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Formation of Pyrazines from Acyloin Precursors under Mild Conditions

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Alkylpyrazines are formed in reactions of acyloins and ammonia under mild conditions and acidic pH. Results from model systems suggest that pyrazines of biogenic origin can be explained by nonenzymic reactions between products of cell metabolism and ammonia.

Alkylpyrazines have been widely investigated as flavor-important trace components in foods. The exact origin of pyrazines remains a mystery, but many model studies suggest that they are minor products of the Maillard reactions of free amino acids and reducing sugars (Maga, 1982). The most prevalent alkylpyrazines, e.g., 2,5-dimethylpyrazine, can reasonably be explained as minor products of the Strecker reaction of amino acids and carbohydrate-derived reductones. The subject of pyrazine formation under relatively high temperature conditions is still under active investigation, and several comprehensive reviews have already appeared (Ohloff et al., 1985; Vernin and Metzger, 1981).

Less attention has been directed toward the chemistry of pyrazine production under biosynthetic conditions. Several reports have appeared describing the natural occurrence of simple pyrazines (McIver and Reineccius, 1986; Gallois, 1984; Kempler, 1983). Tetramethylpyrazine (TMP) formation in bacteria (Demain et al., 1967) has been described as an artifact resulting from mutation-induced dysfunction of *reductoisomerases* in the normal biosynthesis of valine from pyruvate. Thus, α-acetolactate, the normal condensation product of pyruvate, accumulates and eventually undergoes loss of carbon dioxide via *acetolactate decarboxylase* to yield an acyloin, 3-hydroxy-2-butanone (acetoin). Further, it has been suggested that acetoin reacts with ambient ammonia to produce TMP (Kosuge et al., 1971; Demain et al., 1967) although the latter reaction has not been shown to be enzyme catalyzed. Recently, complex alkylpyrazine mixtures resembling those produced in Maillard reactions were reported in fermented cacao (Barel et al., 1985; Gill et al., 1984) and fermented soya products (Liardon and Ledermann, 1980) and in cheese (Liardon et al., 1982). The formation of complex pyrazine mixtures under fermentative conditions can be explained by invoking nonenzyme-catalyzed reactions of a series of biochemically derived acyloins with ammonia. In theory, acyloins are biochemically available from bimolecular reactions of α-keto acids and/or other bioavailable carbonyl compounds. For example, in isoleucine biosynthesis (White et al., 1968) α-ketobutyrate and acetaldehyde combine to form α-aceto-α-hydroxybutyrate, which on decarboxylation could yield another acyloin, 3-hydroxy-2-pentanone.

The purpose of this study was to examine the nature of pyrazine formation from acyloins and ammonia under mild

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reaction conditions, not involving enzymes.

EXPERIMENTAL SECTION

Materials. Acetoin, acetol (1-hydroxy-2-propanone), 2-hydroxycyclohexanone, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, trimethylpyrazine, and tetramethylpyrazine were purchased from Aldrich Chemical Co. 4-Hydroxy-3-hexanone was purchased from K and K Laboratories, Inc. 3-Hydroxy-2-pentanone and octahydrophenazine were synthesized by published procedures [Lawesson and Gronwall (1960) and Rizzi (1972), respectively]. DL-α-Amino-n-butyric acid was purchased from Sigma Chemical Co. Inorganic reagents and solvents were all commercial products of analytical-grade purity.

Methods of Analysis. Reaction mixtures were routinely analyzed by HPLC using an LDC Model III pump and an LDC SpectroMonitor III detector (280 nm) with a 25 × 0.46 cm RP Ultrasphere C-18 ODS column under isocratic conditions at ambient temperature. Solvent was 50/50 v/v methanol–water (1.0 mL/min) for all separations except mixtures containing tetraethylpyrazine, which required 50/50 v/v isopropyl alcohol–water (0.60 mL/min). Quantitation was done on base-line-resolved peaks vs external standards on a Hewlett-Packard 3390A integrator. Replicate analyses using a Rheodyne 7120 injector (20-μL loop) agreed with about 5% precision.

For structure identification and comparison with standards, HPLC equipped with diode array detection (DAD) and GC–MS were used. For HPLC (DAD) we used a Varian 5500 ternary gradient unit with Kratos 757 UV detector (280 nm) and LKB 2140 detectors; the column was an Altex C-18 (5 μm) (35 °C) with 1.50 mL/min flow rate and the following time-programmed solvent composition [solvent A/solvent B, min]: 93/7, initial; 65/35, 40; 50/50, 45; 93/7, final. Solvent A was 0.05 M aqueous ammonium phosphate (adjusted to pH 2.5 with phosphoric acid), and solvent B was acetonitrile.

GC–MS was done with a Kratos MS-30 unit operated in the dual-beam, electron ionization mode (70 eV). Magnet scan speed was 1 s/decade with mass resolution ca. 1000. Source temperature and transfer line temperature were 220 °C. Typical mass measurement precision under these conditions was 15–20 ppm. GC was done with use of temperature-programmed 15 m × 0.25 mm or 30 m × 0.32 mm fused silica columns containing DB Wax under typical head pressures of 7 and 4 psi, respectively, and an injection port temperature of 250 °C. Injection split ratio was 20/1.

NMR spectra on synthesized reference compounds were obtained in $CDCl_3$ solution on a Varian T-60 (60-MHz) spectrometer using tetramethylsilane as internal reference standard (δ 0.00).

Reaction Procedures. Reactions at 22 °C were usually run by combining 3 mol equiv of an ammonium salt/mol of acyloin in glass-stoppered volumetric flasks and storing the mixtures in the dark. Reactions above room temperature were done in flame-sealed glass ampules kept in a thermostated oven. Experiments with acetoin were done with commercial acetoin solution (85% acetoin–15% water). In other experiments water was added to comprise 15% of total reaction weight. High reactant concentrations were used to obtain practical yields of pyrazines that could be quantitated in reasonable time periods.

Tetraethylpyrazine. A mixture containing 4-hydroxy-3-hexanone (3.48 g, 0.030 mol), ammonium acetate (6.94 g, 0.090 mol), and water (0.61 mL) was stored in a closed vial for 94 h at 21 °C. The dark product was treated with 400 g of 25% sodium hydroxide solution and distilled at atmospheric pressure. The aqueous distillate (about 250 mL) was extracted with methylene chloride (3 × 30 mL) and the combined organic phase was dried, evaporated, and vacuum-distilled to provide 2.15 g of crude product, bp 126–130 °C (16 mmHg). Treatment of the product with picric acid in aqueous ethanol gave a crystalline, yellow picrate that was filtered and air-dried. Subsequent decomposition of the picrate with aqueous ammonia followed by ether extraction separated the pyrazine. Solvent removal followed by vacuum distillation gave pure tetraethylpyrazine, 0.29 g (10% yield), as a light yellow oil, bp 100–110 °C (10 mmHg). IR and NMR spectra agreed well with published data (Rizzi, 1972).

2,5-Diethyl-3,6-dimethylpyrazine. A literature synthesis of tetramethylpyrazine (Dakin and West, 1928) was modified by using DL-α-amino-n-butyric acid in place of alanine to obtain the named pyrazine as a colorless oil with a strong camphor-like aroma: 52% overall yield; NMR δ 1.23 (t, J = 8 Hz, CH_3CH_2, 6 H), 2.50 (s, CH_3, 6 H), 2.77 (q, J = 8 Hz, CH_3CH_2, 4 H).

3-(Dimethylamino)-2-butanone. Acetic acid was added dropwise to 10.0 mL of 40% aqueous dimethylamine (0.080 mol) until the mixture attained pH 6.55. The solution was treated with 3.0 mL (0.086 mol) of commercial acetoin (85% 3-hydroxy-2-butanone–15% water) and heated in a sealed ampule at 90 °C for 75 h. The product was diluted 1/10 with 5% aqueous acetic acid and allowed to flow through a short column of Biorad AG 50W-X8 50–100 mesh cation-exchange resin (H^+ form). The column was washed with water to remove acetic acid and neutral compounds and then eluted with 20% aqueous sodium hydroxide to remove bases. The alkaline eluant was extracted with ether to obtain 0.16 g of the amino ketone as an oil: 1.7% yield; NMR δ 1.12 (d, J = 4 Hz, $CH_3CH<$, 3 H), 2.20 (s, $CH_3C{=}O$, 3 H), 2.27 (s, $(CH_3)_2N$, 6 H), 2.97 (m, CH<, 1 H).

Isomerization of 3-Hydroxy-2-pentanone. A 20-mL glass ampule was charged with 0.49 g of 3-hydroxy-2-pentanone and 2.05 g of a mixture composed of acetic acid (64.1%), triethylamine (31.8%), and water (4.1%) (pH 5.6). The container was flame-sealed and stored at 90 °C for 24 h. Later, the reaction mixture was poured into saturated potassium bicarbonate and extracted with ether (4×). The ether solution was quickly washed with 0.4 N HCl to remove triethylamine, dried (Na_2SO_4), and evaporated to afford 0.31 g of a liquid that contained a 4/3 ratio of starting material and its isomer 2-hydroxy-3-pentanone. The isomeric hydroxyketone was indicated by new NMR peaks at δ 1.13 (t, J = 7 Hz, CH_3CH_2), 1.42 (d, J = 6 Hz, $CH_3CH<$), and 2.57 (q, J = 7 Hz, CH_3CH_2) that were distinct from the peaks due to starting material at δ 0.95 (t, J = 5 Hz, CH_3CH_2), 1.73 (m, CH_3CH_2), and 2.23 (s, $CH_3C{=}O$).

RESULTS AND DISCUSSION

At 22 °C 3-hydroxy-2-butanone (acetoin) combined with ammonia to form tetramethylpyrazine (TMP) in a reaction that proved to be highly pH dependent (Table I). After 17.5 h the highest yield of TMP (13.3%) was obtained with the weakly acidic salt, ammonium acetate. Similar reactions with more alkaline or more acidic salts gave only traces of TMP; and no TMP was formed with ammonium hydroxide. At longer reaction times (89.5 h) more TMP was formed and similar yields were obtained with different salts (acetate vs formate) of comparable acidity. The absence of TMP formation with ammonium oxalate was apparently due to low solubility of the salt in the reaction medium.

Reactions of acetoin and ammonium acetate at fixed time and increasing temperature produced higher yields

Table I. Tetramethylpyrazine Formation from 3-Hydroxy-2-butanone and Various Ammonium Salts at 22 °C

ammonium salt (aq pH)[b]	reaction conditions[a] time, h	[acetoin]	% water	TMP yield, %
phosphate (4.48)	17.5	2.3	3.5	<0.001
chloride (5.85)		3.8	6.0	<0.001
acetate (6.88)		3.3	4.7	13.3
acetate	17.5/air[c]	3.0	7.9[d]	13.0
acetate	20.3/N_2	3.0	7.9[d]	12.7
acetate	17.5	0.40	87.4	0.2
acetate	17.5/air	0.40	87.4	0.3
bicarbonate (7.78)	17.5	3.0	4.6	0.001
carbonate (11.24)		2.6	4.0	0.005
citrate (5.14)	89.5	1.3	2.0	6.4
formate (6.45)		3.5	5.4	29.9
oxalate (6.50)[e]		1.9	13.1	<0.001
acetate		3.3	4.7	29.4
hydroxide[f]	17.5	3.6	50.4	<0.001

[a] Three moles of salt/mole of acyloin, air blanket, [acetoin] = initial molal concentration of 3-hydroxy-2-butanone. [b] pH of 1% w/v water solutions. [c] Slow stream of gases bubbled through mixtures. [d] Water added to facilitate mixing. [e] Water solubility of monohydrate is 4% at 16.8 °C. [f] Initial molality of ammonia was 10.76.

Table II. Products of Acyloin Reactions with Ammonium Acetate

entry	acyloin(s)	reaction conditions temp, °C	time, h	pyrazine[a] (yield, %)
1	(a) 3-hydroxy-2-butanone	120	15.5	TMP (58.0)
2		95	15.0	TMP (54.0)
3		70	15.5	TMP (47.0)
4		60	15.3	TMP (47.0)
5		22	17.5	TMP (13.0)
6	(b) 4-hydroxy-3-hexanone	22	160	TEP (10.0)
7	(c) 2-hydroxycyclohexanone	22	160	*OHP (42.2)*
8	(d) 1-hydroxy-2-propanone	22	247	DMP[b] (2.3) TriMP (0.001) *TMP (0.001)*
9	(e) 3-hydroxy-2-pentanone	22	70	*DEDMP (20)*[c]
10	(a) + (d)	22	247	DMP (0) TriMP (20.5) *TMP (29.9)*
11	(a) + (b)	22	138	TMP (41.7) DEDMP[d] (29.0) *TEP (1.6)*
12	(a) + (c)	22	247	TMP (14.8) DMQ (22.0) OHP (25.4)

[a] Key: TMP = tetramethylpyrazine, TEP = tetraethylpyrazine, OHP = octahydrophenazine, DMP = dimethylpyrazines, TriMP = trimethylpyrazine, DEDMP = diethyldimethylpyrazines, DMQ = 2,3-dimethyl-5,6,7,8-tetrahydroquinoxaline (identified by GC–MS library spectrum). [b] Equimolar mixture of 2,5-dimethyl- and 2,6-dimethylpyrazine (isomers resolved by GC–MS only). [c] Equimolar mixture of 2,5-diethyl-3,6-dimethyl- and 2,6-diethyl-3,5-dimethylpyrazines. [d] 2,3-Diethyl-5,6-dimethylpyrazine. In lieu of standard samples, DMQ. 2,3-Diethyl-5,6-pyrazine and 2,6-diethyl-3,5-dimethylpyrazine were quantitated by assuming their UV absorption at 280 nm was equal to that of 2,5-diethyl-3,6-dimethylpyrazine. MS and UV data for tetraalkylpyrazines are given in Table III.

of TMP up to an apparent maximum near 60% (Table II). Consistent maximum yields of less than 100% can be explained by disproportionation, i.e., if pyrazines are being formed from dihydropyrazines via an internal redox process [n(dihydropyrazine) → pyrazine + reduction product(s), $n > 1$] instead of oxidation by molecular oxygen where $n = 1$. Molecular oxygen was not required for pyrazine formation in our system at room temperature, and yields of TMP were not increased by bubbling air through reaction mixtures (Table I). Attempts to identify disproportionation products, e.g., piperazines, were not successful. Dilution of reactants with water (0.4 *m* acetoin) led expectedly to greatly reduced TMP yields, but similar to other reactions, the low yields could not be increased by entrainment of air through the reaction mixtures.

Table III. EI-MS and UV Data for Tetraalkylpyrazines

pyrazine	UV,[a] nm	m/z (%)
2,3-diethyl-5,6-dimethylpyrazine[b]	295	164 (100), 163 (72), 149 (48), 57 (48), 56 (26), 53 (24)
2,5-diethyl-3,6-dimethylpyrazine[b]	296	149 (100), 164 (86), 163 (62), 67 (27), 53 (21), 41 (18)
2,6-diethyl-3,5-dimethylpyrazine[b]	294	163 (100), 164 (89), 149 (30), 67 (27), 53 (26), 41 (18)
tetraethylpyrazine	289	192 (100), 177 (84), 191 (34), 41 (22), 67 (20), 193 (14)
tetramethylpyrazine	299	54 (100), 136 (79), 42 (56), 53 (20), 39 (17), 52 (8)

[a] Values obtained in pH 2.5 buffer are consistent with (300 nm) that reported for protonated tetramethylpyrazine (Demain, 1967). [b] MS data compared well with information recently published (Baltes and Bochmann, 1987).

Effects of acyloin structure on pyrazine formation are shown in Table II. The reaction at 22 °C proved to be general for alicyclic (entries 1–5, 6, and 9) and cyclic (entry 7) acyloins and for the ketol, acetol (entry 8). In each case the pyrazine structures suggested dimerization of the substrate acyloin with incorporation of nitrogen.

Acyloin mixtures were used to probe the reaction mechanism. Coreactions of structurally different acyloins with ammonium acetate (Table II, entries 10–12) produced pyrazine mixtures similar to those obtained in earlier work on Strecker degradations with pairs of α-diketones (Rizzi, 1972). The key difference in the Strecker route to pyrazines lies in the higher activation energy associated with reductive amination involving amino acid decarboxylation. Practically speaking, Strecker degradations require temperatures above 100 °C compared to room-temperature reactions of acyloins and ammonia. Mechanistically, acyloins appear to react with ammonia at weak-acid pH to form α-amino ketones, which react further to form pyrazines.

The intermediacy of α-amino ketones in the acyloin/ammonia reaction was established by reacting acetoin with a secondary amine, dimethylamine, to obtain a stable (tertiary) amino ketone, 3-(dimethylamino)-2-butanone (Gaset et al., 1975) (see the Experimental Section). Because of their structure, tertiary amino ketones cannot react further to form pyrazines.

Diketones were excluded as reaction intermediates since 2,3-butanedione and ammonium acetate failed to produce detectable amounts (<0.001% yield) of TMP under conditions that gave ca. 13% TMP from acetoin (Table I).

Reactions of acyloins containing unlike substituents (Table II, entries 8 and 9) produced pairs of isomeric pyrazines in equal molar yields. These results suggested that a single acyloin isomer could function as a common precursor for two isomeric α-amino ketones and ultimately for two pyrazines (Figure 1). Formation of isomeric amino ketones is supported by results with the Strecker degradation of 2,3-pentanedione (Rizzi, 1972) that led to the same mixture of diethyldimethylpyrazines as described in Table II (entry 9).

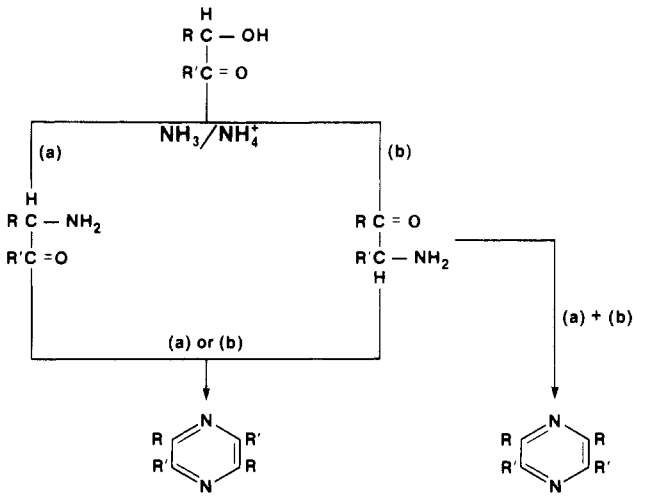

Figure 1. Formation of pyrazines from an acyloin.

The results with acyloins containing unlike substituents can be rationalized three ways: (1) isomerization of the acyloin prior to amination, (2) nonregiospecific acyloin amination, or (3) a combination of (1) and (2). In view of the known acid-catalyzed interconversion of hydroxycarbonyls, in the case of reducing sugars (Mawhinney et al., 1980), we decided to test path (1) in a model system. When 3-hydroxy-2-pentanone was heated at 90 °C in dilute acetic acid buffered at pH 5.6, partial conversion (33.5%) to the isomer 2-hydroxy-3-pentanone took place. Thus path (1) is possibly responsible for isomeric pyrazine formation. Further experiments are needed to test the regioselectivity of the acyloin amination reaction.

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Registry No. 3-Hydroxy-2-butanone, 513-86-0; tetramethylpyrazine, 1124-11-4; tetraethylpyrazine, 38325-19-8; octahydrophenazine, 26468-29-1; 2,5-dimethylpyrazine, 123-32-0; 3-hydroxy-4-hexanone, 4984-85-4; 2-hydroxycyclohexanone, 533-60-8; 1-hydroxy-2-propanone, 116-09-6; 3-hydroxy-4-pentanone, 3142-66-3; trimethylpyrazine, 14667-55-1; 2,5-diethyl-3,6-dimethylpyrazine, 18903-30-5; 2,3-dimethyl-5,6,7,8-tetrahydroquinoxaline, 35149-10-1; 2,6-dimethylpyrazine, 108-50-9; 2,6-diethyl-3,5-dimethylpyrazine, 18940-74-4; 2,3-diethyl-5,6-dimethylpyrazine, 106060-96-2; 4-hydroxy-3-hexanone, 4984-85-4; 3-(dimethylamino)-2-butanone, 10524-60-4; 2-hydroxy-3-pentanone, 5704-20-1; ammonia, 7664-41-7; ammonium acetate, 631-61-8; ammonium bicarbonate, 1066-33-7; ammonium carbonate, 506-87-6; ammonium citrate, 7632-50-0; ammonium formate, 540-69-2.

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