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Note

Origin of the two peaks for 2-keto-3-methylvaleric acid produced by the oximation of the keto acids occurring in maple syrup urine disease

O.A. MAMER*, J.A. MONTGOMERY and V.Y. TAGUCHI

The Mass Spectrometry Unit, McGill University, 1130, Pine Avenue West, Montreal, Quebec H3A 1A3 (Canada)

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(+)-2-Keto-3*S*-methylvaleric acid is the product of the transamination in mammals of L-(+)-isoleucine (2*S*-amino-3*S*-methylvaleric acid). It is also the precursor of 2*S*-methylbutyryl CoA which leads into the conventional *S* pathway of mammalian isoleucine catabolism. Furthermore, the 3*S* chiral center in this keto acid may racemize through non-enzymic keto—enol tautomerism to yield (−)-2-keto-3*R*-methylvaleric acid. This *R* acid can be enzymically reaminated to L-(+)-alloisoleucine (2*S*-amino-3*R*-methylvaleric acid), and is also the precursor of 2*R*-methylbutyryl CoA, the entry point for a recently illuminated *R* pathway of isoleucine catabolism [1]. Thus the *R* and *S* stereomers of 2-keto-3-methylvaleric acid have different catabolic fates and both are physiologically important [2], contrary to the impression conveyed by Langenbeck et al. [3].

A compound having only one center of asymmetry at a carbon atom exists in two forms, *R* and *S* which cannot be distinguished by any physical means except by the observation of the direction of the angle of displacement of transmitted plane polarized light [4]. Several authors have published findings, however, that are interpreted to suggest that the trimethylsilyl (TMS) derivatives of the oximes of (+)-2-keto-3*S*-methylvaleric and (−)-2-keto-3*R*-methylvaleric acids are readily separated by gas chromatography on silicone liquid phases (ref. 5, Figs. 1 and 2; ref. 6, Fig. 1) and on Dexsil 300 as the TMS derivatives of the 3-(*sec.*-butyl)-2-quinoxalinol condensation products of *o*-phenylenediamine and the *R* and *S* keto acids [3]. This is clearly impossible. Enantiomers such as these two keto acids and any possible derivatives which do not introduce a second center of molecular asymmetry (i.e., generation of diastereomers) cannot be chromatographically separated in any racemic or non-chiral system. Gas

*To whom correspondence should be addressed.

chromatography using chiral stationary phases can of course be used to effect separation of enantiomers [7].

The gas chromatographic presentation of a pair of peaks due to the TMS derivatives of 2-ketoxime-3-methylvaleric acid is therefore not related to the relative quantities of the *R* and *S* isomers present for example in the urine and serum of maple syrup urine disease patients as reported by Jakobs et al. [6], but rather is related to the relative proportions of the *E* and *Z* (i.e., *anti* and *syn*) isomers determined by the ketoxime moiety itself. It is interesting to note further that the early eluting member of these two peaks was originally attributed to the L-isomer without substantiating evidence of any kind [5] and that this impossible assignment has been accepted by later authors.

We wish to report here the results of a study that identifies unequivocally the structures of the two oximes of this 2-keto acid.

EXPERIMENTAL

Preparation of oximes

D,L-2-Keto-3-methylvaleric acid (sodium salt, Sigma, St. Louis, Mo., U.S.A.) was converted in 50-mg quantities to its oxime derivatives by two methods: oxime mixture I by the method of Sternowsky et al. [5], and oxime mixture II by the method of Lancaster et al. [8]. Oxime mixture I was obtained in pyridine solution and was converted subsequently to the TMS derivatives as reported [5]. Oxime mixture II was obtained as the crude solid remaining from the evaporated diethyl ether extract. A redissolved aliquot of II was also converted to the TMS derivatives as reported [9]. Analysis of both oxime mixtures was effected on an LKB 9000 mass spectrometer under the following conditions: column, glass (2 m × 6 mm O.D.); 6% OV-101 on Chromosorb W HP (100—120 mesh); 125° isothermal; injector and separator, 280°; ion source, 290°, 70 eV, 60 μA. Under these conditions the derivatives of interest had retention times of approximately 10 min.

Reaction of oxime mixture II with copper(II) sulphate

An aqueous solution (3 ml) of oxime mixture II (10 mg) was made pH 10 with dilute aqueous sodium hydroxide. Saturated aqueous copper(II) sulphate (200 μl) was added and the resulting mixture was allowed to stand at room temperature for 10 min. A fine pale blue precipitate was removed by filtration through a Pasteur pipette packed with glass wool. The blue—green supernatant was acidified with concentrated hydrochloric acid, saturated with sodium chloride and extracted with 3 volumes of diethyl ether. The residue left from the evaporation of the diethyl ether was converted to the TMS derivatives and analyzed as described above.

RESULTS AND DISCUSSION

The bis-TMS derivatives of the *Z* and *E* oximes of 2-keto-3-methylvaleric acid are easily separable by gas chromatography using a 2-m 6% OV-101 column as illustrated in Fig. 1. Peaks D and E are the relative proportions of the *E* and *Z* isomers obtained when the oximation step is carried out in pyri-

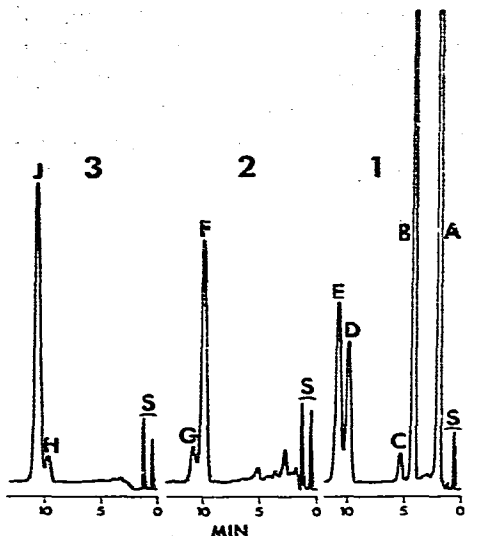


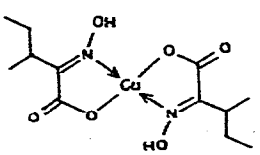
Fig. 1. Gas chromatographic analyses of the TMS derivatives of the isomeric oximes of 2-keto-3-methylvaleric acid. Chromatograms: (1) oxime mixture I prepared in pyridine according to Sternowsky et al. [5], peaks A, B, and C are artifacts produced in this procedure; (2) oxime mixture II prepared in aqueous alkali according to Lancaster et al. [8]; (3) the oxime mixture extracted from the supernatant obtained following the precipitation of the *E* oxime in mixture II with aqueous copper (II) sulphate. Peaks E, G, and J are produced by the *Z* oxime and have the mass spectrum reproduced in Fig. 2. Peaks D, F, and H are due to the *E* oxime and have the mass spectrum reproduced in Fig. 3. The peaks labelled S are solvent and reagent peaks that are gate valve attenuated.

dine according to the method of Sternowsky et al. [5]. Peaks A, B, and C appear to be artifactual, the probable result of the reaction of excess hydroxylamine in pyridine with the silylating reagent.

Peaks F and G are the relative proportions of the oxime isomers formed in aqueous alkaline solution according to the method of Lancaster et al. [8]. Peak G, the oxime that co-elutes with the oxime of 2-ketoisocaproic acid is very much reduced in favour of the isomer that elutes earlier (peak F).

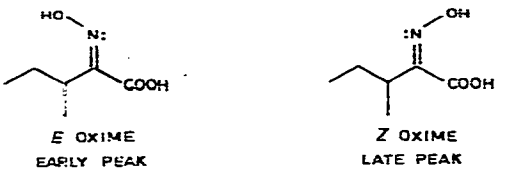
The method of Lancaster et al. [8] therefore bears the advantage over that of Sternowsky et al. [5] in that there is less mutual interference in the determination of 2-keto-3-methylvaleric and 2-ketoisocaproic acids in biological fluids. Secondly, the oximes have been shown to be much more completely extracted from urine and serum when made in situ [8] than the corresponding native keto acids which are oximated by Sternowsky et al. [5] only after extraction.

Treatment of oxime mixture II with an excess of aqueous copper (II) sulphate resulted in the selective precipitation of the *E* oxime as a copper chelate having the probable structure:



Stable chelation requires that the hydroxy group bound to nitrogen be directed away from the carboxyl group (*E*) so that it cannot interfere with coordination between the metal ion and the nitrogen atom. Oximes are known to form chelates involving the nitrogen atom rather than the oxygen atom [10].

The oxime not complexing with copper is therefore the *Z* isomer and corresponds to peak J (Fig. 1), the late eluting member of the pair which co-elutes with the oxime of 2-ketoisocaproic acid.



Peaks E, G, and J have identical mass spectra (Fig. 2) and retention times. The mass spectrum of the *E* isomer (Fig. 3) is very similar to that of the *Z* isomer, the major difference being the relative intensities of the $M^{+\cdot}-CH_3\cdot$ ions (*m/z* 274). The loss of the methyl radical from the ester TMS group in the

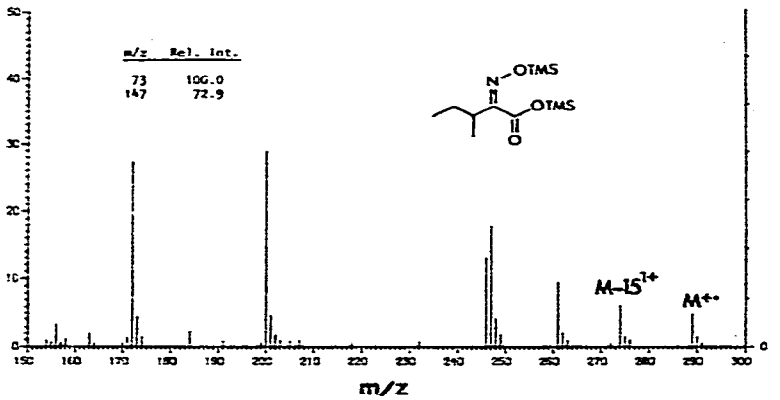


Fig. 2. Mass spectrum of (*Z*)-2-ketoxime-3-methylvaleric acid as the bis-TMS derivative.

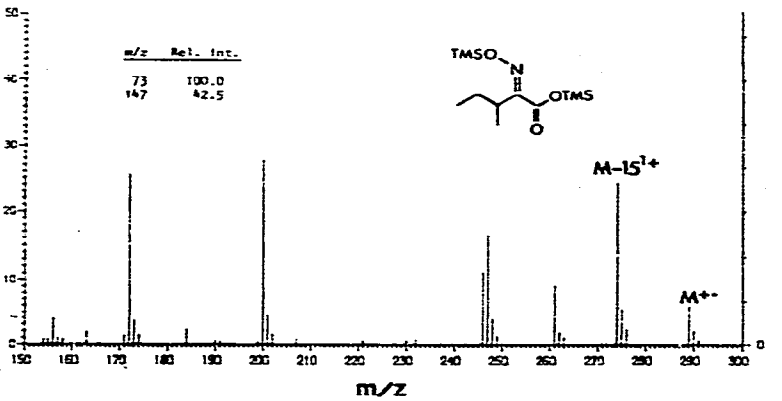
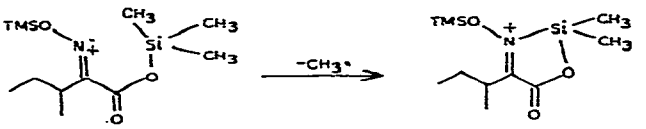


Fig. 3. Mass spectrum of (*E*)-2-ketoxime-3-methylvaleric acid as the bis-TMS derivative.

molecular ion is assisted in the case of the *E* derivative by the formation of a ring-stabilized even-electron fragment ion with the ion center on the nitrogen atom (Scheme 1). The trimethylsiloxy group presents a barrier to such ring formation in the *Z* isomer however, and this stabilizing force is therefore denied to this $M^{+\cdot}-CH_3\cdot$ ion, thus accounting for its reduced intensity relative to the *E* isomer.



SCHEME 1

The 2-keto acids which lack branching substitution on the 3-carbon (e.g., pyruvic, 2-ketobutyric, 2-ketovaleric, 2-ketocaproic, and 2-ketoisocaproic acids) appear to form predominantly a single oxime in pyridine while those with substituent branching on the 3-carbon (e.g., 2-keto-3-methylvaleric and 2-ketoisovaleric acids) form a pair of oximes in comparable yields. In oxime formation, the relative size of the two groups bonded to the carbonyl moiety is a major factor influencing the proportions of *E* and *Z* isomers formed. In the first group of acids, the carboxyl group is larger than the alkyl group unbranched at the 3-carbon, and therefore the *E* isomer predominates. With the second group in aprotic solvents (e.g., pyridine), the bulk associated with 3-carbon branching makes the *E* isomer less favoured and so both the *E* and *Z* oximes are formed in comparable yields. In aqueous alkali however, the carboxylate anion is highly solvated and is effectively much larger than in aprotic solvents thus favouring once again the formation of the *E* oxime which elutes free of 2-ketoisocaproic oxime interference.

ACKNOWLEDGEMENTS

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