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750 MHz ^{1}H NMR spectroscopy characterisation of the complex metabolic pattern of urine from patients with inborn errors of metabolism: 2-hydroxyglutaric aciduria and maple syrup urine disease

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Abstract

750 MHz ^{1}H NMR spectroscopy has been used to characterise in detail the abnormal low molecular weight metabolites of urine from two patients with inborn errors of metabolism. One case of the rare condition 2-hydroxyglutaric aciduria has been examined. There is at present no rapid routine method to detect this genetic defect, although NMR spectroscopy of urine is shown to provide a distinctive pattern of resonances. Assignment of a number of prominent urinary metabolites not normally seen in control urine could be made on the basis of their known NMR spectral parameters including the diagnostic marker 2-hydroxyglutaric acid, which served to confirm the condition. In addition, 750 MHz ^{1}H NMR spectroscopy has been used to characterise further the abnormal metabolic profile of urine from a patient with maple syrup urine disease. This abnormality arises from a defect in branched chain keto-acid decarboxylase activity and results in a build up in the urine of high levels of branched chain oxo- and hydroxy-acids resulting from altered metabolism of the branched chain amino acids, valine, leucine and isoleucine. A number of previously undetected abnormal metabolites have been identified through the use of one-dimensional and two-dimensional J-resolved and COSY 750 MHz ^{1}H NMR spectroscopy, including ethanol, 2-hydroxy-isovalerate, 2,3-dihydroxy-valerate, 2-oxo-3-methyl-*n*-valerate and 2-oxo-isocaproate. NMR spectroscopy of urine, particularly when combined with automatic data reduction and computer pattern recognition using a combination of biochemical markers, promises to provide an efficient alternative to other techniques for the diagnosis of inborn errors of metabolism. © 1997 Elsevier Science B.V.

Keywords: 750 MHz; ^{1}H NMR; Urinalysis; Inborn error of metabolism; 2-hydroxyglutaric aciduria; Maple syrup urine disease

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1. Introduction

High resolution ^{1}H NMR spectroscopy has been used previously to investigate the perturbed metabolic profiles in urine samples from patients with inborn errors of metabolism. Initial studies were performed using 400 MHz spin-echo spectroscopy [1–7], but other studies have included the use of 250 MHz ^{1}H NMR measurements on urine acidified to pH 2.5 [8]. Concomitant with the development of efficient solvent suppression techniques for eliminating the strong water resonance, the need for freeze-drying and the exclusive use of deuterated solvents has been largely removed as a prerequisite for obtaining good quality spectra. In addition, with the advent of commercially available ultra-high frequency instruments for ^{1}H NMR spectroscopy, spectral dispersion and sensitivity has improved dramatically. This has prompted the reinvestigation of the potential of high field ^{1}H NMR spectroscopy for the characterisation of various inborn errors of metabolism [9], which enhances the possibility of using pattern recognition computer methods for classifying such samples [10].

As with all NMR experiments, the exact frequency at which measurements are performed influences signal dispersion, sensitivity and the relaxation properties of the nuclei in the molecules under study. Thus, given the complexity of biofluid matrices, the frequency at which a ^{1}H NMR spectrum is measured has a major effect on the amount of chemical and biochemical information obtained. This information content of biofluid NMR spectra is high both in terms of the number of detectable metabolites and demonstrable dynamic molecular interactions. Despite the powerful array of multiple pulse and multi-dimensional NMR, the very complexity of biofluids precludes a complete assignment of all resonances, although some fluids have been well characterised including blood plasma [11], cerebrospinal fluid [12] and seminal fluid [13]. Urine is one of the simplest fluids in physicochemical terms, but because of the need to maintain homeostasis, it is one of the most complex in composition. Even in normal individuals urine contains hundreds or thousands of compounds from a wide variety of chemical classes of which those in the concentration range of about 50 μM upwards are in the NMR-detectable range [14].

With the recent advent of 750/800 MHz ^{1}H NMR instrumentation with consequent significant improvement in spectral dispersion and sensitivity, there is now the possibility of enhancing the knowledge of biofluid compositions and hence improving the diagnostic potential of ^{1}H NMR spectroscopy. The purpose of this study is to investigate the advantages of ^{1}H NMR spectroscopic measurements at the highest possible observation frequency using the example of human urine from patients with inborn errors of metabolism.

In this study, high resolution 750 MHz ^{1}H NMR spectra have been measured on urine from the patient with the inborn error of metabolism 2-hydroxyglutaric aciduria [9]. This is characterised clinically by non-ketotic hypoglycaemia and metabolic acidosis. Biochemically, changes include the accumulation and excretion of oxidation products of substrates normally oxidised by mitochondrial electron transfer flavin-containing enzymes. The urine can contain abnormal metabolites including various combinations of short chain volatile acids such as isovaleric, 3-hydroxyisovaleric, 2-hydroxyglutaric, 5-hydroxyhexanoic, adipic, suberic, sebacic and dodecanedoic acids. In addition the presence of isovalerylglycine, isobutyrylglycine and 2-methylbutyrylglycine can sometimes be observed. Very low levels of 3-hydroxybutyric and acetoacetic acids are sometimes seen [9]. The detection of 2-hydroxy-glutaric acid distinguishes this condition from glutaric aciduria (glutaryl-CoA dehydrogenase deficiency) in which 3-hydroxyglutaric acid is excreted [9]. Only one example of this disease has been studied previously by NMR spectroscopy (at 250 MHz) and no detailed results on the abnormal urinary metabolite profile were given [8]. NMR spectroscopy, with its ability to measure many biochemical markers simultaneously, offers a potential new methodology for studying this and related metabolic diseases, with great benefits being conferred by the use of high frequency NMR instrumentation.

Maple syrup urine disease (MSUD) is a branched chain keto-aciduria which is caused by a defect in the metabolism of the branched chain amino acids valine, leucine and isoleucine (oxo-acid decarboxylase activity). This defect is one of the more common inborn errors of metabolism with a level very dependent on the type of population being surveyed. Published values for the USA vary from a ratio of 1:760 live births for an inbred Mennonite community to 1: 296 000 for a mobile, urban, predominantly white New England population [9]. The disease is associated with the presence in the urine of the above amino acids and of sweet smelling branched chain oxo- and hydroxy-acids, reminiscent of maple syrup [9]. Earlier ^{1}H NMR studies conducted at lower observation frequencies have shown that it is possible to detect some of the expected metabolites [1,8].

Iles et al. described the first use of NMR spectroscopy to study the urine of a patient with a branched chain ketoaciduria in 1985 [1]. It was shown that resonances from valine, leucine and isoleucine were elevated in the spin-echo spectrum of the urine and that peaks could be observed from branched chain acids not normally observed. These comprised 2-oxo-isocaproate, 2-oxo-3-methyl-*n*-valerate, 2-oxo-isovalerate, 2-hydroxy-3-methyl-*n*-valerate and 2-hydroxy-isovalerate. Not all ^{1}H NMR resonances of all of these compounds were resolved and identification was based on the addition of standard compounds. In addition, elevated levels of glycine were also noted. Lehnert and Hunkler [8] also studied two patients with MSUD and identified a number of diagnostic metabolites namely leucine, 2-oxo-isocaproate, 2-oxo-isovalerate, 3-methyl-2-oxo-*n*-valerate and the corresponding hydroxy acids plus 3-hydroxy-butyrate and lactate. All spectra in this previous study were obtained at pH 2.5.

Ultra-high frequency (750/800 MHz) ^{1}H NMR spectroscopy of biofluids with its unparalleled sensitivity and spectral dispersion promises to offer new insights into the perturbed metabolic profiles associated with inborn errors of metabolism.

2. Experimental

Human urine samples were stored frozen at -70°C until required for NMR analysis. ^{1}H NMR spectra were measured at 750 MHz on a Bruker AMX-750 spectrometer at ambient temperature (298 K) on untreated urine except for the addition of 10% D_2O to provide a field-frequency lock. Typical acquisition parameters were: 128 transients collected into 64 K time domain points over a spectral width of 10 000 Hz leading to an acquisition time of 3.28 s. The water signal was suppressed using a 1-dimensional version of the NOESY sequence [15] or the jump-return $1-\bar{1}$ sequence [16]. The FIDs were processed using Lorentzian-Gaussian resolution enhancement, typically a negative Lorentzian sharpening of 1.5 Hz (LB $= -1.5$) and a Gaussian broadening which had a maximum at 0.35 of the acquisition time (GB $= 0.35$). The data were zero-filled by a factor of two before Fourier transformation.

Two-dimensional J-resolved (JRES) spectra were acquired with water presaturation [17] using, as parameters, 32 transients per increment with 128 increments into 8192 time domain points leading to an acquisition time of 0.51 s. The spectrum width in t_2 was 8064 Hz and in t_1 was 63 Hz. A double-quantum filtered 2-dimensional COSY spectrum was also acquired with water presaturation in phase sensitive mode using the following parameters—acquisition time 0.25 s, spectral width 8064 Hz, time domain points 4096, number of transients per increment 32, 512 increments in t_1, zero-filling by factors of two and four in t_2 and t_1 respectively, TPPI phase sensitive mode, and shifted sinebell squared window functions in t_2 and t_1.

Computer analysis of the spin coupled multiplets of 2-hydroxyglutarate was achieved using PANIC (Bruker Spectrospin, Coventry, UK) on a Bruker Aspect 1000 workstation.

3. Results

The resolution-enhanced one-dimensional 750 MHz ^{1}H NMR spectrum of the urine from the patient with 2-hydroxyglutaric aciduria is shown

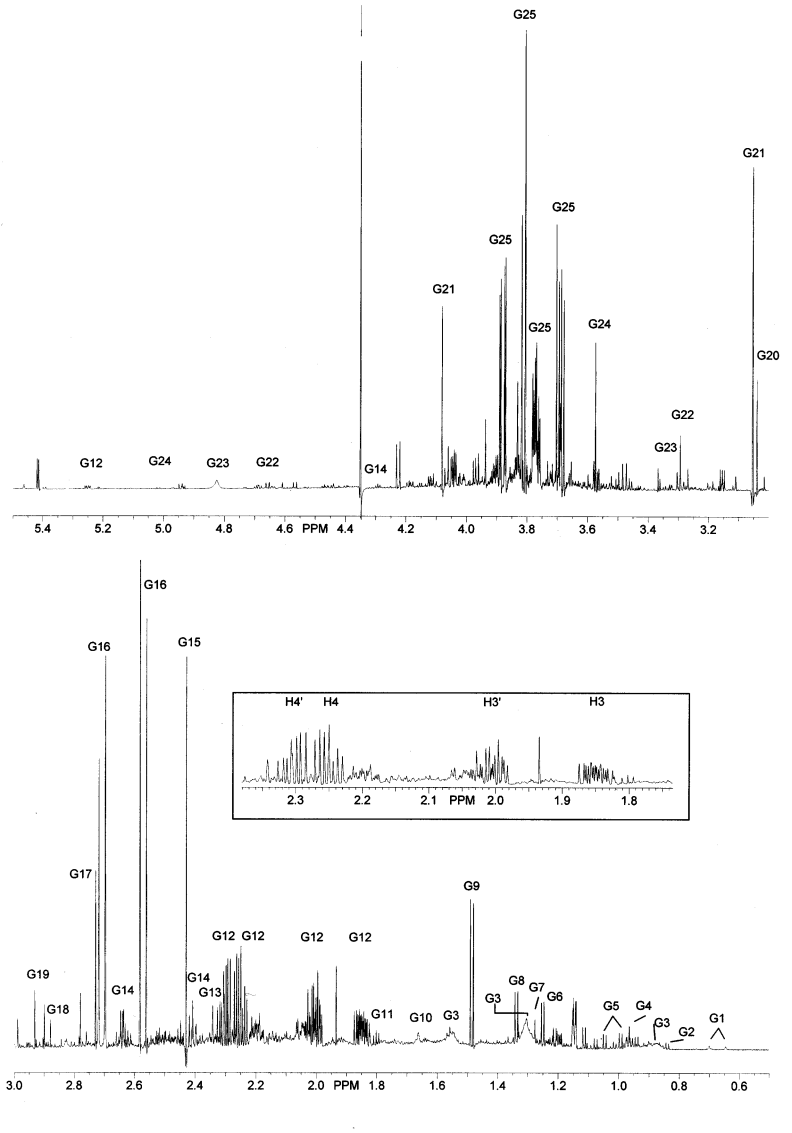

Fig. 1. (a) 750 ^{1}H NMR spectrum of the urine from a patient with 2-hydroxyglutaric aciduria with (b) a vertical expansion with assignments as marked.

in Fig. 1. Many resonances in normal urine [14], blood plasma [11], cerebrospinal fluid [12] and seminal fluid [13] have been assigned previously. In the metabolic disease under study here, high levels of 2-hydroxyglutaric acid are also expected in the urine and signals from this compound can be seen. Thus, the CHOH methine proton appears as a doublet of doublets at δ5.25 showing coupling to the two vicinal methylene protons. These resonate as part of a complex series of signals with the two H3 protons at δ1.85 (H3) and δ2.01 (H3′) and the other methylene protons at δ2.25 (H4) and δ2.31 (H4′). The structure of the molecule is further confirmed by iterative computer analysis of the spin-coupling patterns to yield the values $^{3}J_{23} = 7.7$, $^{3}J_{23'} = 4.0$, $^{3}J_{33'} = 14.1$, $^{3}J_{44'} = 15.0$, $^{3}J_{34} = 5.5$, $^{3}J_{34'} = 10.3$, $^{3}J_{3'4} = 10.4$ and $^{3}J_{3'4'} = 6.1$ Hz. Signals from 3-hydroxyglutaric

acid were not observed in this sample although they would be expected to occur in other types of glutaric aciduria [9].

Apart from the very distinctive resonance pattern arising from 2-hydroxyglutarate, the ^{1}H NMR spectrum of the patient's urine is also notable for the very low levels of the amino acids isoleucine, leucine, valine, tyrosine, phenylalanine and arginine. In addition, no 2-oxo-glutarate or 2-oxo-butyrate was detected. However, a number of broad resonances were detected and these have been assigned to long chain fatty acids such as dodecanoate which are known to be present in this condition. A doublet at δ1.15 has been assigned tentatively to methylmalonate and a broad peak at δ1.67 could arise from adipate. In the carbohydrate region of the spectrum, a high level of mannitol can be detected with four large multiplet resonances at δ3.88, 3.81, 3.77 and 3.69 and this might arise from a dietary or pharmaceutical source. A large singlet is seen at δ4.35 and this is probably from dihydroxyacetone. Also observed is a large doublet at δ5.42 which from its chemical shift and splitting must be a CH group with electronegative substituents, coupled to another CH proton, with the structure RR′CH–CHOH–*X*, where *X* is likely to be NHCOR or OH. The identification of this molecule has not been possible. Small signals are seen at δ9.13 and δ8.84, not shown in the figure, which are as yet unassigned but observed previously in urine from renal failure patients (unpublished results). The use of 750 MHz observation frequency combined with Lorentzian-Gaussian resolution enhancement results in the excellent separation of the two singlets at δ3.04 and δ3.05 arising from creatine and creatinine respectively and would allow precise measurement of their relative proportions. The ^{1}H NMR chemical shifts and peak multiplicities of a number of metabolites detected in the urine sample are summarised in Table 1, which also provides the key to Fig. 1.

The conventional 1-dimensional 750 MHz ^{1}H NMR spectrum, with pre-saturation of the water resonance, of a sample of urine from the patient with MSUD is shown in Fig. 2. This has been assigned as far as possible with the aid of the JRES (Fig. 3) and COSY spectra in a concerted fashion. A metabolite has been considered as assigned when all of the expected resonances and connectivities have been observed. In addition many metabolites have been partially assigned.

The dispersion of 750 MHz NMR spectroscopy coupled with resolution enhancement has given unparalleled resonance discrimination. The biosynthetic pathway for valine is shown in Fig. 4, where the metabolites shown differ from those given in Iles et al. [1]. The methyl signals of valine can be observed at δ0.99 and δ1.04 in the 1-D spectrum, but its resonances are too overlapped to be observed. However, in the region of the ^{1}H NMR spectrum between δ0.80 and δ1.15 there are three resolved triplets and 20 doublets visible in the J-resolved spectrum. For some compounds, reference chemical shifts exist in the literature and these have been assigned as shown in Fig. 2. For example, the large doublet at δ0.84 and the equally large doublet at δ0.97 are both coupled (from the COSY spectrum) to a signal at δ2.03 which in turn is coupled to a resonance at δ3.86. This assigns the spin system to be $(CH_3)_2$–CH–CHOH– and the CH chemical shift at δ3.86 indicates the final substituent as –COOH and identifies the molecule as 2-hydroxy-isovalerate. Further oxidation of 2-hydroxy-isovalerate or loss of water from 2,3-dihydroxy-isovalerate leads to 2-oxo-isovalerate which can be observed in the COSY spectrum with a connectivity at δ1.13/δ3.02 (not shown).

The biosynthetic pathway for leucine is also shown in Fig. 4. The amino acid leucine gives the expected resonances at δ0.94 and δ0.96 for the methyl groups which show a COSY connectivity to a peak at δ1.79 comprising the CH–CH_2 moiety but the further connectivity to the α-CH cannot be resolved. The hydroxy metabolite 2-hydroxy-isocaproate can be detected readily in both the 1-D and COSY spectra with all connectivities apart from the 2-CHOH resolved. The further oxidised species 2-oxo-isocaproate can be detected with all cross-peaks visible in the COSY spectrum. The methyl resonances are at δ0.92 showing a connectivity to the CH at δ2.12 which in turn shows a cross-peak to the CH_2 group at δ2.62, which is visible in the 1-D spectrum between the large citrate peaks.

Table 1
NMR parameters for endogenous urinary metabolites observed in 2-hydroxyglutaric aciduria and in maple syrup urine disease

Identification[a]	Metabolite	Assignment	Multiplicity	^{1}H Chemical shift
G1	Bile acids	CH_3	s	0.63
G1	Bile acids	CH_3	s	0.69
G2, M1	2-hydroxy-isovalerate	δ-CH_3	d	0.83
G3	Dodecanoate etc.	CH_3	br	0.88
M2	2-hydroxybutyrate	CH_3	t	0.89
M3	2-oxo-3-methyl-*n*-valerate	ε-CH_3	t	0.90
M4	2-oxo-isocaproate	ε-CH_3	d	0.92
M1	2-hydroxy-isovalerate	CH_3	d	0.92
G4, M5	Isoleucine	δ-CH_3	t	0.93
M6	Leucine	δ-CH_3	d	0.94
M1	2-hydroxy-isovalerate	δ-CH_3	d	0.96
M6	Leucine	δ-CH3	d	0.96
G5, M7	Valine	CH_3	d	0.99
M5	Isoleucine	γ-CH_3	d	1.02
G5, M7	Valine	CH_3	d	1.05
M3	2-oxo-3-methyl-*n*-valerate	δ-CH_3	d	1.10
M8	2-oxo-isovalerate	CH_3	d	1.13
M9	Ethanol	CH_3	t	1.19
M10	3-hydroxybutyrate	CH_3	d	1.23
G6	Methylmalonate	CH_3	d	1.25
G7, M11	2,3-dihydroxy-isovalerate	CH_3	s	1.28
G3	Dodecanoate etc.	CH_3	br	1.30
G8, M12	Lactate	CH_3	d	1.34
M13	3-amino-isovalerate	CH_3	s	1.36
M3	2-oxo-3-methyl-*n*-valerate	1/2 δ-CH_2	m	1.46
G9	Alanine	CH_3	d	1.49
G3	Dodecanoate etc.	CH_2	br	1.55
G10	Adipate	CH_2	br	1.66
M3	2-oxo-3-methyl-*n*-valerate	1/2 δ-CH_2	m	1.70
G11	GABA	CH_2	t	1.80
G12	2-hydroxy-glutarate	1/2 CH_2	m	1.85
M14	Acetate	CH_3	s	1.93
G12	2-hydroxy-glutarate	1/2 CH_2	m	2.01
M1	2-hydroxy-isovalerate	CH_2	m	2.03
M15	Acetone	CH_3	s	2.21
G12	2-hydroxy-glutarate	1/2 CH_2	m	2.26
G12	2-hydroxy-glutarate	1/2 CH_2	m	2.30
M16	Acetoacetate	CH_3	s	2.24
M17	Oxaloacetate	CH_2	s	2.35
G13, M18	Pyruvate	CH_3	s	2.37
G14	Malate	1/2 CH_2	m	2.41
G15, M19	Succinate	CH_2	s	2.43
M20	Methylamine	CH_3	s	2.49
G16, M21	Citrate	1/2 CH_2	d	2.57
M4	2-oxo-isocaproate	γ-CH_2	m	2.62
G14	Malate	1/2 CH_2	m	2.64
G16, M21	Citrate	1/2 CH_2	d	2.71
G17, M22	Dimethylamine	CH_3	s	2.73
M23	Sarcosine	CH_3	s	2.75
M25	Methylguanidine	CH_3	s	2.81
G18, M26	Trimethylamine	CH_3	s	2.88
G19, M24	Dimethylglycine	CH_3	s	2.93

Table 1 (continued)

Identification[a]	Metabolite	Assignment	Multiplicity	^{1}H Chemical shift
G20, M27	Creatine	CH_3	s	3.04
G21, M28	Creatinine	CH_3	s	3.05
M29	*Cis*-aconitic acid	CH_2	s	3.17
M30	Choline	CH_3	s	3.23
M31	Arginine	CH_2	t	3.25
G22, M32	Betaine	CH_3	s	3.29
G23, M33	TMAO	CH_3	s	3.30
M34	Scyllo-inositol	CH	s	3.37
M35	*Trans*-aconitic acid	CH_2	s	3.45
G24, M36	Glycine	CH_2	s	3.57
M9	Ethanol	CH_2	q	3.67
G25	Mannitol	1/2 CH_2	m	3.69
G25	Mannitol	1/2 CH_2	m	3.77
G25	Mannitol	CH	m	3.81
M1	2-hydroxy-isovalerate	α-CHOH	d	3.86
G25	Mannitol	CH	m	3.88
M27	Creatine	CH_2	s	3.93
M37	Hippurate	CH_2	s	3.95
G21, M28	Creatinine	CH_2	s	4.08
M12	Lactate	CH	q	4.12
G14	Malate	CH	t	4.29
G34	Dihydroxyacetone	CH_2	s	4.35
G22	β-glucose	CH	d	4.65
G23	Water	H_2O	br	4.83
G12	2-hydroxyglutarate	CH	dd	5.25
G25	Urea	NH_2	br	5.80
G26	Uridine	CH	d	5.85
G26	Uridine	CH	d	5.86
G27	Fumarate	CH	s	6.53
G28	4-hydroxyphenyl-lactate	CH (2)	m	6.86
G29	Tyrosine	CH (2)	m	6.89
G29	Tyrosine	CH (2)	m	7.18
G28	4-hydroxyphenyl-lactate	CH (2)	m	7.20
G30	Histidine	CH	s	7.28
G31	Phenylalanine	CH (3)	m	7.35
G31	Phenylalanine	CH (2)	m	7.42
G32	Hippurate	CH (m)	t	7.55
G32	Hippurate	CH (p)	t	7.63
G32	Hippurate	CH (o)	m	7.83
G30	Histidine	CH	s	8.38
G33	Formate	CH	s	8.46

[a] Key for Figs. 1 and 2. G and M refer to 2-hydroxyglutaric aciduria and maple syrup urine disease respectively.

Most of the other peaks in this region of the spectrum, except for those shown in Fig. 2, remain unassigned. A singlet is observed at δ1.27 and this has been assigned to 2,3-dihydroxy-isovalerate based upon measurement of the standard compound. Another singlet is observed at δ1.36 and most likely arises from a similar molecule. One possible suggestion is 3-hydroxy-isovalerate or possibly 3-amino-isovalerate, but authentic standards are not commercially available.

The third branched chain amino acid isoleucine has the biosynthetic pathway also shown in Fig. 4 and it can also be detected in the NMR spectrum of the urine, giving methyl resonances at δ0.93

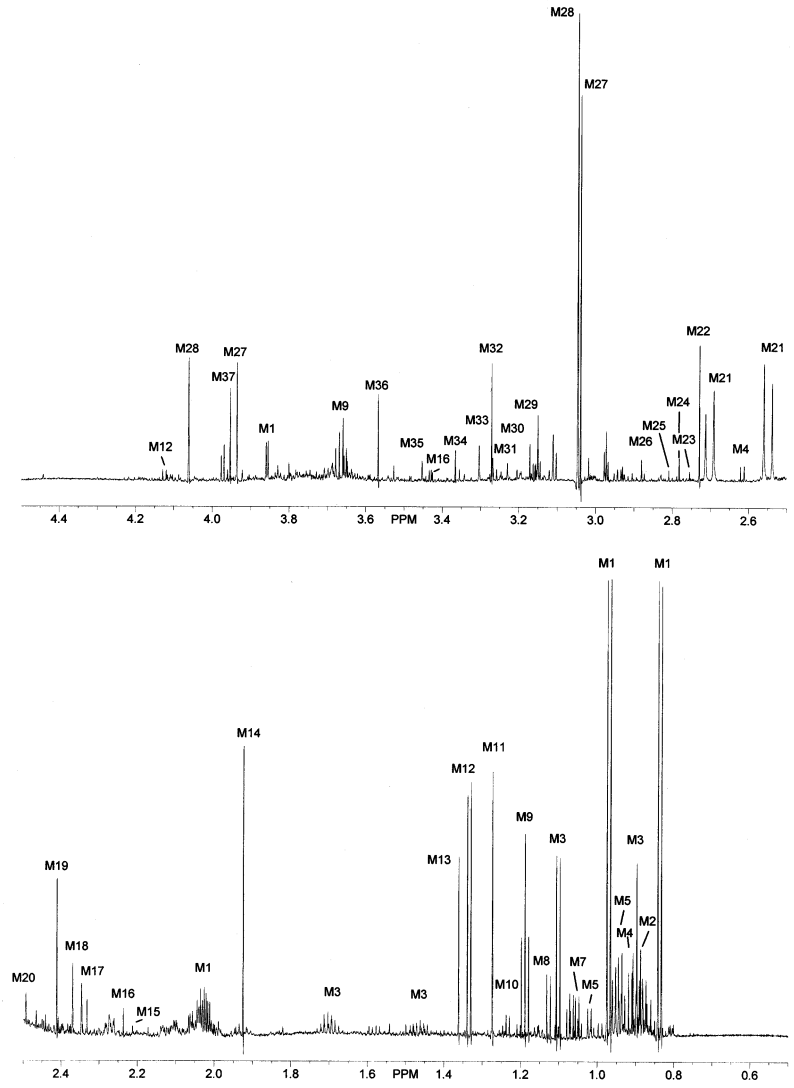

Fig. 2. 750 ^{1}H NMR spectrum of the urine from a patient with maple syrup urine disease.

and δ1.02, but the connectivities further down the chain cannot be resolved. Although the expected 2-hydroxy-3-methyl-*n*-valerate is not observed, its oxidation product 2-oxo-3-methyl-*n*-valerate is observed with all of the expected cross-peaks in the COSY spectrum being resolved. Similarly, the triplet at δ0.90 is coupled to resonances at δ1.47 and δ1.70 and these shifts are consistent with the ethyl group of 2-oxo-3-methyl-*n*-valerate where, because the molecule is chiral, the methylene protons have separate chemical shifts. A methyl doublet of the same intensity as that at δ0.90 is also from the same molecule and this is coupled to signal at δ2.94, confirming the full spin system as $CH_3–CH_2–CH(CH_3)–CO–COOH$.

Other molecules which might be expected include 3-hydroxybutyrate which could be the cause of the doublet resonance at the expected shift of the methyl group at δ1.23 and 3-hydroxypropionate which can be detected by a COSY cross-

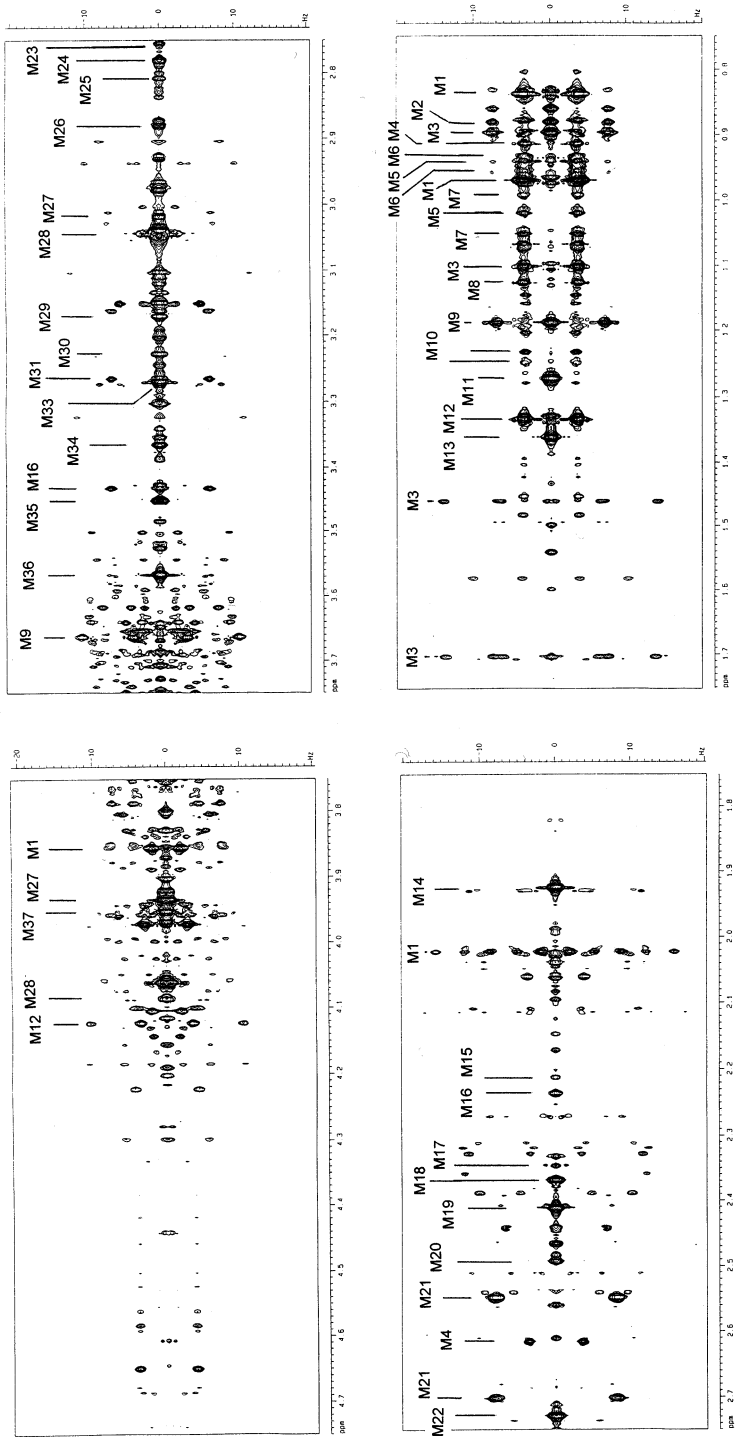

Fig. 3. 750 ^{1}H two-dimensional J-resolved NMR spectrum of the urine from a patient with maple syrup urine disease.

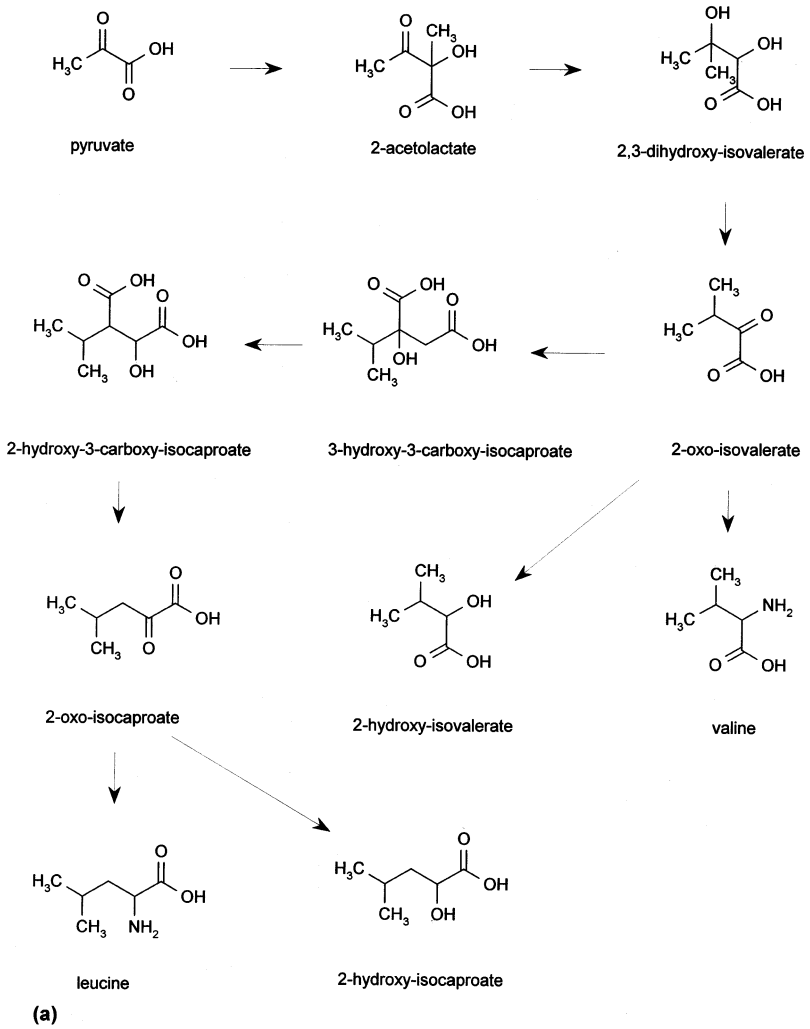

Fig. 4. (a) Biosynthetic pathways for valine and leucine and (b) biosynthetic pathway for isoleucine

peak at δ2.45 and δ3.80. In addition glycine is observed as a singlet at δ3.57 although the expected isovalerylglycine which has a glycyl methylene group resonance at δ3.77 is not detected. The two familiar resonances of lactate are clearly visible. A prominent triplet at δ1.19 is coupled to a quartet at δ3.67 and is thus from an ethyl group, the shifts both being as expected for ethanol which probably originates from fermentation of the sample. The ^{1}H NMR chemical shifts and peak multiplicities of important metabolites detected in the urine sample are summarised in Table 1 along with the key to Fig. 2.

4. Discussion

The region of the ^{1}H NMR spectrum of human urine from δ0.7–4.7 has a particularly rich profile of resonances from low molecular weight com-

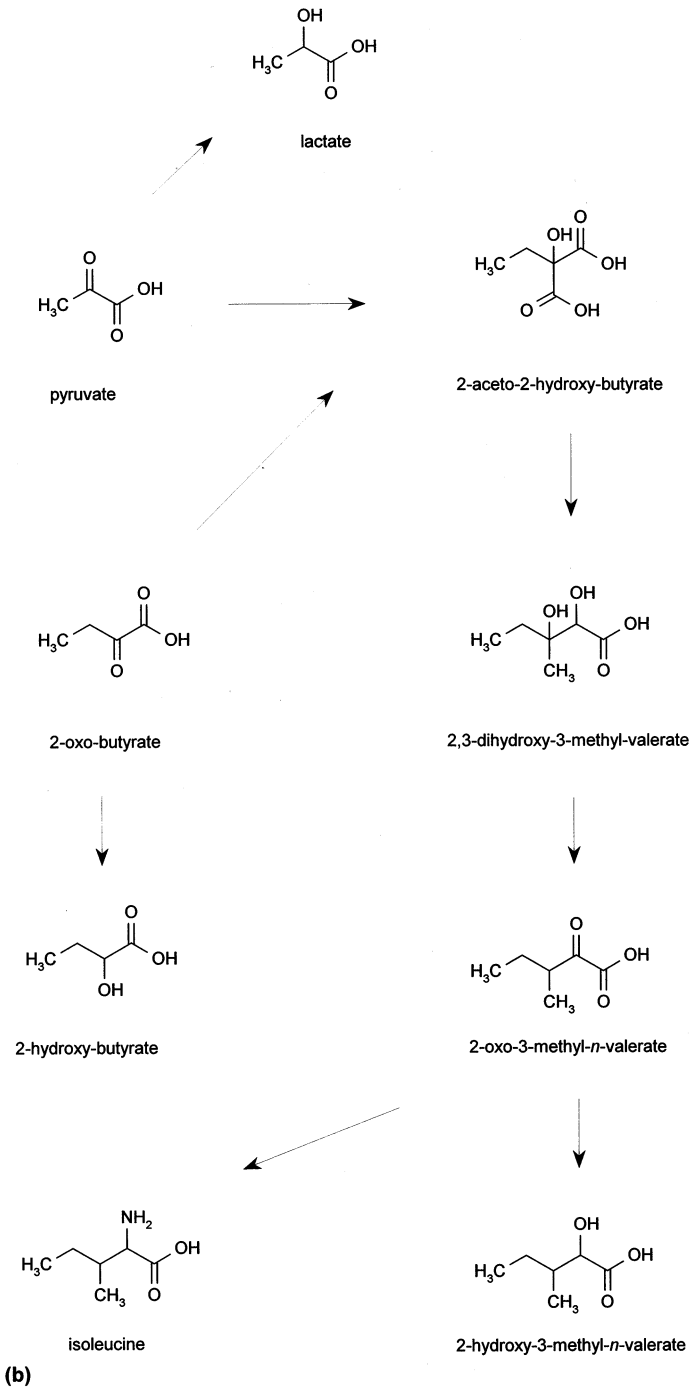
lactate
pyruvate
2-aceto-2-hydroxy-butyrate
2-oxo-butyrate
2,3-dihydroxy-3-methyl-valerate
2-hydroxy-butyrate
2-oxo-3-methyl-n-valerate
isoleucine
2-hydroxy-3-methyl-n-valerate
(b)

Fig. 4(b)

pounds and although there is still extensive peak overlap in many parts of the spectrum, the improved dispersion at 750 MHz ^{1}H observation frequency is useful in that it will provide a less ambiguous set of NMR-based descriptors which can be used as input for computer-based pattern recognition studies for sample classification. This is particularly important in the clinical area where the potential for high resolution NMR spectroscopy for clinical diagnosis has remained largely untapped.

The major benefits of using high frequency, high resolution NMR spectroscopy for clinical diagnosis arise because of the rapidity with which results can be obtained with a spectrum available after about 10 min of data acquisition and with the fact that NMR is a non-destructive technique. NMR spectroscopy, unlike conventional clinical chemical methods, does not require any preselection of the analytes and if the molecules of interest are in free solution in the biofluid and are present at a concentration of greater than about 5 μM, then in principle they should all be detectable simultaneously in a reasonable acquisition time, the major problem being peak overlap and hence ambiguity in resonance assignment and quantitation. The peak overlap problem is minimised by making measurements at the highest possible observation frequency as in the case under study here. In addition, because there is no preselection of analytes, the possibility always exists of discovering entirely new biochemical markers or for uncovering previously unknown biochemical pathway abnormalities.

Pattern recognition methods have proved useful in the diagnosistic analysis of ^{1}H NMR spectroscopic data from biofluids [18–21]. The NMR spectrum can be converted automatically into a series of numerical NMR-based descriptors, which characterise each sample. These values can then be used as input to computer-based pattern recognition methods for sample classification and some preliminary results using this approach on 600 MHz ^{1}H NMR spectra from urine samples from three other types of inborn error of metabolism have already been given [10].

High resolution ^{1}H NMR spectroscopy at very high observation frequencies is ideally suited for automation of the whole process. With a commercial automatic sample changer attached to the spectrometer, it is possible to measure up to 120 samples in an unattended fashion during nights and weekends with minimal human intervention, thereby making the whole process very cost effective despite the high capital cost of the equipment. The cost per sample becomes reasonable and the cost per analyte is very low. Given that each inborn error of metabolism usually gives rise to a distinctive set of resonances for abnormal metabolites in the ^{1}H NMR spectrum of urine, the development of a computer-based expert system for routine diagnosis becomes feasible.

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