bicyclic pyrazines it will assist other research workers in identifying them in other natural products.

ACKNOWLEDGMENT

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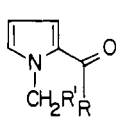
Formation of *N*-Alkyl-2-acylpyrroles and Aliphatic Aldimines in Model Nonenzymic Browning Reactions

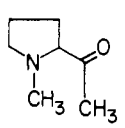
George P. Rizzi

Reactions of α-amino acids with furfural and 2-acetylfuran yielded *N*-alkyl-2-acylpyrroles. The furfural reactions also produced *N*-alkylidenefurfurylamines whose structures were verified by independent syntheses from furfurylamine and aliphatic aldehydes. The pyrroles and aldimines had interesting strong odors, some of which were readily recognized as foodlike.

The *N*-alkyl-2-acylpyrroles (I) are a class of organoleptically interesting substances which have been observed in a wide variety of heat-treated foodstuffs including: tea (Yamamoto *et al.*, 1940a,b), cocoa (Dietrich *et al.*, 1964), and coffee (Stoll *et al.*, 1967). In addition, closely related compounds 2-acetyl-1-methylpyrrolidine (II) and 2-acetyl-1,4,5,6-tetrahydropyridine (III) have been reported among bread volatiles (Hunter *et al.*, 1969).



I



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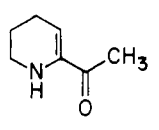

III

In recent years Japanese investigators (Shigematsu *et al.*, 1972) have been studying the mechanism of acylpyrrole formation in foods *via* detailed examinations of model systems. It was suggested (Kato, 1967) that acylalkylpyrroles are formed by interaction of 3-deoxyosuloses (from sugars) and amines or α-amino acids.

In this connection we wish to report our finding that similar acylalkylpyrroles can be formed by reaction of furfural and its homologs with α-amino acids. Our investigation of furfurals stemmed from a consideration of the known wide occurrence of furans in foodstuffs and the possibility of nucleophilic attack by amines at the electrophilic 5 position on the 2-acylfuran nucleus (Figure 1). In the course of our study we also observed aliphatic aldimine products resulting from attack of α-amino acids at the aldehyde function of furfural. The *N*-alkylidenefurfurylamines isolated from our model reactions comprised a class of stable, aroma-rich compounds which heretofore had not been reported in "natural" food systems.

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EXPERIMENTAL SECTION

Materials. α-Amino acids were high-grade commercial materials which were used without further purification; furfural and diethylene glycol dimethyl ether (diglyme) were freshly distilled before use; simple aldehydes, amines, 2-acetylfuran, pyrrole-2-carboxaldehyde, *N*-methylpyrrole-2-carboxaldehyde, and furfurylamine were commercial samples; other compounds were synthesized by methods exemplified below.

Methods of Analysis. Volatile reaction products were separated by a steam distillation–ether extraction procedure described previously (Rizzi, 1972). Acylalkylpyrroles and aldimines were isolated by fractional distillation or preparative gas chromatography (gc) on 5 ft × 0.25 in. stainless steel columns containing 15% Carbowax 20M or 15% neopentyl glycol succinate on 30–60 mesh Chromosorb W (acid washed). Column temperatures of 100–150° and an He flow rate of *ca.* 50 ml/min were used. Quantitative analyses of reaction products were obtained by planimeter integration of gc curves. Aliphatic amines were isolated by bubbling a stream of N_2 through each reaction mixture and through dilute aqueous HCl. The amine hydrochlorides were identified by paper chromato-

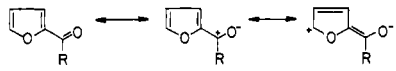


Figure 1. Resonance forms of 2-acylfurans.

graphic comparison with authentic samples (Hrdlicka and Janicek, 1964). Acylalkylpyrroles and aldimines were identified by comparing gc and infrared (ir) spectral data with similar data collected from authentic specimens. The structures of new compounds were established by ir, proton magnetic resonance (pmr), and elemental analyses. Infrared spectra were obtained with a Perkin-Elmer Model 321 instrument. Pmr spectra were taken in CCl_4 solution using a Varian Associates Model HA-100 instrument operating at 100 MHz and employing tetramethylsilane (Me_4Si) as internal reference standard. Chemical shifts are reported in parts per million (ppm expressed in δ units) downfield from Me_4Si.

Reaction of Glycine and Furfural. Glycine (11.25 g, 0.15 mol), furfural (9.6 g, 0.10 mol), and 50 ml of diglyme were combined and refluxed for 2 hr. The reaction mixture which had an ammoniacal odor was steam distilled and extracted with ether to give 3.5 g of crude product. Gc analysis on Carbowax 20M at 150° exhibited a peak at a retention time (T_r) of 12.5 min. The substance was isolated in a cooled capillary tube and shown to be pure *N*-methylpyrrole-2-carboxaldehyde by comparing its T_r and ir spectrum (CS_2 solution) with similar data obtained from an authentic sample (Aldrich Chemical Co.). Other volatiles present in the distillate were: diglyme, T_r 3.3; an unknown material, T_r 4.6; and furfural, T_r 5.6 min.

Reaction of L-Leucine and Furfural. A slurry of L-leucine (13.78 g, 0.15 mol) in 50 ml of diglyme was treated with 9.6 g (0.10 mol) of furfural and refluxed for 2 hr under N_2. The cooled mixture was steam distilled and the aqueous distillate (500 ml) was saturated with sodium chloride and extracted with ether (3 × 100 ml). The combined ether phase was back-extracted with water (5 × 50 ml), washed with saturated brine solution, dried over anhydrous $MgSO_4$, and concentrated to yield 13.7 g of pale yellow liquid. Gc analysis indicated three major products: (A) 12%, not identified but shown not to be *N*-isopentylpyrrole-2-carboxaldehyde; (B) 57%, diglyme; and (C) 29%, *N*-isopentylidenefurfurylamine. Only a trace (0.02%) of furfural was observed. Distillation of the crude product gave 1.73 g (11% yield) of the pure aldimine, bp 90.5–107° (11 mm). A center distillation cut, bp 102–107° (11 mm) (0.463 g, $n^{25}D$ 1.4688), was analyzed.

Anal. Calcd for $C_{10}H_{15}NO$: C, 72.69; H, 9.15; N, 8.48. Found: C, 72.7; H, 9.8; N, 7.8.

The infrared spectrum and gc retention time of the unconjugated aldimine were identical with the authentic substance described below.

***N*-Isopentylidenefurfurylamine.** Furfurylamine (15.57 g, 0.15 mol) was treated dropwise with 16.1 ml (0.15 mol) of isovaleraldehyde while the reaction temperature was maintained near 25° with intermittent ice cooling. After complete addition (*ca.* 10 min) the mixture was stirred 30 min at 25°, treated with NaOH pellets to absorb water, and distilled to afford 19.9 g (80% yield) of *N*-isopentylidenefurfurylamine, bp 92–97° (12 mm), $n^{25}D$ 1.4723. Ir analysis indicated a strong nonconjugated imine absorption band at 6.01 μ; pmr δ 0.96 (d, J = 6 Hz, isopropyl methyls), 1.96 (m, J = 6 Hz, isopropyl methine), 2.12 (t, J = 5 Hz, methylene adjacent to isopropyl group), 4.41 (s, methylene attached to furan ring and imine nitrogen), 6.04 (furan ring, C-3 H), 6.18 (furan ring, C-4 H), 7.24 (furan ring, C-5 H), and 7.62 ppm (t, J = 4.5 Hz, aldimine CH).

Anal. Calcd for $C_{10}H_{15}NO$: C, 72.69; H, 9.15; N, 8.48. Found: C, 72.0; H, 9.4; N, 8.5.

***N*-Furfurylidenisopentylamine.** Isopentylamine (13.07 g, 0.15 mol) and furfural (14.4 g, 0.15 mol) were allowed to react in the same way described above to prepare *N*-isopentylidenefurfurylamine. After distillation 22.1 g (89% yield) of product was obtained which had bp 101–103.5° (12 mm); ir 6.07 μ (conjugated imine); pmr δ 0.88 (d, J = 6 Hz, isopropyl methyls), 1.34–1.88 (complex group of peaks, isopropyl methine and adjacent methylene group), 3.44 (t, J = 7 Hz, methylene adjacent to imine nitrogen), 6.29 (furan ring, C-4 H), 6.65 (furan ring, C-3 H), 7.36 (furan ring, C-5 H), and 7.97 ppm (s, aldimine CH).

Anal. Calcd for $C_{10}H_{15}NO$: N, 8.48. Found: N, 8.5.

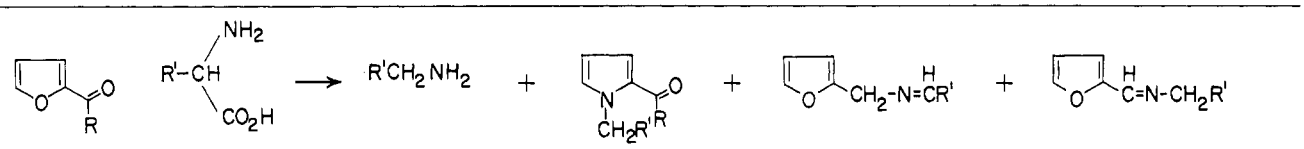
***N*-Ethylpyrrole-2-carboxaldehyde.** A solution of potassium *tert*-butoxide (prepared by dissolving 2.1 g (0.054 g-atom) of K metal in 200 ml of anhydrous *tert*-butyl alcohol) was treated with 4.76 g (0.050 mol) of pyrrole-2-carboxaldehyde and stirred for 15 min at 25°. The solution was treated dropwise with a mixture of ethyl iodide (11.7 g, 0.075 mol) and 25 ml of *tert*-butyl alcohol, stirred for 30 min at 25°, and refluxed for 2 hr. On cooling, water was added and ether extraction was used to separate 5.0 g of crude product which after distillation gave 4.6 g (75% yield) of product: bp 82–86° (12 mm); ir 6.01 μ (aldehyde carbonyl); pmr δ 1.35 (t, J = 7 Hz, *N*-ethyl), 4.28 (q, J = 7 Hz, *N*-ethyl), 6.06 (pyrrole, C-4 H), 6.76 (pyrrole, C-3 H and C-5 H), and 9.47 ppm (s, aldehyde H).

Anal. Calcd for C_7H_9NO: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.8; H, 7.7; N, 11.1.

***N*-Isobutylpyrrole-2-carboxaldehyde.** This compound was prepared from pyrrole-2-carboxaldehyde and isobutyl iodide in the manner described for the *N*-ethyl homolog: ir 5.99 μ (aldehyde carbonyl (CS_2 solution)); pmr δ 0.88 (d, J = 7 Hz, isopropyl methyls), 2.04 (m, J = 6 Hz, methine), 4.04 (d, J = 7 Hz, methylene), 6.07 (pyrrole, C-4 H), 6.76 (pyrrole, C-3 H and C-5 H), and 9.46 ppm (s, aldehyde H).

***N*-Methyl-2-acetylpyrrole.** This compound was pre-

Table I. Products Observed in Reactions of 2-Acylfurans with α-Amino Acids



R	R′				
H	H	*a* (trace)	*b,c* (0.1% yield)	*d*	*d*
H	CH_3	*a* (28% yield)	*d*	*d*	*d*
H	$(CH_3)_2CH$	*a*	*b,c* (trace)	*b,c* (18–23% yield)	*d*
H	$(CH_3)_2CHCH_2$	*d*	*d*	*b,c* (30% yield)	*d*
H	Phenyl	*d*	*d*	*b,c* (~50% rel yield)	*b,c* (~50% rel yield)
CH_3	H	*a* (trace)	*b,c* (trace)	*d*	*d*
CH_3	$(CH_3)_2CH$	*b* (trace)	*b* (trace)	*d*	*d*

[a] Identified by paper chromatography of hydrochloride salt; yield based on weight of isolated salt. [b] Identified by gc retention time. [c] Identified by ir spectrum. [d] Not observed.

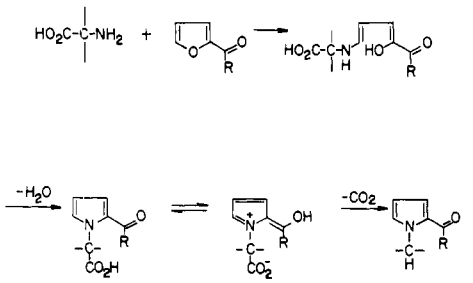


Figure 2. Mechanism for formation of acylalkylpyrroles.

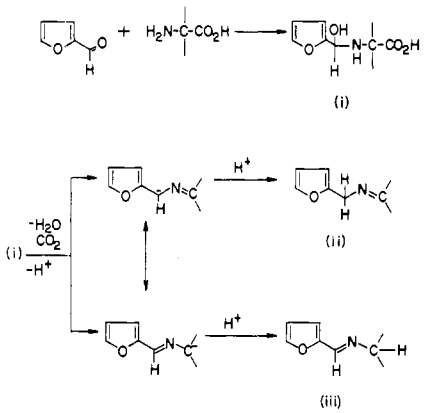


Figure 3. Mechanism for formation of aldimines from furfural and α-amino acids.

pared in a way similar to *N*-ethylpyrrole-2-carboxaldehyde starting from 2-acetylpyrrole and dimethyl sulfate: ir 6.06 μ (carbonyl adjacent to pyrrole ring; spectrum taken in CS_2); pmr δ 2.26 (s, acetyl methyl), 3.82 (s, *N*-methyl), 5.92 (pyrrole, C-4 H), and 6.54–6.82 ppm (broad collection of peaks, pyrrole, C-3 H and C-5 H).

RESULTS AND DISCUSSION

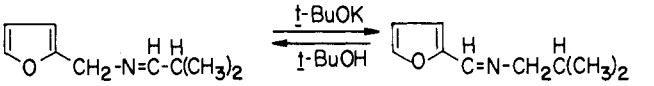
Approximately equimolar amounts of furfural or 2-acetylfuran and α-amino acids were refluxed in dimethylene glycol dimethyl ether (diglyme) for 1–2 hr. Under these conditions browning occurred rapidly and carbon dioxide was evolved. Other reaction products (Table I) included *N*-alkyl-2-acylpyrroles, *N*-alkylidenalkylamines, *N*-arylidenalkylamines, and simple aliphatic amines.

Reactions of furfural and 2-acetylfuran with glycine and L-valine also yielded small amounts of corresponding acylalkylpyrroles. The pyrroles were liquids with pleasant aromas resembling benzaldehyde. A mechanism which explains the formation of acylalkylpyrroles from corresponding furans is shown in Figure 2.

Reactions of acylfurans and amines have been reported to yield acylpyrroles (Dunlop and Peters, 1953), and in the case of α-amino acids the intermediate betaines would be expected to decarboxylate readily (Rizzi, 1970) to yield *N*-alkyl-2-acylpyrroles.

Reactions of furfural with L-valine and L-leucine also produced considerable amounts of aliphatic aldimines. The isolation of imines under our reaction and work-up conditions was surprising in view of the well-known lability of this class toward polymerization and hydrolysis. Evidently certain branched chain aldimines are able to resist decomposition due to steric bulk in the vicinity of the imine double bond. When α-amino acids reacted with furfural the proportions of imine isomers formed varied as to the nature of the α substituent on the amino acid. Reactions of α-monoalkyl amino acids, *e.g.*, L-valine, gave only unconjugated imines (ii, Figure 3) whereas α-monoaryl amino acids, *e.g.*, phenylglycine, gave about equal quantities of both isomers (ii and iii, Figure 3).

Table II. Isomerization of Aldimines with Potassium *tert*-Butoxide in *tert*-Butyl Alcohol



A			B	
Starting material[a]	mol of catalyst/mol of imine	Reflux time, hr	Rel % imines formed[b] A	B
A	0.138	8	9	91
B	0.138	8	4	96
A	0.138	17	0	100
B	0.138	17	0	100
A	0.0138	17	82	18
B	0.0138	17	3	97

[a] A 14% solution of imine in *t*-BuOH was used. [b] Determined by gc analysis, A and B were the only products observed.

Table III. Aroma Properties of Imines

Compound	Aroma sensation
N-Ethylidenefurfurylamine	Amine-like, green[a]
N-Furfurylidenethylamine	Pyridine-like, sharp, irritating[a]
N-Isobutylidenefurfurylamine	Sweet chocolate, floral, rubber[b]
	Chocolate, green, aldehydic[c]
N-Furfurylidenisobutylamine	Chocolate, aldehydic, perfumy[b]
	Chocolate, sweet, rancid[c]
N-Isopentylidenefurfurylamine	Chocolate, aldehydic, fruity[b]
	Chocolate, musty, irritating[c]
N-Furfurylidenisopentylamine	Greenish aldehyde, moldy[b]
	Amine-like, wet wood, rubber[c]
N-Benzylidenefurfurylamine	Benzaldehyde[a]
N-Furfurylidenebenzylamine	Green grain, beany[a]
N-Furfurylidenefurfurylamine	Moldy[a]
N-Benzylidenebenzylamine	Weak benzaldehyde, almond[a]
N-Isobutylidenisobutylamine	Green, herbaceous, musty[b]
	Green apple, green fruit[c]
N-Isopentylidenisopentylamine	Green, aldehyde[b]
	Bitter chocolate, aldehyde[c]

[a] Sniffed neat on a blotter. [b] Sniffed in mineral oil at 1–100 ppm. [c] Sniffed in water at 1–100 ppm.

A mechanism for aldimine formation which is consistent with our data is shown in Figure 3. Attack of the amino acid at the aldehyde carbonyl function followed by loss of water, CO_2, and a proton leads to a resonance stabilized azallylic ion which may reprotonate in two ways to afford isomeric aldimines, ii and iii. The exclusive formation of nonconjugated aldimines with monoalkyl-substituted α-amino acids suggested that the protonation step was proceeding under kinetic control, *i.e.*, that the thermodynamically less stable isomer was being formed. This was shown to be the case by an independent equilibration study of aldimine isomers with potassium *tert*-butoxide (Table II). Thus, it was established that the conjugated aldimine predominates under equilibrating conditions and, hence, it is the thermodynamically more stable of the two isomers.

The possibility that conjugated aldimines were formed but destroyed selectively during work-up was ruled out by

subjecting known mixtures of aldimine isomers to control conditions. In this manner we established that conjugated aldimines would have been detected if 1% had been present after the original reaction. No evidence was found for further reaction between aldimines or pyrrole products and α-amino acids.

The branched chain aldimines obtained from furfural and L-leucine or L-valine were remarkably resistant to isomerization and hydrolysis and they were steam distilled practically quantitatively from aqueous mixtures. In contrast to the results with L-leucine and L-valine, alanine produced ethylamine as the only steam distillable product. Consistent with this observation we found that *N*-furfurylidenethylamine (prepared from furfural and ethylamine) underwent rapid hydrolysis and decomposition during steam distillation attempts. The apparent intermediacy of a conjugated aldimine in the furfural–alanine reaction suggests that the protonation step (Figure 3) may be quite sensitive to the steric size and electronegativity of the α substituent on the amino acid.

Aroma Properties of Aldimines. Aldimines possessed strong odors ranging from biting and unpleasant to mild and foodlike. In view of the unique aromas of aldimines derived from L-valine and L-leucine several analogs were synthesized for organoleptic evaluation (Table III). Subtle differences in structure such as the position of the imine double bond had marked effects on aroma, *e.g.*, *N*-furfurylidenisobutylamine produced an unpleasant aroma under conditions where its isomer smelled more chocolate-like.

We concluded that stable aliphatic aldimines may be playing an important role as isoelectronic analogs of aldehydes in browning reaction flavors. The importance of corresponding cyclic aldimines such as 1-pyrroline (Yoshihawa *et al.*, 1965) and 2-acetyltetrahydropyridines (Buechi and Wuest, 1971) in bread flavor and of trimethyl-3-oxazoline in meat aroma (Chang *et al.*, 1968) has already been well established.

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Quantitative and Qualitative Analysis of Tangerine Peel Oil

Manuel G. Moshonas* and Philip E. Shaw

Seventeen major volatile flavor constituents of cold-pressed tangerine oil were quantitatively and qualitatively analyzed by a gas–liquid chromatograph (glc) with a computing integrator. Quantitative corrections were made for glc detector response factors and high boiling material which would not be eluted from the glc column during the analysis period. The qualitative analysis was extended to include carbonyl constituents, the only group of volatile flavor compounds that had not been systematically investigated in tangerine oil. γ-Elemene is reported as a new citrus component and α- and β-sinensal and thymol methyl ether are reported as new tangerine oil components.

Since cold-pressed citrus oil makes a major contribution to citrus product flavor (Stanley, 1962), knowledge of oil composition is essential to understanding flavor. Individual constituents and their respective quantities are important to desirable flavor and aroma of citrus oils (Nursten and Williams, 1967).

Earlier studies on citrus oils have reported many qualitative analyses (Nursten and Williams, 1967) but relatively few quantitative analyses (Ziegler, 1971). Our laboratory has identified volatile hydrocarbons and alcohols from tangerine, orange, and grapefruit oils (Hunter and Brogden, 1965; Hunter and Moshonas, 1965, 1966). Similarities in these fractions suggested that the carbonyl fraction has the greatest impact on flavor and aroma characterizing each citrus fruit (Moshonas, 1971). Orange and grapefruit carbonyl fractions have been systematically analyzed (Moshonas, 1971; Moshonas and Lund, 1969). Some mandarin (tangerine) oil carbonyls have been reported (Di Giacomo and Calvarano, 1970), but this fraction has not been analyzed systematically. The relatively little quantitative analytical information available on citrus oil components usually involves either groups of compounds (Stanley, 1962) or glc area percent for individual components where glc response factors or percent noneluting (high-boiling) material were not determined (Bernhard, 1960; Kugler and Kovats, 1963; Kita *et al.*, 1969; Ziegler, 1971). Stanley, in 1962, determined quantities of a few individual citrus oil components, and Shaw *et al.*, in 1971, reported weight percent of the 12 main components in Persian lime oil.

The present study reports both qualitative and quantitative analysis of tangerine oil. Qualitative composition of the important flavor fraction, the carbonyls, was determined. Quantitative glc analysis of the 17 major tangerine oil components afforded their weight percentages after glc response factors and percent of high-boiling components

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