Identification of isomeric unsaturated medium-chain dicarboxylic acids in human urine

Shiow-Jen Jin and Kou-Yi Tserng[1]

Research Service, Veterans Administration Medical Center, and Departments of Pharmacology and Medicine, Case Western Reserve University School of Medicine, Cleveland, OH 44106

Abstract Dicarboxylic aciduria caused by enhanced or inhibited fatty acid metabolism is usually described as increased urinary excretion of saturated medium-chain dicarboxylic acids, such as adipic, suberic, and sebacic acids. Besides these saturated acids, increased excretion of unsaturated dicarboxylic acids is also observed. However, the structural identities of these unsaturated dicarboxylic acids are largely unknown. Using synthetic authentic samples, dual capillary column gas–liquid chromatography, and capillary column gas–liquid chromatography–mass spectrometry, we have identified these acids as *trans*-2-hexenedioic, *trans*-3-hexenedioic, *cis*-3-octenedioic, *cis*-4-octenedioic, and *trans*-3-octenedioic acids. The mass fragmentation pathways of these compounds (as trimethylsilyl derivative) are described. We speculate that the metabolic origin of *cis*-3-octenedioic acid is oleic acid through the intermediate *cis*-5-decenedioic acid. We also propose that *cis*-4-octenedioic acid is derived from linoleic acid. The metabolic origin of *trans*-3-octenedioic acid is less certain. Since no corresponding *cis*-hexenedioic acid could be detected in urine, the metabolic degradation of unsaturated dicarboxylic acids appears to terminate at octenedioic acid. *Trans*-2-hexenedioic acid is probably derived from dehydrogenation of adipic acid. —**Jin, S-J. and K-Y. Tserng.** Identification of isomeric unsaturated medium-chain dicarboxylic acids in human urine. *J. Lipid Res.* 1989. **30:** 1611–1619.

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In the analysis of urinary organic acids for genetic screening in disordered fatty acid metabolism, a number of unsaturated dicarboxylic acids with carbon numbers 8, 10, 12, and 14 were generally noted (1). However, the position of the unsaturation and configuration were largely unknown. The total number of isomers of each carbon number was also not known. The unsaturated dicarboxylic acids corresponding to adipic acid, i.e., hexenedioic acids, have never been reported.

Rats treated with hypoglycin exhibited massive dicarboxylic aciduria as a consequence of inhibited fatty acid oxidation (2). Unsaturated dicarboxylic acids, i.e., *cis*- 4-octenedioic, *cis*-4-decenedioic, and *cis*,*cis*-4,7-decenedienoic acids, were the predominant feature of the dicarboxylic aciduria. Kunau and Lauterbach (3) showed that inhibition of oxidation of linoleic and linolic acids by a metabolite of hypoglycin was responsible for the accumulation of these unusual unsaturated dicarboxylic acids. Lindstedt et al. (1) identified *cis*-5-decenedioic and *cis*-5-dodecenedioic acids in a patient with lactic acidosis by comparisons with synthetic authentic samples. In addition, *trans*-3-decenedioic acid and *trans*-3-dodecenedioic acid were implied by GLC retention times.

Urinary organic acid analysis is used as a tool in our study of disorders of energy metabolism. We have observed that the occurrence of unsaturated dicarboxylic acids in urines from patients with dicarboxylic aciduria of largely unknown etiology is varied. Normally, the urine contains very little unsaturated dicarboxylic acids, much less than would be predicted from the free fatty acid pool of linoleic and oleic acids. However, in some of the disorders, the excretion of unsaturated dicarboxylic acids is comparable, and may even surpass that of saturated counterparts, a pattern similar to that described for hypoglycin-treated rats. The identification of these unsaturated dicarboxylic acids and the establishment of their metabolic origin will be extremely useful in elucidation of the metabolic defects in these disorders.

METHODS AND PROCEDURES

Chemicals

Pentadecanoic acid used as an internal standard was supplied by Supelco (Bellefonte, PA). Solvents used for extraction (diethyl ether, ethyl acetate, methanol) were obtained from Fischer Scientific Co. (Pittsburgh, PA). Diethyl ether was stored with ferrous sulfate to remove

Abbreviations: MS, mass spectrometry; GLC, gas–liquid chromatography; BSTFA, bis-trimethylsilyltrifluoroacetamide; TMCS, trimethylchlorosilane.

[1]To whom reprint requests should be addressed at: Research Service, VA Medical Center, 10701 East Boulevard, Cleveland, OH 44106.

peroxide. This step is necessary to remove a contaminating solvent peak that eluted near the urea peak in urinary organic acid analysis. Other solvents were used without further purification. Bis-trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane (BSTFA/TMCS) was purchased from Supelco. Bistrimethylsilylacetamide-d_9 was obtained from MSD Isotopes (Montreal, Canada). 1,3-Cyclohexadiene, 1,4-cyclohexadiene, 1,3-cyclooctadiene, 1,5-cyclooctadiene, and osmium tetroxide were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Synthesis of *cis*-2-hexenedioic acid

A modification of the synthetic procedure of Tanaka (4) was used. To a solution of 1,3-cyclohexadiene (1.1 mmol) in ether (5 ml) and pyridine (0.25 ml) was added osmium tetroxide (1 mmol) in ether (1 ml). After 3 h, the complex was cleaved by adding a solution of sodium bisulfite (4.5 g) in water (10 ml) and pyridine (6 ml). Extraction of the solution with ether (3 × 20 ml) and evaporation of the dried extract gave a crude product which was then recrystallized from ether–hexane 1:1 (v/v) to give *cis*-3-cyclohexene-1,2-diol. The cyclohexenediol was then oxidized with excess Jones reagent as that described by Tanaka (4). 2-Hexenedioic acid was obtained by evaporation of the ether extract and recrystallization from hot toluene. The product, after converting to trimethylsilyl derivative, gave two peaks on gas–liquid chromatography. The predominant isomer was a *cis* form with a ratio of 4 to 1 to that of the *trans* form.

Synthesis of *cis*-3-hexenedioic, *cis*-2-octenedioic, and *cis*-4-octenedioic acids

The same procedure as the preparation of *cis*-2-hexenedioic acid was followed, except that 1,4-cyclohexadiene, 1,3-cyclooctadiene, and 1,5-cyclooctadiene were used as starting materials, respectively. The product from 1,4-cyclohexadiene was the *cis* isomer exclusively. A single *cis* isomer was also obtained for 4-octenedioic acid from 1,5-cyclooctadiene. However, a mixture of *cis* and *trans* isomers (1:2) was produced from the reaction starting from 1,3-cyclooctadiene. The assignment of *cis* and *trans* isomers was based on the subsequent reaction with *p*-toluenesulfinic acid; in this reaction the *cis* isomer was converted to the *trans* isomer while the *trans* isomer was unchanged.

Synthesis of *cis*-3-octenedioic acid

Treatment of *cis*,*cis*-1,4-cyclooctadiene with osmium tetroxide and chromic acid afforded exclusively the *cis* isomer of 3-octene-1,8-dioic acid. The starting material, *cis*,*cis*-1,4-cyclooctadiene, was synthesized based on the partial rearrangement of 1,3-cyclooctadiene during bromination with N-bromosuccinimide as described by Moon and Ganz (5).

Cis to *trans* isomerization

The procedure described by Rakoff (6) using *p*-toluenesulfinic acid isomerization was adopted. Unsaturated dicarboxylic acids were converted to their dimethyl esters with excess diazomethane in methanol–ether 1:1. A mixture of dimethyl ester (1.5 mmol), *p*-toluenesulfinic acid (0.1 mmol), and dioxane (20 ml) was heated in a Teflon-capped vial for 3 h. The cooled solution was neutralized with 1 N NaOH (0.25 ml) and transferred to a separatory funnel with water (2 ml). The aqueous solution was extracted with ether (2 × 5 ml) and the residue remaining after removal of the solvent was hydrolyzed with 5 N NaOH to give the corresponding *trans*-dicarboxylic acid.

Analytical procedures

Synthetic samples. Solutions of synthetic samples were prepared in methanol with a final concentration of 1 mg/ml. An aliquot (20 μl) of this solution and internal standard (1 mg/ml of pentadecanoic acid in methanol) were mixed and evaporated to dryness. The residue was derivatized with BSTFA/TMCS (40 μl) at 90°C for 0.5 h and analyzed by GLC and GLC–MS.

Urine. In a culture tube (16 × 125 mm) with a Teflon-faced screw cap, a urine sample (volume equivalent to 0.05 mg of creatinine and up to a maximum of 1 ml) was diluted with water to a total volume of 1 ml and mixed with 20 μl of pentadecanoic acid (1 mg/ml in methanol) as internal standard. The sample was then acidified with 25 μl of conc. HCl and extracted with a solvent mixture (2 ml) of ethyl acetate diethyl ether 1:1. The top layer was transferred to a disposable culture tube. The bottom aqueous layer was extracted twice more with the same solvent mixture and the combined extract in the culture tube was dried under a gentle stream of air to dryness at 40°C. With the aid of 100 μl of methanol, the residue was transferred to a 1-ml crimp-capped vial. After drying under an air stream, the vial was sealed with a Teflon-faced cap and 40 μl of silylation reagent (BSTFA/TMCS) was introduced into the vial with a micro-syringe. The mixture was heated at 90°C for 0.5 h and then analyzed by GLC and GLC–MS within 24 h of derivatization.

Dual-capillary column gas chromatographic analysis

A Hewlett-Packard 5890A gas chromatograph equipped with a split/splitless Grob-type capillary injector was used. The columns used for separating organic acid trimethylsilyl derivatives were an SPB-1 (bonded dimethylpolysiloxane phase) fused silica capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness) and an SPB-35 (bonded 35% diphenyl: 65% dimethylsiloxane phase) column (30 m × 0.25 mm ID, 0.25 μm film thickness, both from Supelco). These two capillary columns were attached to the injection port with a dual-bore M2A Vespel ferrule (Supelco). The other ends of the columns were connected

to separate flame ionization detectors. Helium was used as carrier gas and maintained at a flow rate of 1 ml/min (column head pressure 20 psi). The total flow of helium through the split injector was adjusted to 50 ml/min to create a split injection ratio of 50 to 1. A flow rate of 30 ml/min of helium was used as make-up gas for flame ionization detectors. The injector was held at 250°C and the detector at 300°C. For the analysis of organic acids, the column temperature was maintained initially at 60°C. Immediately after injection, the temperature was programed to increase at a rate of 4°C/min to a final temperature of 250°C and maintained at this temperature for 4 min before cooling to the initial temperature. The output of the analysis was interfaced through an analog-digital converter (Hewlett-Packard model 18652A) to a Hewlett-Packard 3354 Laboratory Automation System for compound identification, based on retention times. The retention times of organic acids are expressed as methylene unit, MU (7). A mixture of n-paraffins (C12, C14, C16, and C18) in isooctane was analyzed daily for the correction of retention time drift.

The injection of the samples was accomplished with a Hewlett-Packard 7673A automatic injector/sampler for a minimal variation of retention time due to injection technique. In our system, the variation of retention time in a day was typically within ± 0.01 min.

Gas-liquid chromatography-mass spectrometry

A 5985B gas chromatograph-mass spectrometer (Hewlett-Packard, Palo Alto, CA) was used. A shorter (15 M) fused silica capillary column (SPB-1 from Supelco) was used with the temperature program described for GLC. The injection and interface temperature were maintained at 250°C. The column carrier gas (helium) flow rate was kept at 1 ml/min with a split ratio of 20 to 1. A repetitive scanning mode (300 AMU/sec) was used for compound identification. The eluent from the capillary column is repetitively scanned from *m/z* 49 to 550.

Human urine samples

Urine samples from subjects with dicarboxylic aciduria of largely undiagnosed etiologies were provided by Dr. D. S. Kerr of Rainbow Babies and Childrens Hospital, Cleveland, Ohio. These samples were kept at − 20°C until analyzed.

RESULTS

There are four theoretically possible isomers for hexenedioic acids (*cis*-2-hexenedioic, *trans*-2-hexenedioic, *cis*-3-hexenedioic, *trans*-3-hexenedioic) and six octenedioic acids (*cis*-2-octenedioic, *trans*-2-octenedioic, *cis*-3-octenedioic, *trans*-3-octenedioic, *cis*-4-octenedioic, and *trans*-4-octenedioic). All of these isomeric hexenedioic and octenedioic acids were synthesized for comparison with biological samples for compound identification. It was also hoped that the study of their GLC retentions and mass spectral fragmentation patterns would be useful for the determination of double bond positions and configurations of higher homologues, such as decenedioic and dodecenedioic acids. These higher homologues have more potential isomers and the syntheses of these isomers are more complicated. In dual capillary column GLC, the retentions (expressed as methylene unit) are shown in **Table 1**. The 70 eV electron impact mass spectra of these compounds are shown in **Table 2**.

Urine samples were extracted and derivatized as trimethylsilyl derivatives. These derivatized samples were analyzed with repetitive scanning mass spectrometry as well as dual capillary column GLC. As shown by the authentic samples in Table 2, the hexenedioic acids all yield prominent *m/z* 273 (M−15) ion, while octenedioic acids all yield prominent *m/z* 301 ion. Using the mass chromatogram technique, the ions *m/z* 273 and 301, together with total ion current (TI), are displayed in **Fig. 1**. In the vicinity of adipic acid peak (peak 3), four peaks showed mass fragment *m/z* 273. The first two peaks (peaks 1 and 2) are E- and Z-isomers of 3-methylglutaconic acid, assigned by their GLC retention times and mass spectra. By the same technique, the major peak (peak 5) after adipic acid was shown to be *trans*-2-hexenedioic acid. A minor peak (peak 4) eluted immediately after adipic acid was also observed in some urine samples with elevated dicarboxylic acids. A satisfactory mass spectrum of this peak without contaminating mass fragments from the more abundant adipic acid could not be obtained. However, this peak was identified as *trans*-3-hexenedioic acid based on the mass chromatogram of fragments expected from the authentic sample and the retention times by GLC analysis. In some urine samples, another peak with *m/z* 273 in between *trans*-3-hexenedioic and *trans*-2-hex-

TABLE 1. GLC retention indices (MU) of unsaturated dicarboxylic acid di-TMS derivative[a]

Compounds	SPB-1	SPB-35
Cis-2-hexenedioic	14.92	15.95
Trans-2-hexenedioic	15.39	16.52
Cis-3-hexenedioic	15.01	16.00
Trans-3-hexenedioic	15.10	16.18
Adipic[b]	15.02	15.93
Cis-2-octenedioic	16.80	17.95
Trans-2-octenedioic	17.38	18.68
Cis-3-octenedioic	16.72	17.77
Trans-3-octenedioic	16.84	17.97
Cis-4-octenedioic	16.75	17.77
Trans-4-octenedioic	16.84	17.85
Suberic[b]	16.94	17.87

[a]Abbreviations: MU, methylene unit; TMS, trimethylsilyl.
[b]Retention times for adipic and suberic acids are 22.14 and 27.85 min in SPB-1; 22.17 and 27.82 min in SPB-35, respectively.

TABLE 2. Mass spectral fragmentation of dicarboxylic acids

Dioic Acid		Mass Spectra[a]
2-Hexene- (*cis* and *trans*)	*m/z*,	288(0.8), 273(20), 244(0.5), 229(0.8), 213(2), 207(1), 198(12), 183(6), 170(13), 155(22), 147(86), 139(17), 117(6), 111(9), 109(17), 81(31), 75(51), 73(100), 53(3)
3-Hexene- (*cis* and *trans*)	*m/z*,	288(0.2), 273(3), 244(4), 229(0.4), 215(0.4), 213(0.2), 198(1), 183(1), 155(1), 147(47), 139(6), 117(1), 109(6), 82(23), 81(17), 75(24), 73(100), 54(6)
2-Octene- (*cis* and *trans*)	*m/z*,	301(3), 257(3), 226(2), 217(0.8), 211(2), 198(2), 183(6), 167(5), 147(22), 137(21), 136(100), 117(6), 109(24), 108(15), 94(12), 81(24), 75(46), 73(60), 67(5), 55(8)
3-Octene- (*cis* and *trans*)	*m/z*,	316(3), 301(6), 257(7), 226(2), 217(13), 211(2), 204(3), 198(3), 185(12), 183(7), 169(3), 149(10), 147(51), 137(22), 136(52), 129(9), 117(20), 109(13), 108(13), 81(13), 75(45), 73(100), 67(10), 55(17)
4-Octene- (*cis* and *trans*)	*m/z*,	316(0.4), 301(2), 257(0.4), 243(0.1), 226(0.9), 217(2), 211(1), 204(2), 198(2), 185(5), 169(2), 147(15), 137(25), 136(38), 117(23), 109(8), 108(15), 95(4), 81(9), 80(14), 75(45), 73(100), 67(16), 55(6)

[a]Seventy eV electron impact-induced fragmentation. The numbers in parentheses indicate percentage of that ion to the base ion. Usually ions with abundance above 5% are presented; exceptions are those of diagnostic value.

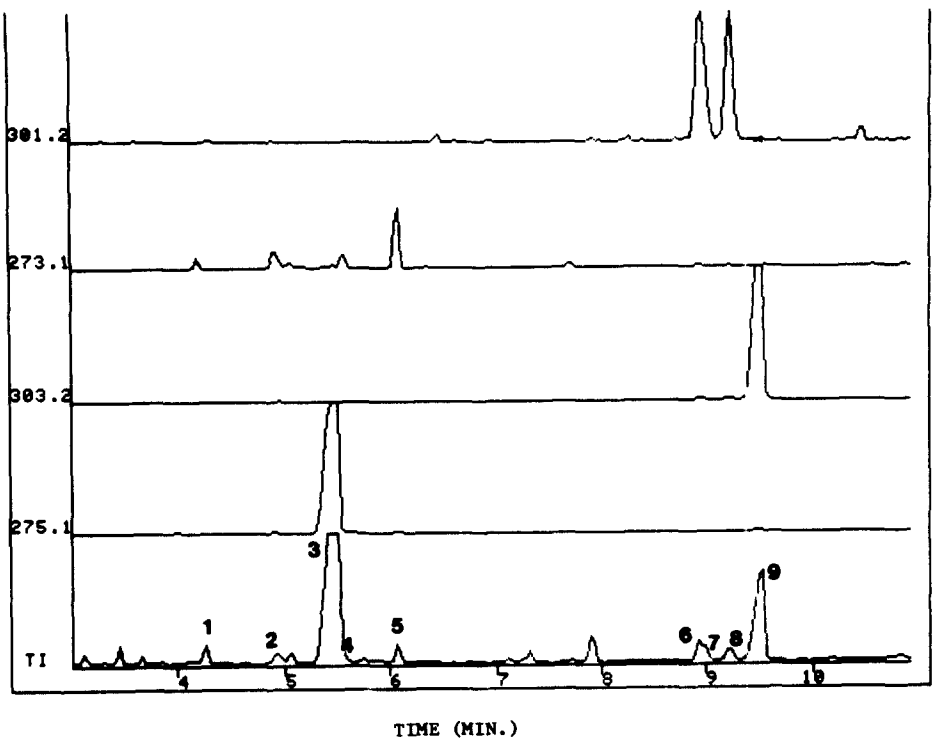

Fig. 1. Mass chromatogram of a urine extract from a patient with undiagnosed nonketotic dicarboxylic aciduria. The sample was analyzed using a gas chromatograph–mass spectrometer with repetitive scanning from *m/z* 49 to 550. The M-15 ions expected for hexenedioic (273), hexanedioic (275), octenedioic (301), and octanedioic (303) acids are shown. Through comparison of mass spectra and retention times in a dual capillary column, the urinary acids are identified as: 1, E-isomer of 3-methylglutaconic; 2, Z-isomer of 3-methylglutaconic; 3, adipic; 4, *trans*-3-hexenedioic; 5, *trans*-2-hexenedioic; 6, *cis*-3-octenedioic; 7, *cis*-4-octenedioic; 8, *trans*-3-octenedioic; and 9, suberic acids. The gas chromatograph (SPB-1) was programmed from 110°C to 250°C with a 4°C/min rate increase. A 20 to 1 split ratio was used for analysis.

enedioic acids present. This peak has been identified as 4-hydroxycyclohexane-1-carboxylic acid (8).

Three peaks (peaks 6, 7, and 8 in Fig. 1) that eluted before suberic acid on the SPB-1 column showed a mass fragment *m/z* 301. Mass spectral fragmentations indicate that these are isomeric octenedioic acids. Mass spectral fragmentations alone were of only partial help in assigning the position and configurations of the double bond. Based on the retention time on SPB-1, peak 6 appears to be *cis*-3-octenedioic acid and peak 7, *cis*-4-octenedioic acid. Their identities were confirmed by their retentions on SPB-35 (**Fig. 2**); both isomers showed identical retentions and the total area of peak 6 and peak 7 on SPB-1 equaled the area of peak 6 + 7 on SPB-35. The close resemblance of their mass spectra (**Fig. 3**) to those of authentic samples further established the identities. Based on the retention on SPB-1, peak 8 could be either *cis*-2-octenedioic, *trans*-3-octenedioic, or *trans*-4-octenedioic acid. Fortunately, these three isomeric octenedioic acids are well separated on SPB-35. Based on its retention on SPB-35, this peak was assigned as *trans*-3-octenedioic acid. Its identity was confirmed by comparing its mass spectrum to that of an authentic sample. Overall, the three isomeric octenedioic acids were identified as *cis*-3-octenedioic, *cis*-4-octenedioic, and *trans*-3-octenedioic acids. No other isomeric octenedioic acids could be detected.

DISCUSSION

Using capillary column GLC and mass chromatographic techniques, we found that urinary unsaturated dicarboxylic acids include hexenedioic, octenedioic, decenedioic, and dodecenedioic acids. For a definite identification of these compounds, comparison with synthetic

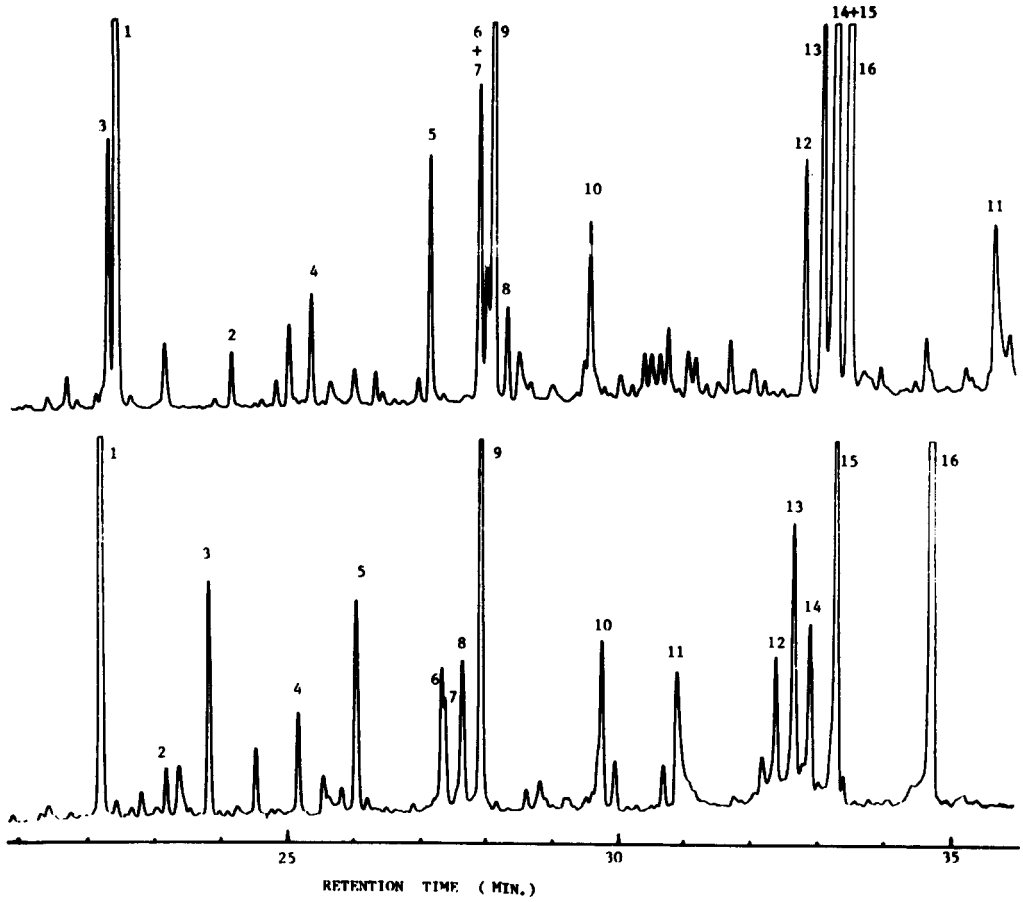

Fig. 2. Dual capillary column chromatogram of a urine extract from a patient with medium-chain acyl-CoA dehydrogenase deficiency. The upper chromatogram was from an SPB-35 column, and the lower from SPB-1. Column temperature was from 60 to 250°C with a 4°C/min rate increase and a 1 ml/min helium flow. A 50 to 1 split ratio injection was used. The peaks were identified as: 1, adipic; 2, *trans*-2-hexenedioic; 3, 7-hydroxyoctanoic; 4, pimelic; 5, *p*-hydroxyphenylacetic; 6, *cis*-3-octenedioic; 7, *cis*-4-octenedioic; 8, *trans*-3-octenedioic; 9, suberic; 10, aconitic; 11, hippuric; 12, *cis*-5-decenedioic; 13, *cis*-4-decenedioic; 14, *trans*-3-decenedioic (?); 15, sebacic; and 16, pentadecanoic (internal standard) acids.

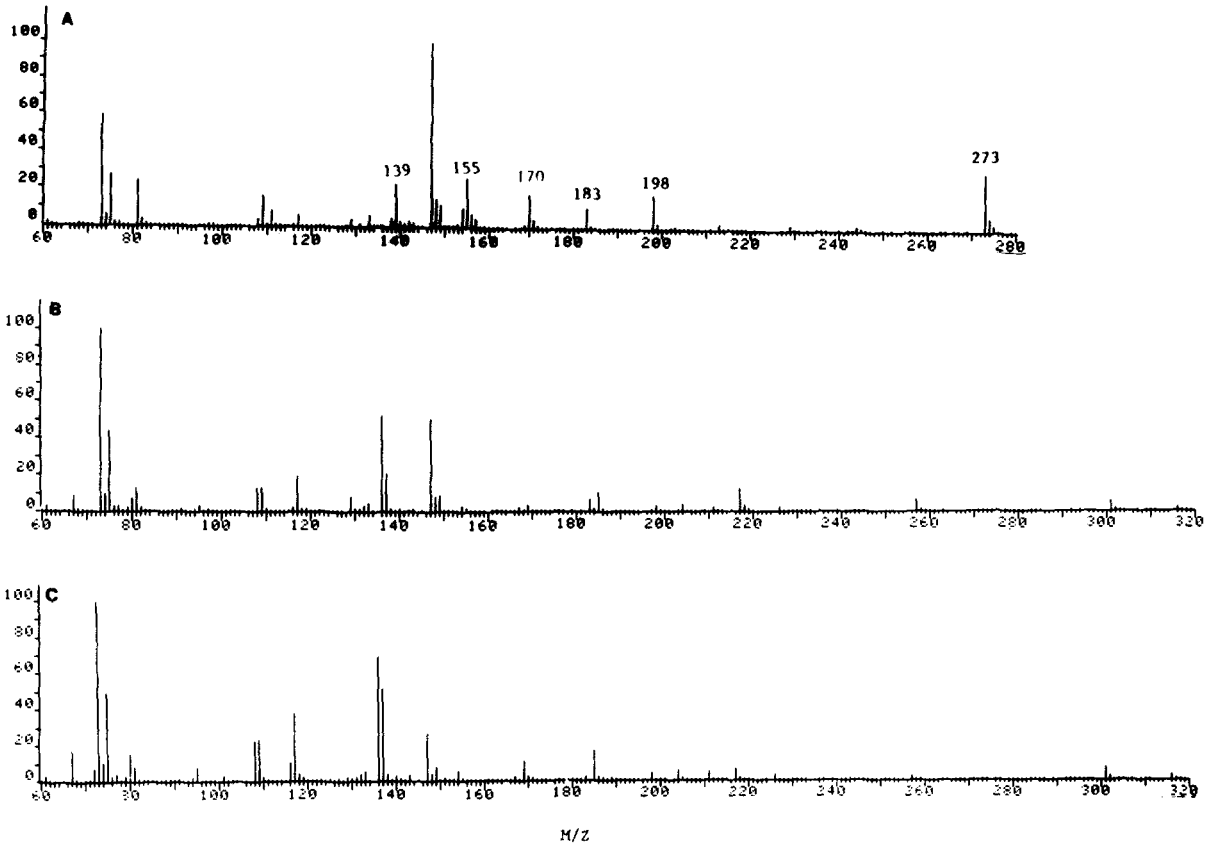

Fig. 3. Seventy eV electron impact mass spectra of medium-chain unsaturated dicarboxylic acids (as trimethylsilyl derivatives) from human urine: A, *trans*-2-hexenedioic acid; B, *cis*-3- and *trans*-3-octenedioic acids; and C, *cis*-4-octenedioic acid.

authentic samples is essential. The isomeric hexenedioic and octenedioic acids can be readily synthesized by osmium tetroxide oxidation of the corresponding cycloalkadiene to a monounsaturated cyclic diol. The oxidation of this diol with chromic acid yields the corresponding acid.

In human urine, *trans*-2-hexenedioic acid was identified. Its metabolic origin could be from the dehydrogenation of adipic acid (9). In addition, *trans*-3-hexenedioic acid was also identified. This acid could be produced from the beta-oxidation of *trans*-3-octenedioic acid.

A total of three isomeric octenedioic acids was identified. They were identified as *cis*-3-octenedioic, *cis*-4-octenedioic, and *trans*-3-octenedioic acids. The metabolic origin of *cis*-3-octenedioic acid could be oleic acid as depicted in **Fig. 4**. In route A, the immediate precursor is *cis*-5-decenedioic acid. In route B, the immediate precursor is *cis*-3-decenedioic acid. Since *cis*-5-decenedioic acid has been identified in human urine (1), it is likely that route A is the preferred pathway. The metabolic origin of *cis*-4-octenedioic acid is likely to be linoleic acid through the stepwise omega- and beta-oxidations of the metabolic intermediate, *cis*-4-decenoic acid (3). The metabolic origin of *trans*-3-octenedioic acid is less certain. It could possibly arise from the metabolic degradation of *trans*-9,10-octadecenoic acid, an unnatural fatty acid contained in partially hydrogenated oils, in a fashion similar to that depicted for oleic acid in Fig. 4.

GLC retention times of isomeric unsaturated hexenedioic and octenedioic acids

In all of the acids studied, *cis* isomers always have shorter retention times than their *trans* counterparts on both SPB-1 and SPB-35 columns. This is to be expected since *cis* configuration renders the molecules less interactive with the stationary phase than the *trans* isomer as a result of potential intramolecular hydrogen bonding in the *cis* forms. For hexenedioic acids, the *cis*-3 isomer has a lower retention than the *cis*-2 isomer. This is probably due to the fact that the *cis*-3 isomer is more symmetrical and more compact than the *cis*-2 isomer resulting in less interaction with the stationary phase. The same relationship also holds for *trans*-3 and *trans*-2 hexenedioic acids.

Using this rationalization, we expected octenedioic acids to have the following elution sequence: *cis*-4 > *cis*-3 > *cis*-2. The actual data show the sequence as *cis*-3 ≥ *cis*-

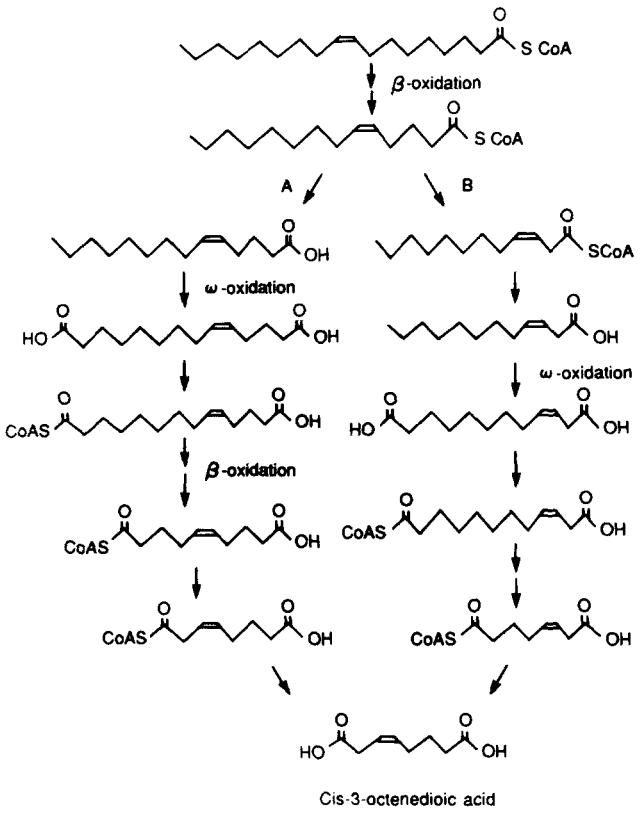

Fig. 4. Postulated metabolic origin of urinary *cis*-3-octenedioic acid. Oleoyl-CoA is metabolized by beta-oxidation to *cis*-5-tetradecenoyl-CoA. From this point, it can proceed through route A. After omega-oxidation and subsequent beta-oxidation from the newly created carboxyl terminus, *cis*-5-decenedioic and *cis*-3-octenedioic acids are produced. Alternatively, *cis*-5-tetradecenoyl-CoA can continue through route B. After beta-oxidation as well as sequential omega- and beta-oxidation, it can be transformed to *cis*-3-decenedioic and *cis*-3-octenedioic acids. Since *cis*-5-decenedioic acid has been identified in the urine, it is likely that route A is the preferred pathway for the generation of *cis*-3-octenedioic acid.

4 > *cis*-2. The slight deviation of the retention from the argument of molecular symmetry could result from the further interaction between carboxyl groups. In going from DC6 to DC8, the carboxyl groups from *cis*-4-octenedioic acid appear to have more crowded environment which tends to distort the whole molecule to a less compact form, hence a longer retention than for the *cis*-3 isomer.

For SPB-35, besides the interaction with the aliphatic chain, the interaction of the double bond with the aromatic group on the stationary phase is expected to render the retention of unsaturated compound longer than the saturated counterparts. This was indeed observed for *cis*-hexenedioic acids. For the SPB-35 phase, the retention sequence of octenedioic acids is *cis*-3 = *cis*-4 > *trans*-4 > suberic > *cis*-2 > *trans*-3 > *trans*-2. The general retention sequence was almost the same as in SPB-1. The lower retention of *cis*-3, *cis*-4 and *trans*-4 than the saturated suberic acid is probably due to the shielding of double bond by the alkyl chains, carboxyl, and trimethylsilyl groups from interaction with the phenyl group on the stationary phase.

Mass spectral fragmentations

Cis and *trans* hexenedioic acids have exactly the same mass spectral fragmentations, indicating the rapid *cis-trans* transformation that occurred in the mass spectrometer ion source. The mass spectra of *cis*-2 and *cis*-3 hexenedioic acids have essentially the same fragments indicating that migration of the double bond probably occurs. However, the contribution of mass fragments to the overall spectra is different between these two isomers indicating that the degree of isomerization is not rapid enough to produce equal contribution of mass fragments. The possible origin of these mass fragments is shown in **Fig. 5**. The postulated routes were confirmed from the mass shift of the TMS-d_9 derivatives. Overall, 2-hexenedioic acid has more prominent peaks at *m/z* 273, 198, 183, 170, and 155. These ions are produced from two fragmentation pathways shown in heavy lines in Fig. 5. The greater abundance of these ions in 2-hexenedioic acids is probably due to the greater abundance of the molecular ion (*m/z* 288) and the M−15 ion (*m/z* 273) as a result of charge stabilization through conjugation of the double bond with the carboxyl group. The other contributing factor could be the mechanism of $(CH_3)_3SiOH$ (TMSOH) elimination. The transfer of the hydrogen atom from the alkyl chain to the siloxyl, $(CH_3)_3SiO$, could occur preferentially from

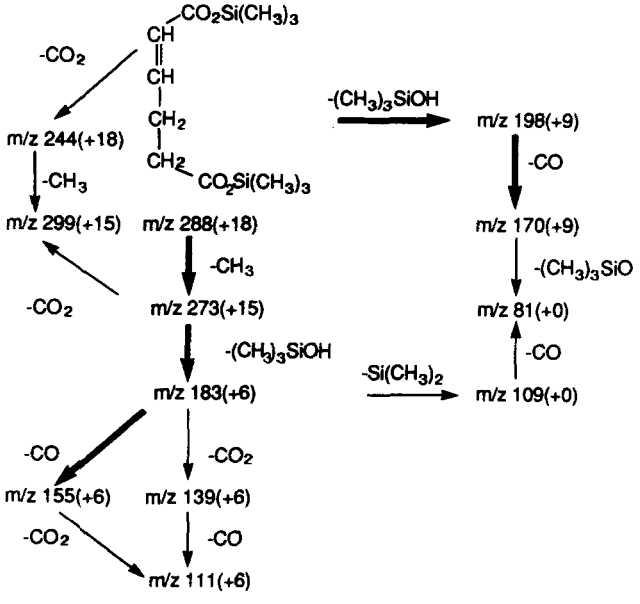

Fig. 5. Postulated fragmentation pathways for the electron impact-induced mass spectra of hexenedioic acid bis-trimethylsilyl derivatives. The heavy lines indicate the preferred fragmentation pathways for the 2-isomer of hexenedioic acids. The numbers in parentheses indicate the mass shift found in the bis-trimethylsilyl-d_9 derivative.

hydrogens at carbon 4 since this transfer requires a favored six-member ring intermediate. In 2-hexenedioic acids these hydrogens are allylic hydrogens that are known to be more reactive than others; therefore, the elimination of TMSOH is facilitated in these compounds.

The increase in chain length by two carbons to octenedioic acids renders the mass fragmentations among isomeric compounds less distinguishable since the abstraction of hydrogen for the elimination of TMSOH could proceed through either carboxyl group with an equally favored six-member ring intermediate for most of the isomers. Besides, the electron impact-induced isomerization of the double bond might be more extensive as the alkyl-chain length increases (10, 11). The general fragmentation pathways for octenedioic acids are depicted in **Fig. 6** together with mass shifts observed from di-TMS-d_9 derivative. Nevertheless, some noticeable variations in fragmentation pathways exist for these isomers.

Mass fragments that can be used for identification purposes are the ion pairs *m/z* 183 versus 185 and *m/z* 136 versus 137. The fragment *m/z* 136 is generated from the successive loss of two molecules of TMSOH, while *m/z* 137 is produced from the loss of TMSOH and TMSO groups. The 2-isomer has the lowest ratio between *m/z* 137 versus 136, and the 4-isomer has the highest ratio. The ratios observed for 3-isomers are intermediate. Actually, the abundance of *m/z* 137 stays almost constant in all three isomers; the difference in the ratios to *m/z* 136 is due to the variation in the abundance of *m/z* 136 ions. The 2-isomer has the most abundant *m/z* 136 (as base peak), followed by the 3-isomer and the 4-isomer. The reason could be due to the relative activity of abstraction of hydrogen from the alkyl chain. A decreased abstraction activity is expected in going from the 2-isomer to the 3-isomer to the 4-isomer.

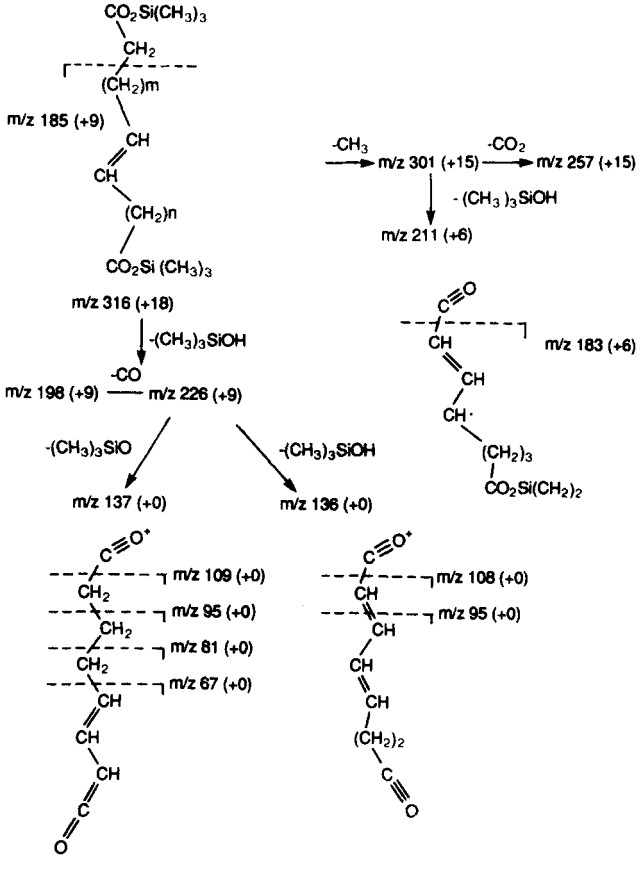

Fig. 6. General fragmentation pathways postulated for octenedioic acid bis-trimethylsilyl derivatives. The numbers in parentheses indicate the mass fragment shift observed in the bis-trimethylsilyl-d_9 derivative.

2-Octenedioic acids are predominant in *m/z* 183 and 4-octenedioic acids are predominant in *m/z* 185, while 3-octenedioic acids contain both *m/z* 183 and 185 with a slightly higher abundance of *m/z* 185. A postulated mechanism for the production of these two ions is shown in Fig. 6. The fragment *m/z* 185 is produced by beta-cleavage (β to the carboxyl group) to eliminate $CH_2CO_2Si(CH_3)_3$. Apparently, the production of this ion is not favored in the 2-isomer since a double bond exists in that position. Instead, the 2-isomer uses an alternative route to eliminate TMSOH through a six-membered intermediate to produce *m/z* 211. Elimination of CO from this fragment would produce *m/z* 183. This route is favored in 2-isomers as already discussed. In contrast, the unavailability of the hydrogen on carbon 4 in the 4-isomer renders this route unfavorable. In addition, beta-cleavage to produce *m/z* 185 is most favored in 4-isomers since this cleavage is facilitated by an allylic position to the double bond (12).

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