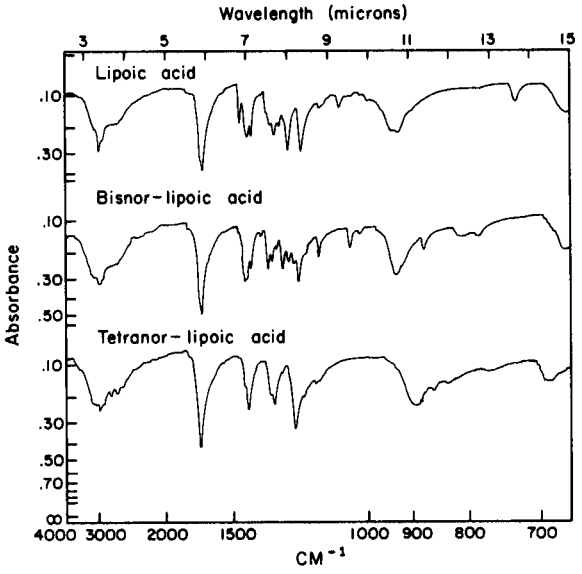


Figure 2. Ir spectra of lipoic acid and its analogs in potassium bromide pellets.

Uv absorption spectra of the three analogs are shown in Figure 3. In addition to the carboxylic absorption maximum in the short-wavelength region (< 250 nm), all three compounds have a disulfide absorption maximum at 330 nm, with molar extinction coefficients: lipoic acid, 150; bisnor-lipoic acid, 160; tetranor-lipoic acid, 159. An absorption at 330 nm is a characteristic of the dithiolane ring, while aliphatic dialkyl disulfides with a CSS-SSC dihedral angle near 90° have an absorption maximum at 250 nm. A simple relationship between the uv absorption displacement toward longer wavelengths and the reduction of CSS-SSC dihedral angles has been observed and supported by energy calculations (11). The CSS-SSC dihedral angle in the dithiolane ring was thus estimated to be approximately 27°. From x-ray crystallographic data, the di-

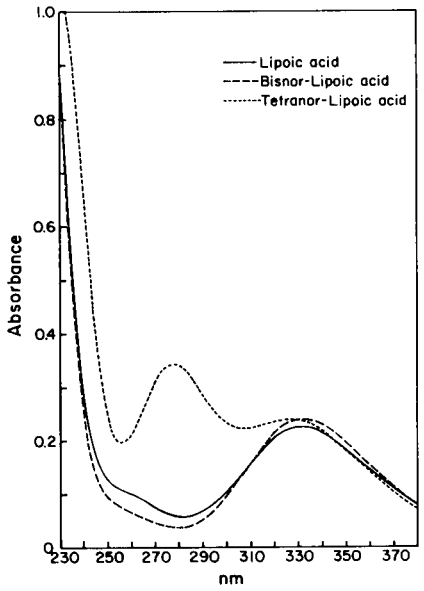


Figure 3. Uv absorption spectra of lipoic acid and its analogs (1.5 x 10^{-3} *M* in 95% ehtanol).

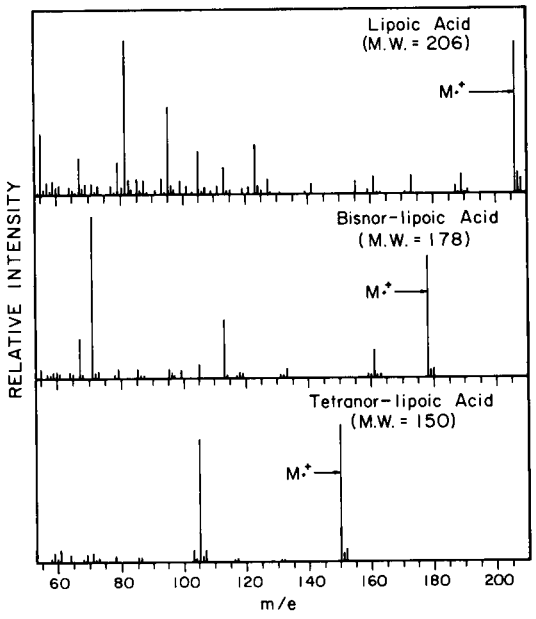


Figure 5. Mass spectra of lipoic acid and its analogs.

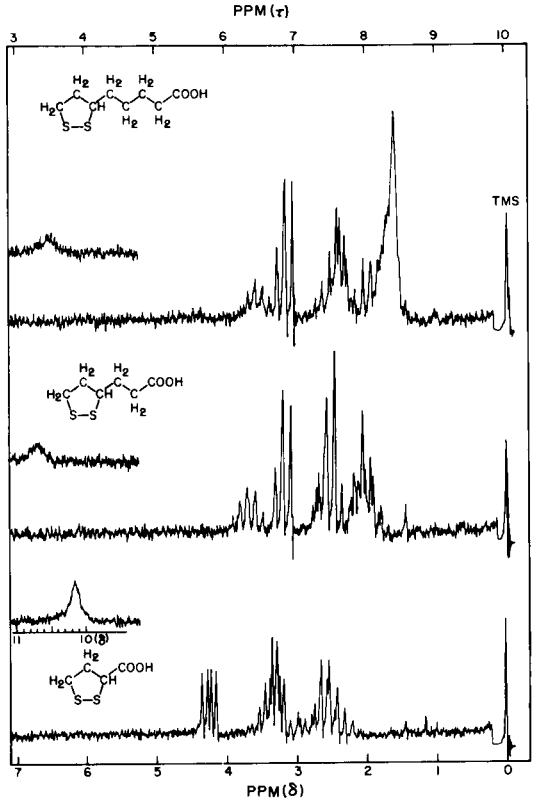


Figure 4. Pmr spectra of lipoic acid and its analogs (0.2 *M* in deuterated chloroform containing 1% tetramethylsilane).

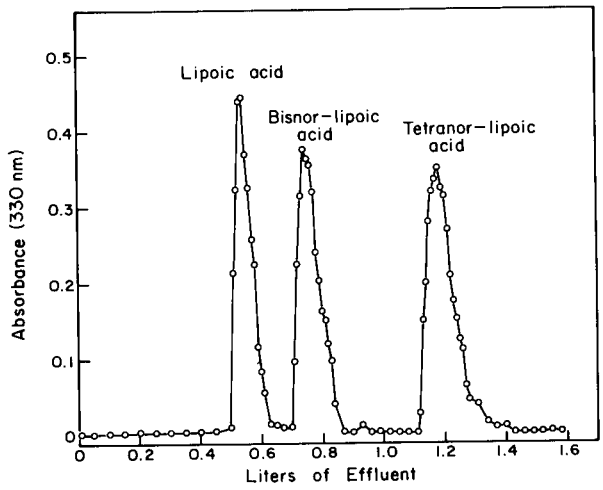


Figure 6. Sephadex LH-20 column chromatography of lipoic acid and its analogs.

hedral angles were found to be 26.6° in 1,2-dithiolane-4-carboxylic acid (12), a compound which is an isomer of tetranor-lipoic (1,2-dithiolane-3-carboxylic) acid, and 35° in lipoic acid (13). On the other hand, tetranor-lipoic acid has another stronger absorption maximum at 280 nm, with a molar extinction coefficient of 250. This absorption maximum is missing in lipoic, bisnor-lipoic, and also 1,2-dithiolane-4-carboxylic acids (14), and must be due to a strong interaction between the disulfide bond and adjacent carboxyl group. Therefore, the conformation, or,

TABLE I

Nmr Details of Lipoic Acid and Analogs

Compound	Protons on carbon no. (a)	Chemical shift (δ)
Lipoic acid	3,4,5	1.6
	7	1.8-2.0
	2	2.2-2.6
	8	2.9-3.3
	6	3.4-3.7
	carboxylic	10.5
Bisnor-lipoic acid	3,5	1.8-2.2
	2	2.4-2.7
	6	3.1-3.3
	4	3.4-3.9
	carboxylic	10.7
Tetranor-lipoic acid	3	2.2-2.8
	4	3.1-3.6
	2	4.2-4.4
	carboxylic	10.2

(a) Numbered from the carboxyl carbon as 1.

TABLE II

R_f Values from Paper and Thin-Layer Chromatography of Lipoic Acid and its Analogs

Compound	Paper (a)	Thin-layer (b)
Lipoate	0.50	0.72
Bisnor-Lipoate	0.40	0.66
Tetranor-lipoate	0.30	0.63

(a) Whatman No. 1 with a solvent of butanol: ethanol: ammonium hydroxide (8:2:2, v/v); compounds detected by $Na_2Fe(CN)_5$ NO-NaCN spray. (b) MN Silica gel N-HR with a solvent of chloroform:methanol:formic acid (8:1:1, v/v); compounds detected by iodination.

more specifically, the CSS-SSC dihedral angle, of the tetranor-lipoic acid may not be the same as for other dithiolane-containing compounds, which absorb only at 330 nm and have a dihedral angle of approximately 27°.

This unique structural property of tetranor-lipoic acid is further evidenced by the proton magnetic resonance spectra of the three analogs in deuterated chloroform (Figure 4). The assignments of protons to peaks at various chemical shift positions (δ, ppm) in the three spectra are summarized in Table I. From lipoic acid to tetranor-lipoic acid, as the carboxyl group approaches the dithiolane ring, a general trend of downfield shifting of chemical shift positions is observed, except for the carboxylic proton of tetranor-lipoic acid, which shows an unusual upfield shift, with a peak that is sharpened at 10.2 δ (ppm). A hydrogen-bonded proton in tetranor-lipoic acid can thus be suggested. Constructs of molecular models allow the possibility of hydrogen bonding through the carboxylic proton and the γ-sulfur atom (sulfur 1 in 1,2-dithiolane-3-carboxylic acid).

The mass spectra of lipoic acid and analogs (Figure 5) show a high intensity of molecular ion peaks, *i.e.* 206 for lipoic acid, 178 for bisnor-lipoic acid, and 150 for tetranor-lipoic acid. Isotopic peaks attributable to sulfur, M+1 and M+2, are present in all three spectra. No especially common patterns of fragmentation are observed for the three compounds, though a peak at m/e = 105, representing the 1,2-dithiolane ion, is observed in all three spectra.

The R_f values from paper and thin-layer chromatography of lipoic acid and analogs are summarized in Table II. Paper chromatography with a solvent of butanol:ethanol:ammonium hydroxide (8:2:2, v/v) produced more distinguishable R_f values of the three analogs and, hence, a better separation.

The results of Sephadex LH-20 column chromatography of lipoic acid and its analogs are shown in Figure 6. The three analogs in the effluent from the Sephadex LH-20 column were distinctly separated and their identities surmised by correspondence of molecular sizes and solubilities with the sequence of elution. The R_f values from paper chromatography, using the known pure compounds as standards, confirmed the identification. The recovery of the compounds was calculated to be 90 to 95% on the basis of known molecular extinction coefficients at 330 nm. Chromatography on other types of columns, e.g. silica gel, aluminum oxide, anion exchangers, and Amberlite XAD-2 has also been attempted, but the results were not as satisfactory.

EXPERIMENTAL

Syntheses of Lipoic Acid Analogs.

Bisnor-lipoic acid was synthesized from monomethyl succinate (Aldrich Chemical Company) after the method of Kumagaya and Kasuga (15). The total yield was about 17%. Purified by repeated recrystallizations from cyclohexane, yellow crystals of bisnor-lipoic acid melt at 56° (b.p. 150° at 0.2 mm Hg). Some analytical characteristics not previously reported for the isolated and stable intermediates in the synthesis are summarized in Table III.

Tetranor-lipoic acid was synthesized from γ-butyrolactone (Aldrich Chemical Company) according to Claeson (16) as modified by Wladislaw (17). The total yield was about 20%. Purified by recrystallization from a cyclohexane:benzene (1:1, v/v) solvent mixture, the light yellow crystals melt at 81° (b.p. 160° at 0.7 mm Hg). A summary of some analytical characteristics not previously reported for the stable intermediates in the synthesis is shown in Table IV.

Spectrometric Analyses.

Ir spectra were obtained either on sodium chloride plates for liquid samples or in potassium bromide pellets for solid samples in

TABLE III

Analytical Characteristics of Intermediates in the Syntheses of Bisnor-Lipoic Acid

Compound	m.p.	b.p. (mm Hg)	R_f on TLC (a)	Principal absorption by Ir (μ)	δ (ppm) by Pmr
Monomethyl succinate	53-56°				2.4 3.3 9.4
6-Chloro-4-hexano-lactone		103-104° (0.5)	0.79	3.4 5.65 8.5 9.6	1.5-1.8 2.4-2.8 3.4-3.9
6-Benzylmercapto-4-hexanolactone		178° (0.1)	0.82	3.4 5.65 8.5 9.7 14.25	1.6-2.4 3.4 4.0-4.4 7.1
4,6-Dibenzylmercapto-hexanoic acid	55-57		0.78	3.4 5.8 8.0 14.35	0.9-2.8 3.6 7.2

(a) Thin-layer plates: MN-polygram Sil gel N-HR; solvent system: chloroform:methanol:formic acid (8:1:1, v/v/v). Detection was by iodine vapor.

TABLE IV

Analytical Characteristics of Intermediates in the Synthesis of Tetranor-Lipoic Acid.

Compound	m.p.	b.p. (mm Hg)	Principal absorption by IR (μ)	δ (ppm) by Pmr
γ-Butyrolactone	206°		3.4 5.6 8.6 9.6 10.1	1.9-2.6 4.29
Methyl 2,4-dibromobutyrate		85° (0.6)	2.9 5.8 7.0 7.8 8.6	
Methyl 2,4-dibenzylmercapto-butyrate		202° (0.2)	3.4 5.75 6.7 6.85 8.7 13.0 14.3	1.8-2.1 2.9-3.1 3.3-3.7 7.2
2,4-Dibenzylmercaptobutyric acid		260° (0.2)	3.3 5.85 6.7 6.9 7.8 9.35 9.75 13.0 14.3	1.8-2.2 3.5 3.8 7.25

a Perkin-Elmer Infracord spectrophotometer. Uv spectra of 1.5 x 10^{-3} *M* solutions of lipoic acid and analogs in 95% ethanol were taken with a Perkin-Elmer Model 356 spectrophotometer. Pmr spectra of 0.2 *M* solutions of samples in deuterated chloroform containing 1% tetramethylsilane were obtained with a Varian A60A (60 MHz)-nmr spectrometer at ambient temperature. Mass spectrum analyses were carried out by the Cornell High Resolution Mass Spectral Facility, Department of Chemistry, Cornell University.

Chromatographic Analyses.

Both paper and thin-layer chromatographic techniques have been described (9). The Sephadex LH-20 (Pharmacia Fine Chemicals) column was prepared after the method described by Downey *et al.* (18) by transferring a suspension of 100 g. of Sephadex LH-20 in chloroform (AR grade, Mallinckrodt Chemical Works) into a glass chromatography column. The dimensions of a tightly packed column were 2.8 x 35 cm. A mixture of lipoic, bisnor-lipoic, and tetranor-lipoic acids (30 mg. each) was eluted with chloroform at a flow rate of 1 ml./minute. The effluent was collected in 10-ml. fractions. The presence of lipoic acid and analogs in the effluent was detected and estimated by the absorbance at 330 nm with a Beckman DU spectrophotometer.

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