Formation of 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (Sotolone) from 4-Hydroxy-L-isoleucine and 3-Amino-4,5-dimethyl-3,4-dihydro-2(5*H*)-furanone

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The proposed formation of 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolone) from 4-hydroxy-L-isoleucine (**1**) and the corresponding lactone 3-amino-4,5-dimethyl-3,4-dihydro-2(5*H*)-furanone (**2**) by thermally induced oxidative deamination was corroborated. The formation of sotolone was studied in model systems by reacting **1** or **2** with different carbonyl compounds in a phosphate buffer at pH 5 at 100 °C for 1 h. The amount of sotolone was quantified by stable isotope dilution assays using $^{13}C_2$-labeled sotolone as internal standard and GC-MS operating in the selected ion monitoring mode. In general, α-ketoaldehydes were found to be more reactive than α-diketones. Methylglyoxal gave rise to about 64 μg sotolone per mg **1** (7.4 mol %) compared to less than 1 μg (<0.1 mol %) when reacted with 2,3-pentanedione. Using **2** as the starting material, the yields were increased to 274 μg (35.9 mol %) and 5.4 μg (0.7 mol %), respectively. The optimum pH of the reaction with HIL was 5, representing the best compromise between the lactonization step and the amino−carbonyl reaction. Significant amounts of sotolone were generated only at temperatures higher than 70 °C. The yield increased over a period of 10 h to about 210 μg/mg **1** (23.8 mol %). The Strecker degradation of **1**, resulting in 3-hydroxy-2-methylbutanal, was a competitive reaction to the formation of sotolone.

Keywords: *3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolone); 4-hydroxy-L-isoleucine; oxidative deamination; Strecker degradation; quantification by isotope dilution assay; model system studies; synthesis*

INTRODUCTION

3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolone) is a powerful flavor compound found in several foods and spices. It contributes significantly to the burnt/sweet note of cane sugar (Tokitomo et al., 1980), aged sake (Takahashi et al., 1976), and coffee (Blank et al., 1992), to the spicy/curry note of fenugreek (Girardon et al., 1986), lovage (Blank and Schieberle, 1993), and condiments (Blank et al., 1993), as well as to the typical nutty/sweet flavor of botrytized wines (Masuda et al., 1984) and flor-sherry wines (Dubois et al., 1976; Martin et al., 1992).

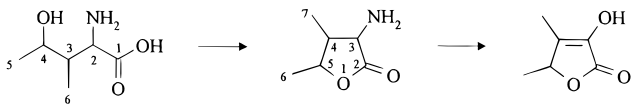
The flavoring potential of sotolone is due to its low threshold value of 0.02 ng/L air (Blank et al., 1992). The following threshold values were reported in water: detection/nasal 0.3 μg/L (Blank et al., 1993); detection/retronasal 0.01 μg/L (Tokitomo, 1980), 0.036 μg/L (Wild, 1988), 1−5 μg/L (Sulser et al., 1972); recognition/nasal 70 μg/L (Rödel and Hempel, 1974), 30 μg/L (Blank et al., unpublished results).

In sugar manufacturing, the formation of sotolone was explained via an amino−carbonyl reaction of pyruvate and α-ketoglutarate, the latter originating from glutamate (Kobayashi, 1989). In aged sake, sotolone was produced by condensation of α-ketobutyrate and acetaldehyde, both being acid decomposition products of threonine (Takahashi et al., 1976). The concentration of acetaldehyde and sotolone was positively correlated to the nutty/sweet flavor of flor-sherry wines (Martin et al., 1992).

The presence of sotolone in fenugreek oleoresin was first reported by Rijkens and Boelens (1975). The authors proposed sotolone as a character-impact compound of fenugreek (*Trigonella foenum-graecum* L.). Girardon et al. (1986) confirmed the presence of sotolone in fenugreek on the basis of MS data and synthesis. Blank et al. (1993) reported 4−25 mg/kg of sotolone in fenugreek seeds from different geographical origins.

Girardon et al. (1986) have pointed out the structural similarity between sotolone and 4-hydroxy-L-isoleucine (HIL) which is the most abundant free amino acid in fenugreek seeds (Fowden et al., 1973; Sauvaire et al., 1984). The authors postulated that this unusual amino acid could be the precursor of sotolone in fenugreek. This hypothesis has recently been supported by the fact that only the 4*S* enantiomer of sotolone is present in fenugreek (Sauvaire et al., 1993). This is in good agreement with the stereochemistry of HIL isolated from fenugreek which was shown to be 2*S*,3*R*,4*S* (Alcock et al., 1989).

Blank et al. (1993) have recently formulated a pathway for the formation of sotolone via thermally induced oxidative deamination of HIL (**1**). As shown in the simplified scheme, acid-catalyzed cyclization of HIL leads to the corresponding lactone (**2**) which reacts with an α-dicarbonyl. Rearrangement of the resulting Schiff



1 **2** Sotolon

base and subsequent hydrolysis gives rise to sotolone. The aim of this study was to verify this hypothesis and to study the parameters influencing the formation of sotolone from **1** and **2**.

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EXPERIMENTAL PROCEDURES

1. Materials and Reagents. The following chemicals and materials were obtained commercially: sotolone (Aldrich, Neu-Ulm, Germany), diethyl 2-methyl-3-oxobutanedioate, L-isoleucine, Dowex 50WX8 (20−50 mesh, Na^+ form), methylglyoxal (40% in water), phenylglyoxal, propionaldehyde, phenylacetaldehyde, 2,3-butanedione, and 2,3-pentanedione (Fluka, Buchs, Switzerland); [1,2-^{13}C]-acetaldehyde (99% purity, Cambridge Isotope Lab., Andover, MD); TLC plate silica gel 60 F254 (Merck, Darmstadt, Germany). The rotation perforator was from Normag (Weinheim, Germany). The deuterated solvents (D_2O, DMSO-d_2, CD_3OD) were from Dr. Glaser AG (Basel, Switzerland). Other solvents and chemicals were of analytical grade from Merck.

2. Synthesis. *3-Amino-4,5-dimethyl-3,4-dihydro-2(5H)-furanone hydrochloride (**2·HCl**).* Compound **2·HCl** was synthesized by photochemical chlorination of L-isoleucine in concentrated HCl and subsequent hydrolysis according to Faulstich et al. (1973) and Hasan (1986). A solution of L-isoleucine (21 g) in concentrated HCl (400 mL) was cooled to 0 °C. Chlorine gas was then bubbled through the solution for 6 h while it was irradiated in a photochemical reactor using a high-pressure Hg vapor lamp (380 nm). The reaction mixture was heated under reflux for 4 h and then concentrated to dryness. Water (50 mL) was added to the residue, and the pH was adjusted to 8.7 with Na_2CO_3 (28 g) at 5 °C. After dilution with water (200 mL), the sample was continuously extracted with Et_2O (100 mL) for 24 h using a rotation perforator. The organic phase was dried over anhydrous Na_2SO_4. Dry HCl gas was passed into the solution until complete precipitation was achieved. The precipitate was filtered and dried over KOH. The brown product (4.8 g) was dissolved in methanol and precipitated with Et_2O to give 3.1 g of the compound **2·HCl** as a diastereomeric mixture of the isomers (3*S*,4*R*,5*R*) and (3*S*,4*R*,5*S*) (overall yield 12%).

Elemental analysis: Found C (43.25), H (7.53), N (8.43), Cl (21.66); $C_6H_{12}NO_2Cl$ requires C (43.51), H (7.30), N (8.46), Cl (21.41).

MS-EI, *m/z* (rel int): 129 (9, M^+), 85 (29), 70 (100), 57 (31), 56 (29).

^{1}H-NMR (DMSO-d_6, internal TMS): Apart from a D_2O exchangeable slightly broadened signal at 9.01 ppm representing about 3 (total) protons, subspectra of two isomers in an approximate mole ratio of 59:41 were obtained. Major component: δ 4.766 (1H, q d, J = 6.3, 4.6 Hz, H-5), 4.591 (1H, d, J = 7.4 Hz, H-3), 2.800 (1H, m, ≥10 lines, H-4), 1.271 (3H, d, J = 6.5 Hz, CH_3-6), 0.866 (3H, d, J = 7.3 Hz, CH_3-7). Minor component: δ 4.524 (1H, d, J = 8.3 Hz, H-3), 4.491 (1H, q d, J = 6.5, 3.0 Hz, H-5), ca. 2.523 (1H, m, mostly under solvent signal, H-4), 1.367 (3H, d, J = 6.5 Hz, CH_3-6), 1.076 (3H, d, J = 7.2 Hz, CH_3-7). When the unavoidable milieu-induced shift effects are taken into account, these data are in good agreement with those of Hasan et al. (1976) and Hasan (1986) for the (3*S*,4*R*,5*R*) and the (3*S*,4*R*,5*S*) isomers, respectively.

*4-Hydroxy-L-isoleucine (HIL, **1**).* HIL (**1**) was synthesized by the base-catalyzed opening of the lactone function of **2·HCl** on a cation exchange resin (Hasan et al., 1976; Alcock et al., 1989). The column (24 × 200 mm) was packed with Dowex 50WX8 (150 g), converted to its NH_4^+ form with NH_4Cl (2 mol/L), and washed with water to remove the chloride ions. Compound **2·HCl** (1.5 g) dissolved in water (30 mL) was added layered to the column. Chloride ions were removed by washing with water (500 mL). Compound **1** was eluted with ammonia (0.5 mol/L) to obtain six fractions each of 500 mL. The presence of the amino acid was checked by TLC using butanol/acetic acid/water in the ratio 4/1/1 (v/v/v) as the mobile phase and detected with ninhydrin. The ninhydrin-positive fractions were concentrated at 37 °C and lyophilized to obtain 1.2 g of a colored product. Recrystallization from ethanol (90%, 50 mL) and hot filtration gave 700 mg of a white powder (4.8 mmol, 53% yield).

Elemental analysis: Found C (48.74), H (9.00), N (9.47); $C_6H_{13}NO_3$ requires C (48.97), H (8.90), N (9.52). MS-EI, *m/z* (rel int): 132 (2), 129 (10), 102 (63), 74 (65), 58 (100); MS-PCI (NH_3) *m/z* (rel int): 147 (100, M^+), 148 (49), 165 (6).

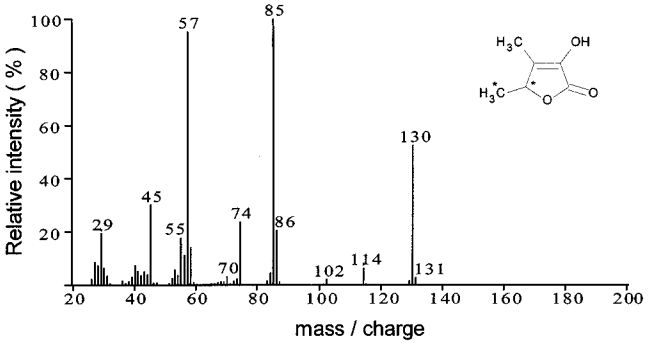

Figure 1. Electron impact (EI) mass spectrum of [5,6-^{13}C]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone ($^{13}C_2$-sotolone). The MS-EI spectrum of the corresponding unlabeled sotolone is *m/z* (% relative abundance) 83 (100), 55 (95), 128 (60, M^+), 43 (55), 57 (45), 29 (40), 27 (30), 39 (25), 72 (25), 85 (25).

^{1}H-NMR (D_2O/internal TSP). Two subspectra representing two isomers in a mole ratio of about 59:41 were again obtained, the minor of which was nearly identical with the data published for the (2*S*,3*R*,4*S*) isomer (Alcock et al., 1989): δ 3.908 (1H, d, J = 4.4 Hz, H-2), 3.865 (1H, d q, J = 8.0, 6.4 Hz, H-4), 1.933 (1H, m, 16 lines, H-3), 1.256 (3H, d, J = 6.3 Hz, CH_3-5), 0.970 (3H, d, J = 7.1 Hz, CH_3-6). The major component was therefore attributed to the (2*S*,3*R*,4*R*) isomer: δ 4.070 (1H, q d, J = 6.4, 2.8 Hz, H-4), 3.825 (1H, d, J = 4.0 Hz, H-2), 2.144 (1H, m, 16 lines, H-3), 1.220 (3H, d, J = 6.4 Hz, CH_3-5), 1.074 (3H, d, J = 7.3 Hz, CH_3-6).

[5,6-^{13}C]-3-Hydroxy-4,5-dimethyl-2(5H)-furanone ($^{13}C_2$-Sotolone). $^{13}C_2$-Sotolone was synthesized by condensation of diethyl 2-methyl-3-oxobutanedioate and [1,2-^{13}C]-acetaldehyde followed by lactonization and subsequent decarboxylation under strongly acidic conditions as described for the corresponding unlabeled compound (Rödel and Hempel, 1974). The reaction was carried out on a micro-scale using a Sovirel test tube (20 mL) which was modified with a double jacket to cool the upper part. Diethyl 2-methyl-3-oxobutanedioate (22 mmol) was placed in the tube, to which labeled acetaldehyde (21.7 mmol) and dry pyridine (40 mmol) were added at 0 °C. The mixture was heated in an oil bath at 120 °C for 2 h, while the upper part of the tube was kept at +5 °C using a cryostat. After adding HCl (37%, 7 mL), acetic acid (99%, 6 mL), and water (9 mL), the solution was refluxed for 4 h. The solution was diluted with water (300 mL), and the pH was adjusted to 8 with NaOH (10 mol/L). The target compound was extracted with Et_2O (100 mL) overnight using a rotation perforator. The solvent was removed by distillation, and the residue was dissolved in methanol (100 mL). The amount of labeled sotolone was determined by GC-MS measuring a 1:1 mixture of the labeled and unlabeled sotolone used as internal standard resulting in 323 mg of labeled sotolone (12% yield). The MS spectrum of the compound shown in Figure 1 indicates the incorporation of two ^{13}C atoms. The position of the ^{13}C atoms was verified by NMR by comparison with the proton and carbon spectra of unlabeled sotolone.

^{1}H-NMR (CD_3OD, internal TMS; moderate resolution enhancement after zero filling was required to extract the coupling constants (0.089 Hz/point resolution), the *italicized* data are due to coupling with the two ^{13}C atoms); δ 4.835 (*d* q *d* q, J = *152.1*, 6.6, *3.2*, 1.2 Hz, H-5), 1.870 (*d* d *d*, J = *4.3*, 1.2, *0.7* Hz, CH_3-7), 1.362 (*d* d *d*, J = *128.5*, 6.6, *4.6* Hz, CH_3-6).

^{13}C-NMR (CD_3OD, internal TMS, proton decoupled, spectral width 20 000 Hz, FID 64 K data points, zero filling, resolution 0.305 Hz/data point); dominating signals from the ^{13}C substituted sites: δ 78.94 (d, J = 38.7 Hz, C-5), 18.98 (d, J = 38.7 Hz, C-6). Small signals from unsubstituted sites: 171.81 (d, J = 1.8 Hz, C-2), 138.87 (d d, J = 4.7, 1.5 Hz, C-3), 134.37 (d d, J = 41.6, 1.3 Hz, C-4), 9.08 (d d, J = 3.7, 0.6 Hz, C-7); all shifts were within 0.1 ppm of those of natural abundance sotolone (D. H. Welti and F. Arce Vera, unpublished data).

3. Instrumental Analysis. *Preparative Gas Chromatography.* Labeled sotolone was purified for NMR analysis by preparative GC using a MCS Gerstel gas chromatograph

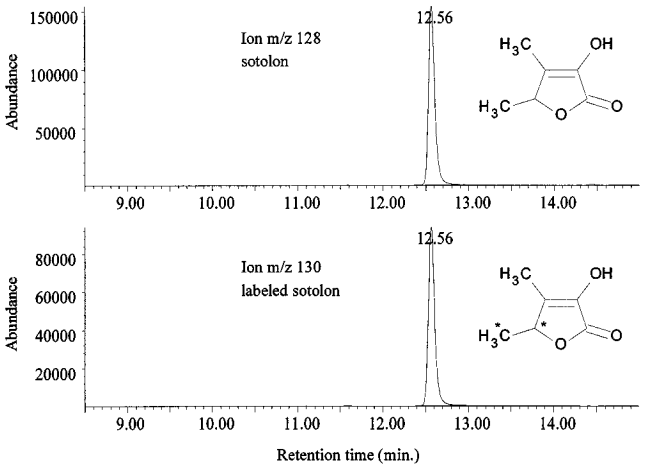

Figure 2. Quantification of sotolone by stable isotope dilution assays using $^{13}C_2$-sotolone as internal standard. The mass chromatograms were recorded by GC-MS operating in the selected ion monitoring (SIM) mode to measure the molecular ions *m*/*z* 128 of sotolone and *m*/*z* 130 of the doubly-labeled sotolone standard.

(Mülheim, Germany). Two DB-Wax widebore capillaries were employed (J&W Scientific), both 15 m × 0.53 mm with a film thickness of 1 μm. Helium was used as carrier gas (13 psi). The oven was programmed as follows: 60 °C (1 min), 10 °C/min up to 200 °C (3 min). Nine injections resulted in about 3 mg of sotolone trapped in a glass tube at 50 °C.

Nuclear Magnetic Resonance (NMR) Spectroscopy. All NMR spectra were acquired on a Bruker AM-360 spectrometer equipped with a quadrinuclear 5 mm probe head (QNP), at 360.13 MHz for ^{1}H and at 90.56 MHz for ^{13}C under standard conditions. The probe temperature was 21 °C for the proton spectra and slightly higher for the carbon spectra due to heteronuclear composite pulse decoupling. The frequency resolution for the proton spectra was 0.231 Hz/point. The shifts are cited in parts per million from the respective internal standards.

Mass Spectrometry (MS). a. Qualitative Analysis. Electron impact (EI) and positive chemical ionization (PCI) mass spectra of the synthesized compounds **1** and **2** were obtained at a resolution of 1000 on a Finnigan MAT 8430 mass spectrometer. The samples were directly introduced into the ion source heated at 180 °C. The acquired mass range was 20−500 Da for both EI and PCI experiments. Ammonia was used as reagent gas for chemical ionization. Labeled sotolone and 3-hydroxy-2-methylbutanal (**9**) were introduced via a Hewlett-Packard HP-5890 gas chromatograph using the cold on-column injection technique. The samples were analyzed on the FFAP capillary column (J&W Scientific, 30 m × 0.25 mm, film thickness 0.25 μm) using helium as carrier gas (10 psi). Temperature program: 50 °C (2 min), 4 °C/min to 180 °C, 10 °C/min to 240 °C (10 min).

b. Quantitative Analysis. Sotolone was quantified by stable isotope dilution assays using $^{13}C_2$-sotolone as internal standard (Blank et al., 1993). Quantification experiments were performed with a Hewlett-Packard HP-5971 GC-MS equipped with a DB-Wax capillary column (J&W Scientific): 30 m × 0.25 mm, film thickness 0.25 μm. Helium was used as carrier gas (10 psi). The sample (2 μL) was introduced via splitless injection at 250 °C. Temperature program: 20 °C (0.5 min), 30 °C/min to 100 °C, 4 °C/min to 145 °C, 70 °C/min to 220 °C (10 min). The compounds were recorded by electron impact ionization at 70 eV. The temperature of the ion source was 190 °C. Sotolone and the labeled internal standard ($^{13}C_2$-sotolone) were detected by selected ion monitoring (SIM) of their molecular ions *m*/*z* 128 and 130, respectively. The traces of the ions were recorded as shown in Figure 2, and the concentration of sotolone was calculated from the peak areas using a calibration factor of 1.1. The calibration curve was established with standard mixtures containing defined amounts of labeled and unlabeled sotolone in different ratios following the procedure described by Guth and Grosch (1990). A good linearity was found in the concentration range 3−150 μg/mL (r^2 = 0.999). Samples for establishing the calibration curve and for quantifying sotolone were injected twice.

Table 1. Formation of Sotolone from 4-Hydroxy-L-isoleucine (HIL, 1)[a]

carbonyl	sotolone[b] (μg/mg HIL)	yield (mol %)
2,3-butanedione	0.34 ± 0.03	<0.1
2,3-pentanedione	0.30 ± 0.03	<0.1
methylglyoxal[c]	64.2 ± 0.3	7.4
phenylglyoxal	22.2 ± 0.3	2.5
propionaldehyde	1.00 ± 0.06	0.1
phenylacetaldehyde	0.24 ± 0.03	<0.1

[a] HIL control experiment (without carbonyl) yielded 0.04 μg sotolone/mg HIL (<0.01 mol %). [b] Data are the means of at least two experiments, each of them injected twice. [c] Methylglyoxal control experiment (reaction without HIL) yielded 0.07 μg of sotolone.

Table 2. Formation of Sotolone from 3-Amino-4,5-dimethyl-2-oxotetrahydrofuran (2)[a]

α-dicarbonyl	sotolone (μg/mg **2**)	yield (mol %)
methylglyoxal	274.4 ± 3.4	35.9
2,3-pentanedione	5.4 ± 0.3	0.7

[a] Control experiment (reaction without carbonyl) yielded 0.03 mol % sotolone.

4. Preparation of Samples for Quantitative Analysis. HIL (**1**, 2−10 mg) and **2·HCl** (2−10 mg) were dissolved in a phosphate buffer (2−10 mL, 0.1 mol/L, pH 5.0). The carbonyl reactant was added so that the molar ratio of precursor to carbonyl was 1:10. The solution was boiled for 1 h in a glass tube closed with a screw cap. After rapidly cooling down the reaction mixture, water (50 mL) and then the internal standard ($^{13}C_2$-sotolone, 25.8−64.5 μg) were added. The sample was saturated with NaCl, and the pH was adjusted to 4 with HCl (1 mol/L). Sotolone was continuously extracted with Et_2O for 8 h using a rotation perforator. The extract was dried over Na_2SO_4 and concentrated to about 1 mL on a Vigreux column (1 m × 1 cm). All experiments were performed in duplicate.

RESULTS AND DISCUSSION

1. Verification of the Hypothesis. *4-Hydroxy-*L*-isoleucine (HIL, **1**) as Precursor.* The formation of sotolone from HIL was studied by reacting HIL with different mono- and α-dicarbonyl compounds in a phosphate-buffered model system (0.1 mol/L, pH 5.0) at 100 °C for 1 h. The results summarized in Table 1 show that both 2,3-butanedione and 2,3-pentanedione formed only low amounts of sotolone. Higher yields were achieved when using methylglyoxal (7.4 mol %) and phenylglyoxal (2.5 mol %) as reactant, producing about 70−200 times more sotolone than the corresponding reaction with 2,3-butanedione. The yields obtained with propionaldehyde and phenylacetaldehyde were low (≤0.1 mol %), i.e. about 60−90 times less compared to the corresponding α-ketoaldehydes.

*3-Amino-4,5-dimethyl-3,4-dihydro-2(5H)-furanone (**2**) as Precursor.* The efficiency of the lactone of HIL was studied by reacting **2·HCl** with α-dicarbonyls. As shown in Table 2, significantly higher amounts of sotolone were generated from **2·HCl** as compared to **1**. Using methylglyoxal, the yields were increased by a factor of about 5 to 35.9 mol %. The lactone **2** was found to be a better precursor than the amino acid.

Formation of Sotolone (Figure 3). The data reported above confirm the hypothesis of the formation of sotolone by thermally induced oxidative deamination of HIL (**1**) (Blank et al., 1993). Acid-catalyzed cyclization of HIL leads to the corresponding lactone (**2**) which

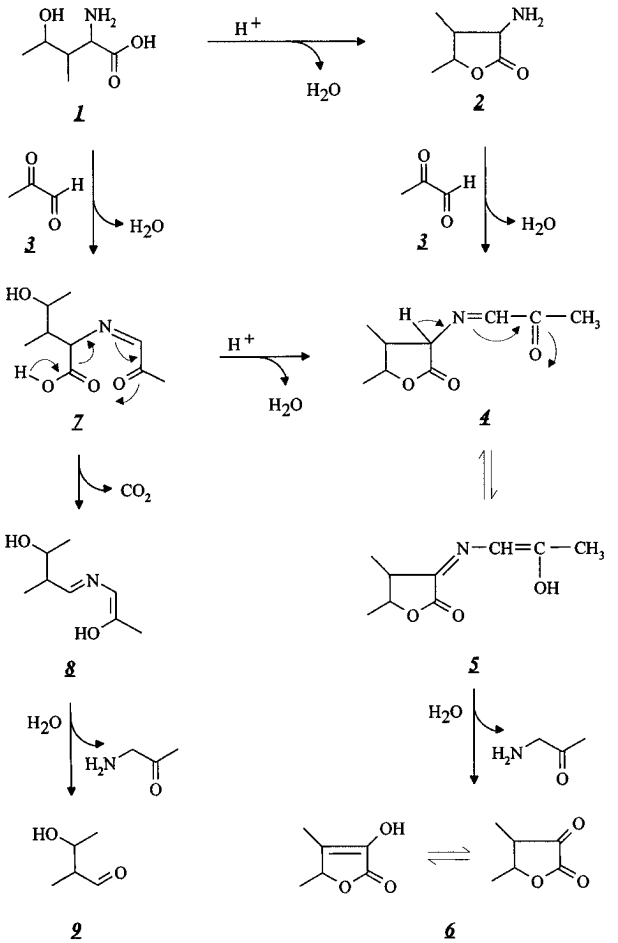

Figure 3. Formation of sotolone (**6**) and 3-hydroxy-2-methylbutanal (**9**) from 4-hydroxy-L-isoleucine (**1**) and 3-amino-4,5-dimethyl-3,4-dihydro-2(5*H*)-furanone (**2**) using methylglyoxal (**3**) as the carbonyl reactant (more details in the text).

reacts with an α-dicarbonyl (**3**) to form the Schiff base **4**. Rearrangement of **4** and subsequent hydrolysis of **5** give rise to sotolone (**6**). The data show that α-dicarbonyls are capable of generating sotolone from both HIL (**1**) and the lactone **2**. However, the latter is more efficient in producing sotolone.

The lactonization step (**1** → **2**) is apparently an important parameter of the reaction and is favored under acidic conditions. The lactonization yields were about 50% at pH 3 and 10% at pH 5 (Blank et al., unpublished results, 1996). Furthermore, α-ketoaldehydes (**3**) are much more efficient than α-diketones, indicating that the reactivity of the carbonyl compounds is another crucial parameter, particularly for the formation of the Schiff base **4**. This is in agreement with the data obtained with α-keto acids and ascorbic acid which generate only low amounts of sotolone from HIL (Blank et al., 1995).

The fact that Strecker-inactive carbonyl compounds, such as propionaldehyde and phenylacetaldehyde, are also able to transform HIL into sotolone indicates an alternative formation pathway. Aldol condensation products, e.g. 2,4-dimethyl-2,4-heptadienal, may function as active components in the transamination of HIL. Similar reactions, i.e. reductive amination of conjugated carbonyls, have been reported by Rizzi (1976). Hence, condensation products of methylglyoxal may also be involved in the formation of sotolone.

The lower yields obtained with HIL can be explained by a partial Strecker degradation of the amino acid (**1**)

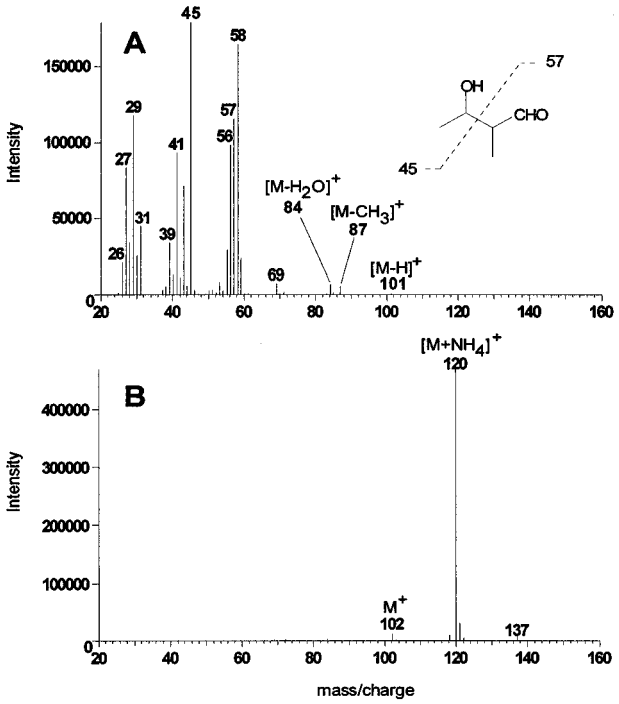

Figure 4. Mass spectra of 3-hydroxy-2-methylbutanal (**9**) eluted as the second peak of a diastereomeric mixture on an FFAP (retention index: 1560): (A) electron impact spectrum and (B) spectrum by chemical ionization using ammonia as reagent gas.

Table 3. Formation of Sotolone from HIL (1) and the Corresponding Lactone 2 As Affected by the pH[a]

α-dicarbonyl	pH	from HIL (**1**) (μg/mg)	yield (mol %)	from **2** (μg/mg)	yield (mol %)
methylglyoxal	3	42.8 ± 3.2	5.0	178.2 ± 8.5	23.0
methylglyoxal	5	64.2 ± 0.3	7.4[b]	274.4 ± 3.4	35.9
methylglyoxal	6	16.5 ± 1.3	1.9	311.2 ± 12.5	40.2
methylglyoxal	7	2.3 ± 0.3	0.2	48.6 ± 2.3	6.3

[a] Data are the means of at leas two experiments, each of them injected twice. [b] The reaction performed at pH 5 without using phosphate resulted in 2.8 mol % sotolone.

in the presence of an active α-dicarbonyl, i.e. methylglyoxal (**3**). As shown in Figure 3, the amino−carbonyl reaction of **1** and **3** results in the Schiff base **7** which may cyclize to **4** or decompose to **8** via decarboxylation. The Strecker aldehyde of HIL is released from **8** by hydrolysis, i.e. 3-hydroxy-2-methylbutanal (**9**). Indeed, this compound was tentatively identified by GC-MS (Figure 4) as a diastereomeric mixture. Both isomers showed nearly identical EI and CI spectra, respectively.

In the sample based on HIL and methylglyoxal, the ratio of formed Strecker aldehyde (**9**) to sotolone was about 1:2 (Blank et al., unpublished results, 1996). Hence, the Strecker degradation of HIL is a competitive reaction to the formation of sotolone. In contrast, only traces of **9** were detected in the sample containing the lactone **2**, i.e., about 50 times less than in the reaction with HIL. The formation of sotolone from **2** is the favored reaction, most likely due to the blocked carboxyl group.

2. Parameters Affecting the Formation of Sotolone from HIL (1) and the Corresponding Lactone (2). *Influence of pH (Table 3).* It is well-known that both lactonization and the formation of the Schiff base are strongly dependent on the pH of the reaction medium. To find the optimum pH, methylglyoxal was reacted with HIL and the lactone **2·HCl**, respectively.

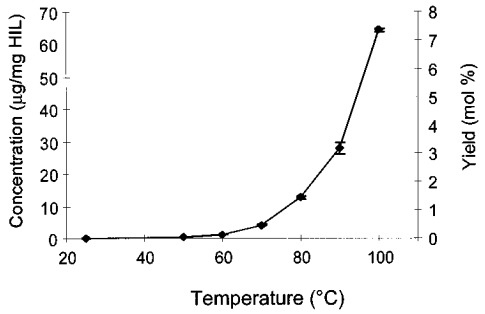

Figure 5. Formation of sotolone from 4-hydroxy-L-isoleucine (HIL, **1**) as a function of the reaction temperature (reaction with methylglyoxal in a phosphate buffer of pH 5.0 for 1 h).

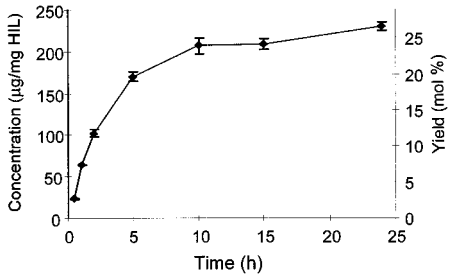

Figure 6. Formation of sotolone from 4-hydroxy-L-isoleucine (HIL, **1**) depending on the reaction time (reaction with methylglyoxal in a phosphate buffer of pH 5.0 at 100 °C).

Table 4. Formation of Sotolone from HIL As Affected by the Concentration of Methylglyoxal

molar ratio HIL/methylglyoxal	sotolone (μg/mg HIL)	yield (mol %)
1:1	24.0 ± 0.3	2.7
1:2	28.8 ± 0.9	3.3
1:5	41.3 ± 2.1	4.7
1:10	64.2 ± 0.3	7.4
1:20	62.9 ± 1.1	7.2

The reaction of methylglyoxal and HIL was favored at pH 5 which apparently is the best compromise between the lactonization step and the reactivity of the amino group to form the Schiff base (Figure 3). Once the lactone **2** is formed, the amino−carbonyl reaction was favored at pH 5−6. In general, the yields obtained with the lactone **2** were significantly higher compared to the amino acid, particularly at pH 6 (40.2 mol %). The reaction of HIL and methylglyoxal at pH 5 performed in water yielded 2.8 mol % sotolone compared to 7.4 mol % when using the phosphate-buffered system. This suggests a catalytic effect of phosphate on the formation of sotolone from HIL.

Influence of Reaction Temperature and Time. The sotolone yields from HIL and methylglyoxal were strongly dependent on both the reaction time and temperature. Significant amounts were generated only above 70 °C (Figure 5). At a constant temperature of 100 °C, the yield of sotolone continuously increased over a period of 10 h and flattened off thereafter (Figure 6). About 23−210 μg sotolone was generated from 30 min to 10 h which corresponds to 2.7 and 23.8 mol %, respectively.

Amount of α-Dicarbonyl (Table 4). The efficiency of transforming HIL into sotolone can be enhanced by using higher amounts of the carbonyl reactant. Increasing the concentration of methylglyoxal 5-fold almost doubled the amount of sotolone produced. However, the concentration of sotolone did not increase linearly with the amount of the α-dicarbonyl.

CONCLUSIONS

The proposed formation of sotolone from 4-hydroxy-L-isoleucine (HIL, **1**) via thermally induced oxidative deamination was substantiated. The lactone of HIL, 3-amino-4,5-dimethyl-3,4-dihydro-2(5*H*)-furanone (**2**), was found to be a better precursor compared to the amino acid. α-Ketoaldehydes were more effective in generating sotolone from both HIL and the corresponding lactone **2** than α-diketones. The reactivity of the dicarbonyl and the lactonization step are important parameters, particularly for the formation of the Schiff base. The transformation yields from HIL into sotolone greatly depend on the reaction conditions, such as temperature, time, pH, and the amount of the dicarbonyl. Best results were obtained by boiling methylglyoxal and HIL for 10 h at pH 5 (about 24 mol %). Alternatively, due to the inhibition of the Strecker degradation of the amino acid by the lactone form, even better results (about 40 mol %) were achieved in a shorter time (1 h) at pH 6 using **2** as precursor.

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