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Studies on the Metabolism of Unsaturated Fatty Acids. III.[1] Structural Determination of *cis*- and *trans*-Isomers of 3- or 2-Alkenoic Acids by Nuclear Magnetic Resonance Spectroscopy using a Chemical Shift Reagent

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Nuclear magnetic resonance spectroscopy with a lanthanide chemical shift reagent was applied for the structural determination of *cis*- and *trans*-isomers of 3-alkenoic acids, prepared as substrates for some enzyme reactions related to fatty acid metabolism. The configuration of N-acetylcysteamine derivatives of *cis*-2-alkenoic acids was also determined by the same technique.

Keywords——NMR spectra of 2- and 3-alkenoic acids; NMR spectra of N-acetylcysteamine derivatives; tris(heptafluorobutanoylpivaloylmethanato)europium^{III}; *cis*-2-alkenoic acids; *cis*-3-alkenoic acids; *trans*-3-alkenoic acids

In our previous investigations, we separated a new reductase from *Candida*[3] and *Escherichia coli*.[1] The enzyme requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor and acts specifically on *cis*-2-alkenoyl-coenzyme A (CoA), being different from NADPH-dependent *trans*-2-enoyl-CoA reductase, which is involved in the chain elongation system. It was also found that *cis*-3-dodecenoyl-CoA was efficiently reduced to dodecanoyl-CoA in the presence of *Candida* extracts. It remains to be clarified whether the reduction proceeds directly or whether it is a coupled reaction with *cis*-3-, *trans*-2-enoyl-CoA isomerase.

Furthermore, it has been observed that considerable amounts of a *cis*-2-alkenoic acid are non-enzymatically converted to the *trans*-isomer[4] during preparation of the CoA derivative by the mixed anhydride method, in which a Michael addition of the thiol compound to the double bond is also possible. Therefore, it is necessary to determine the structures of *cis*-2-alkenoic acids and their thiol esters before their use as substrates to investigate the properties of the above-mentioned reductase and isomerase, especially in connection with the chain length specificity.

Nuclear magnetic resonance (NMR) spectroscopy is a suitable and convenient technique for the analysis of these substrates. Its application is, however, limited in the analysis of long chain fatty acids because of the coincident chemical shifts of the successive methylene protons in the chain. These protons afford broad and overlapping signals, so that the determination of the coupling constants is difficult without the use of a chemical shift reagent,[5] at least with generally available instruments.

This paper deals with the application of NMR spectroscopy to the structural determination of 2- and 3-alkenoic acids and N-acetylcysteamine esters, which are model compounds of CoA

1) Part II: M. Mizugaki, T. Unuma, and H. Yamanaka, *Chem. Pharm. Bull.*, **27**, 2334 (1979).
2) Location: *Aobayama, Sendai 980, Japan.*
3) K. Ishidate, M. Mizugaki, and M. Uchiyama, *J. Biochem.*, **74**, 279 (1973); K. Ishidate, M. Mizugaki, and M. Uchiyama, *Chem. Pharm. Bull.*, **22**, 2685 (1974).
4) M. Mizugaki, Y. Ito, T. Hoshino, T. Shiraishi, and H. Yamanaka, *Chem. Pharm. Bull.*, submitted.
5) D. Swern and J.P. Wineburg, *J. Am. Oil Chemists' Soc.*, **48**, 371 (1971); J.P. Wineburg and D. Swern, *J. Am. Oil Chemists' Soc.*, **49**, 267 (1972).

Table I. NMR Spectral Data for *cis*-3-Alkenoic Acids (A) and the *trans*-Isomers (B)

	$-\overset{5}{C}H_2-$	$-\overset{4}{C}H=\overset{3}{C}H-$	$-\overset{2}{C}H_2-$	COOH
A				
Octenoic	1.80—2.45(2H, m)[a]	5.35—5.85(2H, m)	3.18(2H, d, J=4.5 Hz)	11.45(1H, s)
Decenoic	1.80—2.45(2H, m)	5.45—5.71(2H, m)	3.08(2H, d, J=4.5 Hz)	11.45(1H, s)
Dodecenoic	1.70—2.40(2H, m)	5.40—5.65(2H, m)	3.07(2H, d, J=4.5 Hz)	11.90(1H, s)
Tetradecenoic	1.85—2.45(2H, m)	5.43—5.68(2H, m)	3.08(2H, d, J=4.5 Hz)	11.32(1H, s)
Hexadecenoic	1.75—2.38(2H, m)	5.40—5.63(2H, m)	3.06(2H, d, J=5.0 Hz)	11.25(1H, s)
B				
Hexenoic	1.76—2.30(2H, m)	5.40—5.63(2H, m)	2.99(2H, d, J=4.5 Hz)	11.83(1H, s)
Octenoic	1.81—2.30(2H, m)	5.37—5.62(2H, m)	2.98(2H, d, J=4.5 Hz)	11.72(1H, s)
Decenoic	1.82—2.20(2H, m)	5.38—5.64(2H, m)	2.99(2H, d, J=4.5 Hz)	11.93(1H, s)
Dodecenoic	1.80—2.23(2H, m)	5.38—5.60(2H, m)	3.00(2H, d, J=4.5 Hz)	11.87(1H, s)
Tetradecenoic	1.83—2.20(2H, m)	5.41—5.65(2H, m)	3.01(2H, d, J=4.5 Hz)	10.70(1H, s)

a) Abbreviations: s=singlet, d=doublet, m=multiplet.

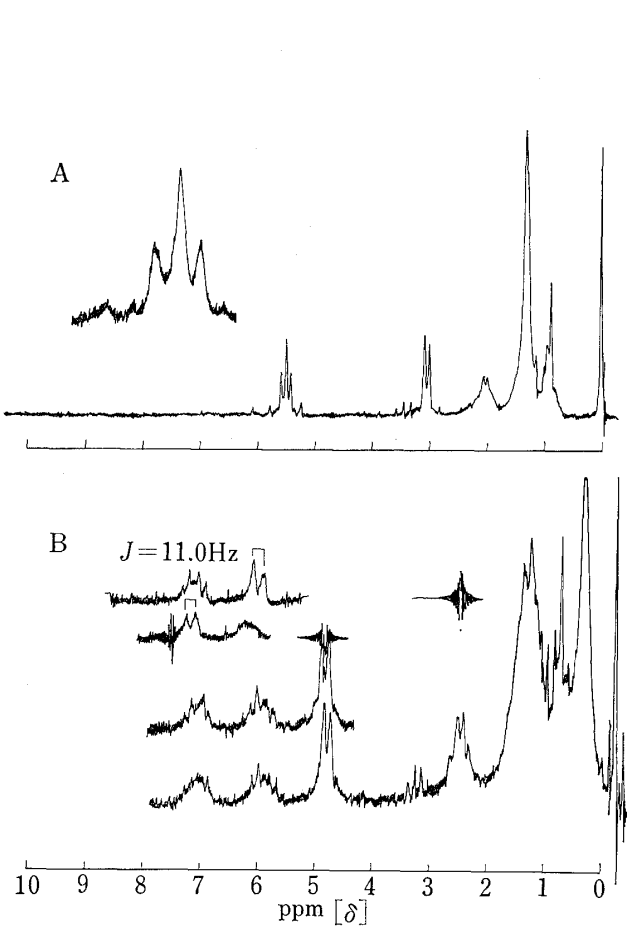

Fig. 1. NMR Spectra of *cis*-3-Decenoic Acid in the Absence (A) and in the Presence (B) of $Eu(fod)_3$

A: The sample (18.8 mg) was dissolved in CCl_4 (0.3 ml).
B: $Eu(fod)_3$ was added to the sample at a molar ratio of 0.3.

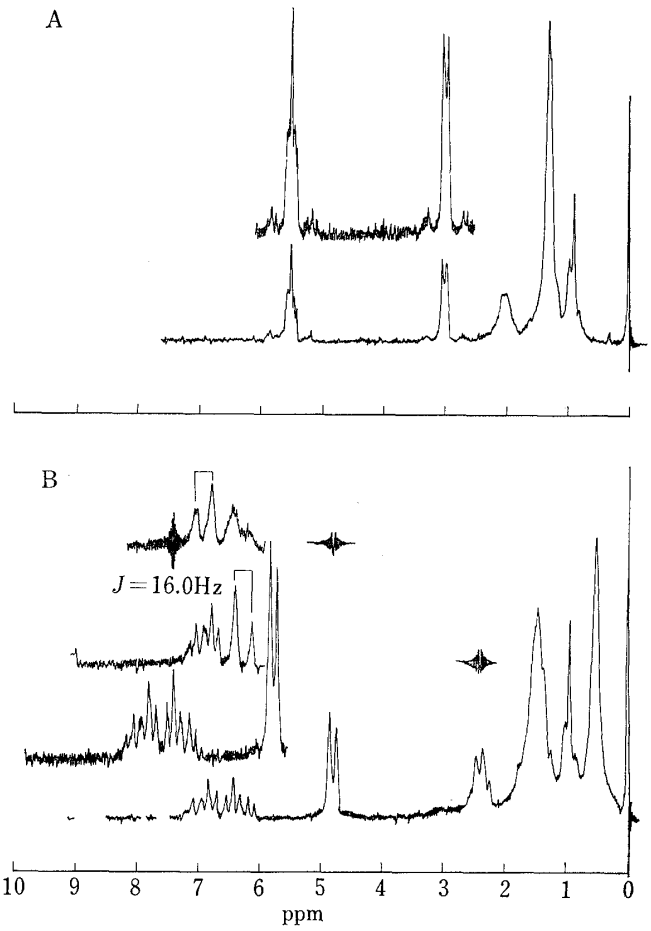

Fig. 2. NMR Spectra of *trans*-3-Decenoic Acid in the Absence (A) and in the Presence (B) of $Eu(fod)_3$

A: The sample (18.7 mg) was dissolved in CCl_4 (0.3 ml).
B: $Eu(fod)_3$ was added to the sample at a molar ratio of 0.3.

derivatives, using tris(heptafluorobutanoylpivaloylmethanato)europium^{III}(Eu(fod)$_3$) as a chemical shift reagent, if necessary. The NMR spectra of the *cis*- and *trans*-isomers of various 2-alkenoic acids showed characteristic signals due to 2-olefinic protons, and the coupling constants of these signals were easily estimated to be 12 and 16 Hz, respectively.

On the other hand, it is difficult to determine the coupling constants of the olefinic protons in 3-alkenoic acids, as shown in the table. The data for *cis*- and *trans*-isomers of 3-decenoic acid are illustrated in Fig. 1-A and Fig. 2-A as examples. The illustrated NMR spectra are in good agreement with the 3-alkenoic acid structure, but no information on the configuration of the double bond was obtained.

Thus, the effects of a lanthanide reagent, Eu(fod)$_3$, on the NMR spectra of *cis*-3-alkenoic acids were tested. Incremental additions of Eu(fod)$_3$ to the sample led to the tentative assignment of signals. While signals for two olefinic protons were observed at 5.3—5.8 ppm as a multiplet in the absence of Eu(fod)$_3$, the signal for one olefinic proton on the γ-position appeared at 5.7—6.6 ppm and the signal for another olefinic proton on the β-carbon appeared at 6.9—7.6 ppm in the presence of the shift reagent at a molar ratio of 0.3 with respect to the substrate (Fig. 1-B).

Irradiation of the broad doublet due to α-methylene protons at 4.9—5.3 ppm changed the broad β-CH signal (6.9—7.6 ppm) to a doublet. Decoupling the δ-methylene proton multiplet at 2.5—3.1 ppm changed the multiplet at 5.7—6.6 ppm to a doublet. From these data, the coupling constant was determined to be 11.0 Hz (Fig. 1-B); this value is consistent with the usual *cis* coupling of olefinic protons.

Similarly the coupling constant of *trans*-3-decenoic acid was determined to be 16.0 Hz in the presence of Eu(fod)$_3$ at a molar ratio of 0.3 relative to the substrate by the decoupling method (Fig. 2-B).

This technique was then applied to the structural determination of 2-alkenoyl-NAC derivatives, which were prepared as model compounds of the coenzyme A esters. Details of the preparation and purification of the NAC derivatives will be reported and discussed elsewhere.

The NMR spectrum of *trans*-2-octenoyl-NAC (Fig. 3-A) shows signals assignable to olefinic protons at 6.19 and 7.05 ppm with a coupling constant of 16.0 Hz. On the other hand, in the

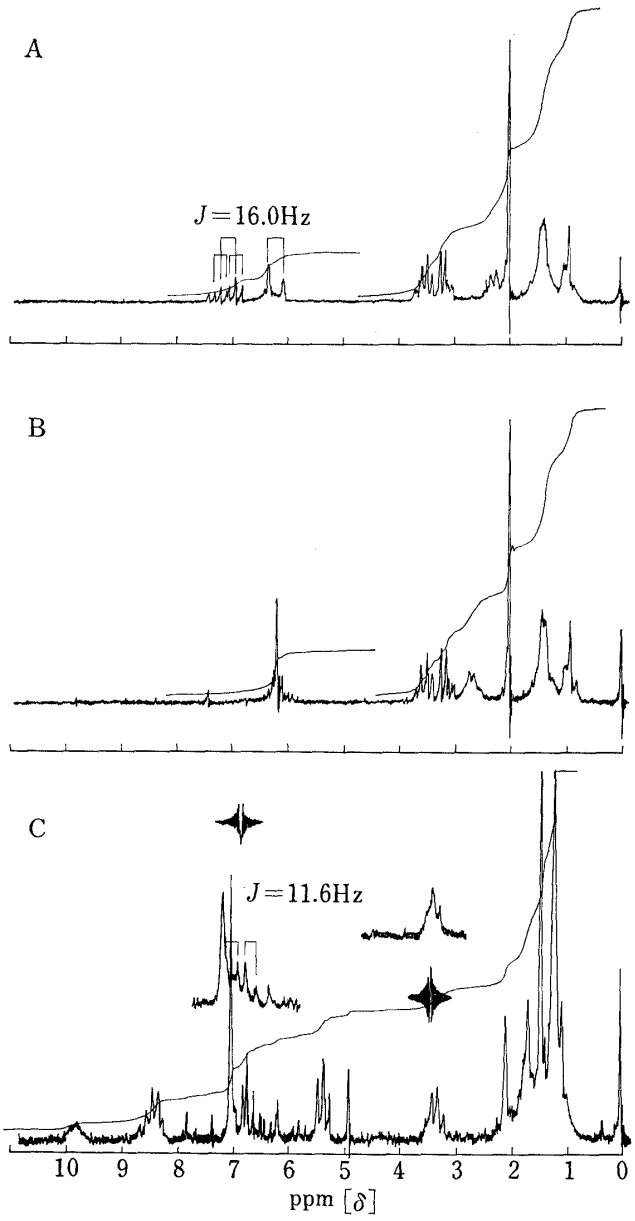

Fig. 3. NMR Spectra of 2-Octenoyl-NAC

A: *trans*-2-Octenoyl-NAC (37 mg) was dissolved in $CDCl_3$ (0.3 ml).
B: *cis*-2-Octenoyl-NAC (27 mg) was dissolved in $CDCl_3$ (0.3 ml).
C: Eu(fod)$_3$ was added to the *cis*-isomer at a molar ratio of 0.7.

case of *cis*-2-octenoyl-NAC, it was difficult to determine the coupling constant due to overlapping of the olefinic proton signals (Fig. 3-B). Therefore, we tried to distinguish the olefinic protons by using $Eu(fod)_3$. The NMR spectrum in the presence of the reagent at a molar ratio of 0.7 with respect to the sample showed a signal for one olefinic proton at the β-position at 6.5 ppm and the signal for another olefinic proton at the α-position at 6.8 ppm (Fig. 3-C). The coupling constant was determined to be 11.6 Hz by decoupling experiments, supporting the *cis*-configuration.

Although the *cis*- and *trans*-isomers of 3-decenoic acid and NAC esters of *cis*-2-octenoic acid and its *trans*-isomer have been presented as examples, the same techniques using NMR spectroscopy with a chemical shift reagent can be applied to other 3-alkenoic acids and thiol ester of 2-alkenoic acids to obtain structural information.

Experimental

NMR Measurement——NMR spectra were taken at 60 MHz on a JEOL JNM-PMX 60 NMR spectrometer. Chemical shifts are expressed as δ (ppm), using tetramethylsilane (TMS) as an internal standard. All NMR spectra were measured at 35°.

Fatty Acids——All acids, prepared by the methods described below, were finally purified by column chromatography on silica gel treated or untreated with silver nitrate. The purity of each acid was greater than 98%, as judged by gas-liquid chromatography.

***trans*-3- and *trans*-2-Alkenoic Acids**——The *trans*-3- and *trans*-2-isomers of hexenoic, octenoic, decenoic, dodecenoic, and tetradecenoic acids were synthesized by a Knoevenagel condensation of malonic acid with aldehydes in the presence of triethanolamine (the 3-isomers) or pyridine (the 2-isomers) according to the report of Boxer and Linstead.[6]

***cis*-2-Alkenoic Acids**——These acids were synthesized by partial hydrogenation over Lindlar's catalyst[7] from the corresponding 2-alkynoic acids, which were prepared by a Grignard reaction of 1-alkyne with carbon dioxide.[8]

***cis*-3-Alkenoic Acids**——*cis*-3-Octenoic, decenoic, dodecenoic, tetradecenoic and hexadecenoic acids were synthesized by partial hydrogenation over Lindlar's catalyst from the corresponding 3-alkynoic acids which were prepared by a Grignard reaction of 1-alkyne with ethylene oxide followed by oxidation by chromium trioxide.[9]

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6) S.E. Boxer and R.P. Linstead, *J. Chem. Soc.*, **1931**, 740.
7) H. Lindlar, *Helv. Chim. Acta.*, **35**, 446 (1952).
8) J.A. Knight and J.H. Diamond, *J. Org. Chem.*, **24**, 400 (1959).
9) W. Stoffel, "Methods in Enzymology," Vol. XIV, ed. by J.M. Lowenstein, Academic Press, New York, 1969, p. 99.