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Model reactions of roast aroma formation: X. Amino acid-specific products after roasting of tryptophan with reducing sugars and sugar degradation products

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In a series of model reactions, mixtures of L-tryptophan with D-glucose, D-xylose and furfuraldehydes were roasted at 220°C. Under the same conditions, L-tryptophan, D-fructose-L-tryptophan (Amadori product) and the azomethines *N*-(furfurylidene) tryptamine and *N*-(5-methylfurfurylidene)tryptamine were treated. The volatile products were investigated by HRGC/MS and compared with synthetic standards measured by ^{1}H NMR spectroscopy. Besides the well-known 'standard roast aroma products', which include sugar degradation products and *N*-containing heterocyclic compounds, a total of 50 tryptophan-specific Maillard products with an indole residue were characterized. Among them, β-carbolines and indolyl-pyrrolyl-ethanes were the most important. Possible mechanisms of formation are discussed.

INTRODUCTION

Interactions between reducing sugars and amino acids, peptides or proteins produce a wide spectrum of compounds that influence foodstuffs during heating. These reactions are well known as 'Maillard' or non-enzymatic browning reactions. Changes in odour, flavour and colour result.

To investigate the formation of volatile Maillard compounds we carried out some model reactions of single, selected amino acids with sugars or sugar degradation products under conditions of roasting (e.g. coffee), cooking (e.g. soup) and heating on an autoclave (Baltes & Bochmann, 1987 *a–e*; Baltes & Mevissen, 1988; Kunert-Kirchhoff & Baltes, 1990 *a,b*; Wilken & Baltes, 1990). In this paper we report results obtained after reaction of tryptophan with sugars and some sugar degradation products.

MATERIALS AND METHODS

Roasting

Equimolar amounts (0·01 mol each) of L-tryptophan and D-glucose or D-xylose were mixed and spread on sand (to 8·0 g). After addition of 0·8 g sodium phosphate buffer, pH 5·8 (0·4 mol/litre), the mixture was poured into a reaction tube covered with a heater band and connected with the cooling traps of the reaction apparatus (Baltes & Bochmann, 1987*a*). In the same manner furfuraldehyde or 5-methyl-furfuraldehyde were mixed and roasted with L-tryptophan. Other experiments were carried out with D-fructose-L-tryptophan (0·01 mol), *N*-(furfurylidene)tryptamine or *N*-(5-methylfurfurylidene) tryptamine (0·003 mol of each), which were roasted alone. The roasting was carried out by heating the tube at a rate of 20°C/min to a final temperature of 220°C and holding for 10 min. The volatile products were carried over to the cooling traps in a nitrogen stream of 50 ml/min. The water-containing condensates of the cooling traps were combined, ether was added and the acids eliminated with $NaHCO_3$ solution. The ether fraction was dried over Na_2SO_4 and concentrated carefully on a Vigreux column.

D-Fructose-L-tryptophan dihydrate

This was synthesized by the method of Heyns and Noack (1964).

Preparation of *N*-(furfurylidene) tryptamine and *N*-(5-methylfurfurylidene)tryptamine

The synthesis of the azomethines was carried out

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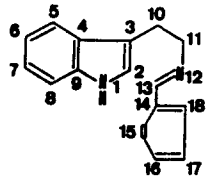
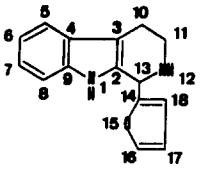
N-(Furfurylidene)tryptamine



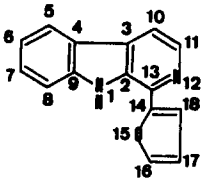
δ = 3·20 (t, J = 7 Hz; CH$_2$ — 10); 3·92 (td, J = 7 + 1 Hz; CH$_2$ — 11); 6·46 (dd, J = 3 + 2 Hz; CH — 17); 6·68 (d, J = 3 Hz; CH — 18); 7·02 (d, br., J = 2 Hz; CH — 2); 7·12 (td, J = 7·5 + 1 Hz; CH — 7); 7·20 (td, J = 7·5 + 1 Hz; CH — 6); 7·36 (d, J = 7·5; CH — 8); 7·51 (d, J = 2 Hz; CH — 16); 7·66 (d, J = 8 Hz; CH — 5); 7·96 (s, br., CH — 13); 8·04 (s, br., NH — 1)

1-(Furyl-2)-1,2,3,4-tetrahydro-β-carboline



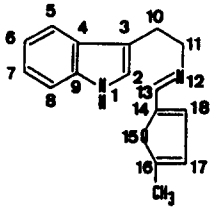
δ = 1·81 (s, br., NH — 12); 2·83 (m, CH$_2$ — 10); 3·17 (ddd, J = 13·6 + 5·5 Hz; CH — 11); 3·34 (dt, J = 13 + 5·5 Hz; CH — 11); 5·31 (s, br., CH — 13); 6·21 (d, br., J = 3 Hz; CH — 18); 6·35 (dd, J = 3 + 2 Hz; CH — 17); 7·12 (td, J = 7·5 + 1 Hz; CH — 7); 7·17 (td, J = 7·5 + 1 Hz; CH — 6); 7·30 (d, J = 7·5 Hz; CH — 8); 7·43 (dd, J = 2 + 1 Hz; CH — 16); 7·53 (d, br., J = 7·5 Hz; CH — 5) 7·85 (s, br., NH — 1)

1-(Furyl-2)-β-carboline



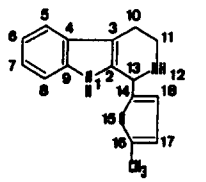
δ = 6·63 (dd, J = 4 + 2 Hz; CH — 17); 7·27 (m, CH — 7); 7·30 (d, br., J = 3 Hz; CH — 18); 7·56 (m, CH — 6, CH — 8); 7·67 (dd, J = 2 + 1 Hz; CH — 16); 7·85 (d, J = 5 Hz; CH — 10); 8·11 (d, J = 8 Hz; CH — 5); 8·45 (d, J = 5 Hz; CH — 11); 9·49 (s, br., NH — 1)

N-(5-Methylfurfurylidene)tryptamine



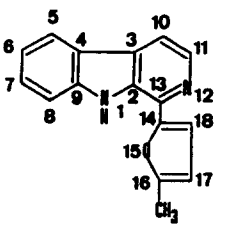
δ = 2·38 (s, CH$_3$); 3·17 (t, J = 7 Hz; CH$_2$ — 10); 3·88 (t, J = 7 Hz; CH$_2$ — 11); 6·07 (dq, J = 3 + 1 Hz; CH — 17); 6·54 (d, J = 3 Hz; CH — 18; 7·02 (d, br., J = 2·5 Hz; CH — 2)); 7·12 (td, J = 7·5 + 1 Hz; CH — 7); 7·20 (td, J = 7·5 + 1 Hz; CH — 6); 7·36 (d, br., J = 7·5 Hz; CH — 8); 7·66 (d, br., J = 7·5 Hz; CH — 5); 7·86 (t, J = 1 Hz; CH — 13); 7·98 (s, br., NH — 1)

1-(5-Methylfuryl-2)-1,2,3,4-tetrahydro-β-carboline



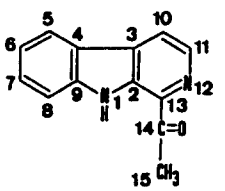
δ = 2·30 (s, CH$_3$); 2·83 (m, CH$_2$ — 10); 3·15 (ddd, J = 13; 6 + 5·5 Hz; CH — 11); 3·34 (dt, J = 13 + 5·5 Hz; CH — 11); 5·25 (s, br., CH — 13); 5·91 (d, br., J = 3 Hz; CH — 17); 6·06 (d, J = 3 Hz; CH — 18); 7·11 (td, J = 8 + 1 Hz; CH — 7); 7·17 (td, J = 8 + 1 Hz; CH — 6); 7·30 (d, J = 8 Hz; CH — 8); 7·53 (d, J = 8 Hz; CH — 5); 7·81 (s, br., NH — 1)

1-(5-Methylfuryl-2)-β-carboline



δ = 2·56 (s, CH$_3$); 6·26 (dq, J = 3 + 1 Hz; CH — 17); 7·19 (d, 3 Hz; CH — 18); 7·30 (ddd, J = 8·7 + 1·5 Hz; CH — 6); 7·58 (ddd, J = 8·7 + 1 Hz; CH — 7); 7·62 (dd, J = 8 + 1·5 Hz; CH — 8); 7·84 (d, br., J = 8 Hz; CH — 10); 8·14 (dd, J = 8 + 1 Hz; CH — 5); 8·44 (d, J = 5 Hz; CH — 11); 9·25 (s, br., NH — 1)

1-Acetyl-β-carboline

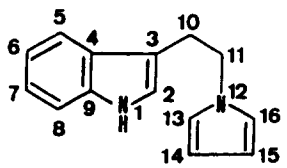


δ = 2·91 (s, CH$_3$); 7·34 (m, CH — 7); 7·60 (m, CH — 6, CH — 8); 8·16 (d, J = 5 Hz; CH — 10); 8·16 (d, J = 8 Hz; CH — 5); 8·55 (d, J = 5 Hz; CH — 11)

ray—treatment:

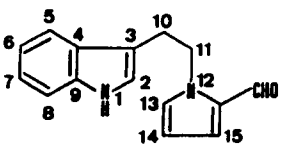
at δ = 8·16	s, 8·55;	change at 7·60 and 7·34
δ = 7·34		change at 7·60 and 8·16
δ = 7·60		change at 7·34 and 8·16

(Indolyl-3)-(pyrrolyl-1)ethane



δ = 3·23 (t, J = 7·5 Hz; CH$_2$ — 10); 4·19 (t, J = 7·5 Hz; CH — 11); 6·14 (m, centr., CH — 14; CH — 15); 6·65 (m, centr., CH — 13, CH — 16); 6·81 (d, J = 2·5 Hz; CH — 2); 7·15 (td, J = 7·5 + 1 Hz; CH — 7); 7·23 (td, J = 7·5 + 1 Hz; CH — 6); 7·38 (d, J = 7·5 Hz; CH — 8); 7·58 (d, J = 7·5 Hz; CH — 5); 7·98 (s, br., NH — 1)

(Indolyl-3)-(2-formylpyrrolyl-1)ethane



δ = 3·20 (t, J = 7 Hz; CH$_2$ — 10); 4·57 (t, J = 7 Hz; CH$_2$ — 11); 6·11 (dd, J = 4 + 2·5 Hz; CH — 13); 6·69 (s, br., CH — 15); 6·84 (d, br., J = 2·5 Hz; CH — 2); 6·96 (dd, J = 4 + 2 Hz; CH — 14); 7·14 (td, J = 7·5 + 1 Hz; CH — 7); 7·20 (td, J = 7·5 + 1 Hz; CH — 6); 7·36 (d, br., J = 7·5 Hz; CH — 8); 7·67 (d, br., J = 7·5 Hz; CH — 5); 8·41 (s, br., NH — 1); 9·60 (d, J = 0·5 Hz; CHO)

Fig. 1. ^{1}H NMR data of synthetic standards

according to Severin and Heidenhain (1966): 2·0 g tryptamine and 6·0 ml furfuraldehyde (6·0 ml 5-methylfurfuraldehyde) were refluxed in benzene (300 ml) for 2 h (4 h). Yield: about 60% of theoretical amount.

Preparation of 1-(furyl-2-)-1,2,3,4-tetrahydro-β-carboline, 1-(furyl-2)-β-carboline, 1-(5-methylfuryl-2-),1,2,3,4-tetrahydro-β-carboline, 1-(5-methylfuryl-2)-β-carboline, 1-ethyl-, 1-propyl- and 1-acetyl-β-carboline

The syntheses of the tetrahydro-β-carbolines were carried out according to Severin and Heidenhain (1966) by treating the corresponding azomethine (1·0 g each, see above) with conc. hydrochloric acid. A typical preparation yielded approximately 50% of the theoretical amount of each tetrahydro-β-carboline. 1-(Furyl-2)-β-carboline and the methyl homologues were obtained by dehydrogenation of the tetrahydro-β-carbolines with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). Tetrahydro-β-carboline (2·1 mmol) was dissolved in benzene (50 ml) and, under stirring, 4·4 mmol DDQ in benzene were added. After refluxing (5 h), the mixture was stirred overnight and subsequently treated with the same volume of an $NaHCO_3$ solution and ether. The benzene/ether phase was evaporated to dryness, and the residue dissolved in ether. The syntheses of 1-ethyl- and 1-acetyl-β-carboline were carried out according to Akabori and Saito (1930) by reaction of tryptamine with propanoic aldehyde or methylglyoxal. 1-Propyl-β-carboline was synthesized by the method of Späth and Lederer (1930) by reaction of tryptamine with butanoic acid. For dehydration, see above.

Preparation of (indolyl-3)-(furyl-2)methane

The synthesis of (indolyl-3)-(furyl-2)methane was carried out according to Brown and Sawatzky (1956) by reaction of 0·01 mol furan and 0·01 mol 3-indolylmethanol in 5 ml conc. hydrochloric acid.

Preparation of (indolyl-3)-(pyrrolyl-1)ethane

The synthesis of (indolyl-3)-(pyrrolyl-1)ethane was carried out according to Murahashi *et al.* (1974) by reaction of 0·06 mol *cis*-2-buten-1,4-diole, 0·03 mol tryptamine and 0·15 g palladium black.

Preparation of (indolyl-3)-(2-formylpyrrolyl-1)ethane

The synthesis of (indolyl-3)-(2-formylpyrrolyl-1)ethane was carried out by reaction of 2 mmol (indolyl-3)-(pyrrolyl-1) ethane, 20 mmol, *N,N*-dimethylformamide and 2 mmol phosphoroxichloride (Vilsmeyer formylation).

Preparation of indoles

The synthesis of 3-allylindole was carried out according to Brown *et al.* (1952). 1,3-Dimethylindole, 1,2,3-trimethylindole, 2,3-diethylindole, 3-methyl-2-propylindole, 2-methyl-3-propylindole and 3-ethyl-2-methylindole were synthesized according to Fitzpatrick and Hiser (1957) after production of the corresponding phenylhydrazone from phenylhydrazine (*N*-methyl-*N*-phenylhydrazine) and carbonyl compound. 3-Ethylindole, 3-propylindole and 3-butylindole were synthesized by reduction of the corresponding *p*-toluene sulfonic ester with lithium aluminium hydride.

HRGC/MS

Column 1
60 m J & W fused silica column DB Wax 60N, 0·25 mm i.d.; temperature: 40°C for 6 min, then increased to 210°C at 2°C/ min, 210°C isotherm.

Column 2
30 m J & W fused silica column DB-5, 0·25 mm i.d.; temperature programme: 150°C for 6 min, then increased to 300°C at 2°C/min, 300°C isotherm.

Carrier gas
Helium; 2 ml/min; split 1:5.

HRGC/MS system:
Finnigan MAT 4500 with INCOS data system.

Electron impact (EI)
Transfer line temperature: 240°C (direct coupling); ion source 120°C, approx. 1×10^{-6} torr, 70 eV; cycl. scan: 0·8 s; mass range: 35–350 amu.

Chemical ionization (CI) reactant gas
CH_4, 0·7 torr; mass range 80–350 amu. Exact mass determination (Wittkowski *et al.*, 1983, 1984): mass range: 45–350 amu; conditions as EI, with FC 43 used as calibration gas. Retention index values, calculated according to van den Dool and Kratz (1963). A standard of *n*-alkanes C_8–C_{26}; C_{28} and C_{30} was added to the volatiles.

^{1}H NMR spectroscopy

Bruker WM 400 NMR spectrometer, solvent: $CDCl_3$, tetramethylsilane (Me_4Si) as internal standard. Figure 1 shows the ^{1}H NMR data of synthetic standards.

Infrared spectroscopy

Pye Unicam SP 1100, KBr, 4000–400 cm^{-1}.

D-Fructose-L-tryptophan dihydrate

The crystallized compound was identified by elemental analysis ($C_{17}H_{22}N_2O_7 \times 2\ H_2O$; theoretical composi-

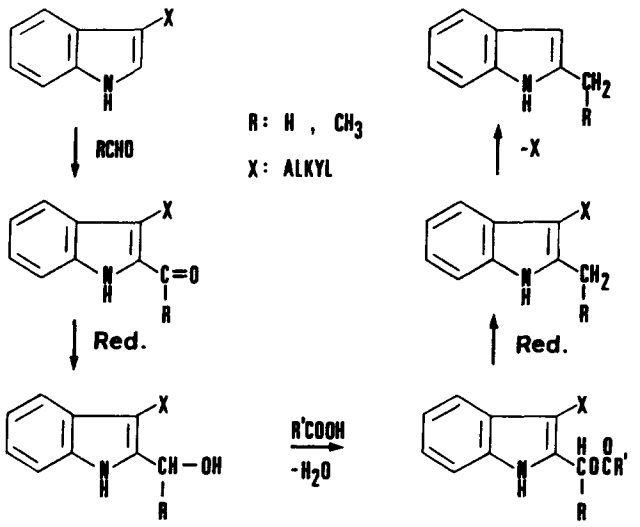


Fig. 2. Assumed reaction pathway to explain the formation of 2- and 2,3-alkylated indoles by Maillard reaction of L-tryptophan with monosaccharides.

tion (%): C = 50·74, H = 6·51, N = 6·96; found (%): C = 50·75, H = 6·40, N = 6·96) and infrared spectroscopy ($\tilde{\nu}$: 1,2-disubstituted benzene (750), C–O–C (1100), NH deformation (1570), C=O (1630) cm^{-1}).

RESULTS AND DISCUSSION

Equimolar amounts of L-tryptophan and monosaccharides were roasted at 220°C for 10 min. The Amadori rearrangement product (fructose-L-tryptophan), mixtures of furfuraldehydes with tryptophan or the corresponding azomethines (*N*-furfurylidene-tryptamines) were treated in the same manner. After extraction of the volatiles with ether, acids were eliminated with $NaHCO_3$ solution and the complex mixture of components analysed by HRGC/MS. Structure identifications were based on their mass-spectrometric data, obtained by electron impact, chemical ionization, H/D isotope exchange and precise mass determination in a quadruple mass spectrometer (Wittkowski *et al.*, 1983, 1984). A total of 311 volatiles were identified. Besides pyrazines, pyrroles, furans and other well-known roast aroma compounds, we identified 50 amino acid-specific products possessing an indole system. In order to prove these structures, we synthesized 24 of them and compared their MS and ^1H NMR data.

Indoles

On roasting tryptophan alone or in a mixture with sugars, very unpleasant odours appear. Indeed, we identified several indoles among the volatile compounds. These structures are typical for a pyrolytic degradation of the amino acid combined with reactions occurring with reactive sugar degradation products. As well as monoalkyl indoles substituted in the 3-position, we identified some compounds containing substituents in the 2- or 2,3-positions. To explain a substitution in the 2-position of an indole we postulate an attack of aliphatic aldehydes, which is favoured at this point. The acyl derivatives formed react: on the one hand by ring enlargement to yield β-carbolines; on the other hand they can be transformed by reduction to the corresponding 2-alkanol derivatives, which can form esters after reaction with carbonic acids. By reductive ester scission, 2-alkylindoles are formed. Figure 2 shows the assumed reaction pathway.

The formation of 1-alkylated, 1,2- and 1,3-dialkylated as well as 1,2,3-trialkylated indoles shows that the secondary amino group in the indole moiety is also included in the non-enzymatic browning reaction.

Table 1 shows the mass spectrometric data of the identified indole structures with an aliphatic residue. Besides alkylated indoles, we identified some compounds containing heterocyclic substituents in the 2- or 3-position. The structures of (indolyl-2)-(furyl-2)methane, (indolyl-3)-(furyl-2)methane, (indolyl–2)-(furyl-2)ethylene, (indolyl-3)-(furyl-2)ethylene and (indolyl-3)-(pyrrolyl-1) ethane were identified as roast aroma ingredients for the first time. Indolyl-pyrrolylethane was obviously formed by the nucleophilic attack of tryptamine at the electrophilic C_5 atom of the furan ring, following dehydration (Leditschke, 1952). The formation of (indolyl-3)-(2-formylpyrrolyl-1)ethane via *N*-(furfurylidene)tryptamine is shown in Fig. 3.

The formation of indolyl-furylethanes is assumed to proceed via a reaction of 3-(2'-hydroxyethyl)indole with the favoured 2-position of furans. The formation of the isomeric indolyl-furylmethanes also makes probable an attack of furfuraldehydes at the 2- or 3-position of 1H-indole.

Table 2 shows the mass spectrometric data of the identified indole structures with a heterocyclic residue.

β-carbolines

Among the volatiles, we detected 11 compounds with pyrido-indole structures (β-carbolines). Alkaloids with the β-carboline structure evoke a variety of actions in biological systems. 1,2,3,4-Tetrahydro-β-carboline, in particular possesses potent pharmacological activity which inhibits 5-hydroxytryptamine uptake. In higher concentrations β-carbolines retard the absorption of dopamine and noradrenaline and are inhibitors of monoamine oxidase A (Kari *et al.*, 1980). Norharman (β-carboline) and harman (1-methyl-β-carboline) are well known ingredients of tobacco smoke (Poindexter *et al.*, 1962). Neither compound is mutagenic, but both are active co-mutagens to a number of compounds (Nagao *et al.*, 1977).

During our experiments, β-carbolines were formed by reaction of tryptamine with carbonyl compounds.

Table 1. Indoles with an aliphatic residue from Maillard reaction of tryptophan with sugars

Compound	*M*	RI[a]	*m/z* (relative intensity)	Ref.[b]
Tryptamine	160	<1755>	130(100) 131(56) 160(17) 77(8) 103(7) 132(5) 12(3) 128(3)	R
3-(2'-Hydroxyethyl)indole	161	<1765>	130(100) 161(21) 131(10) 77(7) 103(6) 102(4) 129(3) 128(3)	R
3-Indolyl-acetonitrile	156	<1813>	155(100) 156(76) 130(49) 128(14) 101(11) 77(9) 129(9) 157(8)	R
1-Methylindole	131	1958	131(100) 130(77) 89(19) 77(16) 63(16) 65(16)	R
1-Ethylindole	145	1973	130(100) 145(52) 89(15) 63(13) 77(12) 39(11)	R
1,3-Dimethylindole	145	2032	144(100) 145(68) 77(14) 72(11) 71(10) 115(8)	S
1,2-Dimethylindole	145	2126	144(100) 145(97) 39(15) 77(14) 115(12) 51(11)	R
1,2,3-Trimethylindole	159	2243	158(100) 159(91) 144(51) 78(19) 79(18) 115(17) 77(14)	S
1H-Indole	117	2440	117(100) 90(48) 89(35) 63(20) 39(17) 59(15)	R
3-Methylindole	131	2489	130(100) 131(59) 77(18) 65(15) 51(14) 103(8)	R
2-Methylindole	131	2490	130(100) 131(78) 65(19) 77(17) 51(13) 39(11)	R
2-Ethylindole	145	2549	130(100) 145(44) 131(12) 65(10) 77(10) 59(9)	P
2,3-Dimethylindole	145	2563	144(100) 145(85) 130(50) 77(15) 51(12) 39(11)	R
3-Ethylindole	145	2573	130(100) 145(39) 77(12) 65(11) 39(9) 131(9)	S
2-Ethyl-3-methylindole	159	2580	144(100) 159(52) 143(18) 158(11) 145(10) 77(9) 130(9)	P
3-Ethyl-2-methylindole	159	2606	144(100) 159(32) 143(11) 72(10) 145(10) 115(8)	S
2,3-Diethylindole	173	2612	158(100) 173(33) 143(26) 159(12) 115(10) 77(9)	S
3-Methyl-2-propylindole	173	2623	144(100) 173(27) 143(12) 145(12) 77(7) 115(7)	S
3-Propylindole	159	2643	130(100) 159(23) 77(13) 131(9) 103(7) 51(6)	S
2-Methyl-3-propylindole	173	2667	144(100) 173(19) 145(10) 143(9) 77(7) 115(5)	S
3-Allylindole	157	2722	130(100) 157(64) 156(40) 77(39) 129(22) 128(21)	S
3-Butylindole	173	2749	130(100) 77(13) 131(13) 173(12) 103(7) 39(4)	S

[a] RI, modified retention index on column 1; <RI>, modified retention index on column 2.
[b] P, proposed structure according to MS data; S, standard synthesized; R, standard available.

Intramolecular cyclization of the resulting azomethines and subsequent dehydrogenation might have produced the thermally very stable β-carboline structures. The missing of 3-indolylacetaldehyde (Strecker aldehyde of tryptophan) in all tryptophan roasting experiments as well as the large amount of 3-(2'-hydroxyethyl)indole seems to indicate a redox reaction yielding this compound, by binding the hydrogen, as well as β-carboline and pyrazine formation. Two of three possible pathways of β-carboline formation are shown in Fig. 4.

Table 2. Indoles with an herocyclic residue

Compound	*M*	RI[a]	*m/z* (relative intensity)	Ref.[b]
(Indolyl-2)-(furyl-2)methane	197	<1850>	197(100) 168(67) 196(62) 167(38) 84(30) 130(22) 77(18) 117(15)	P
(Indolyl-3)-(furyl-2)methane	197	<1903>	197(100) 168(74) 196(57) 167(33) 84(32) 130(21) 117(20) 115(15)	S
(Indolyl-2)-(5-methyl furyl-2) methane	211	<1934>	168(100) 211(82) 210(45) 43(37) 167(35) 130(31) 77(20) 129(18)	P
(Indolyl-3)-(5-methyl-furyl-2) methane	211	<1990>	168(100) 211(75) 210(36) 167(29) 130(24) 43(21) 77(15) 169(11)	P
(Indolyl-3)-(furyl-2)ethane	211	<1992>	130(100) 211(14) 77(12) 131(9) 103(7) 81(5) 51(4)	P
(Indolyl-3)-(5-methyl furyl-2) ethane	225	<2077>	130(100) 131(10) 77(7) 95(7) 225(6) 103(4) 43(4)	P
(Indolyl-3)-(pyrrolyl-1) ethane	210	<2097>	130(100) 210(16) 131(10) 77(7) 80(7) 143(6) 103(6) 53(5) 105(4)	S
(Indolyl-3)-(2-methyl pyrrolyl-1)ethane	224	<2180>	130(100) 224(16) 143(15) 94(14) 131(11) 77(8) 103(5) 144(4)	P
(Indolyl-3)-(furyl-2)ethylene	209	<2252>	209(100) 180(96) 152(25) 77(23) 181(19) 210(15)	P
(Indolyl-3)-(2,5-dimethyl pyrrolyl-1)ethane	238	<2271>	130(100) 108(33) 238(17) 143(16) 144(11) 131(10) 96(10) 77(8)	P
(Indolyl-3)-(5-methyl furyl-2) ethylene	223	<2360>	180(100) 223(74) 43(28) 152(19) 181(13) 208(11)	P
(Indolyl-3)-(2-formylpyrrolyl-1)ethane	238	<2372>	143(100) 130(96) 77(17) 144(12) 103(12) 238(12) 115(11) 39(11)	S
(Indolyl-3)-(2-formyl 5-methyl pyrrolyl-1)ethane	252	<2479>	143(100) 130(58) 144(12) 252(8) 131(6) 109(3) 122(2)	P

[a] RI, modified retention index on column 1; <RI>, modified retention index on column 2.
[b] P, proposed structure according to MS data; S, standard synthesized; R, standard available.

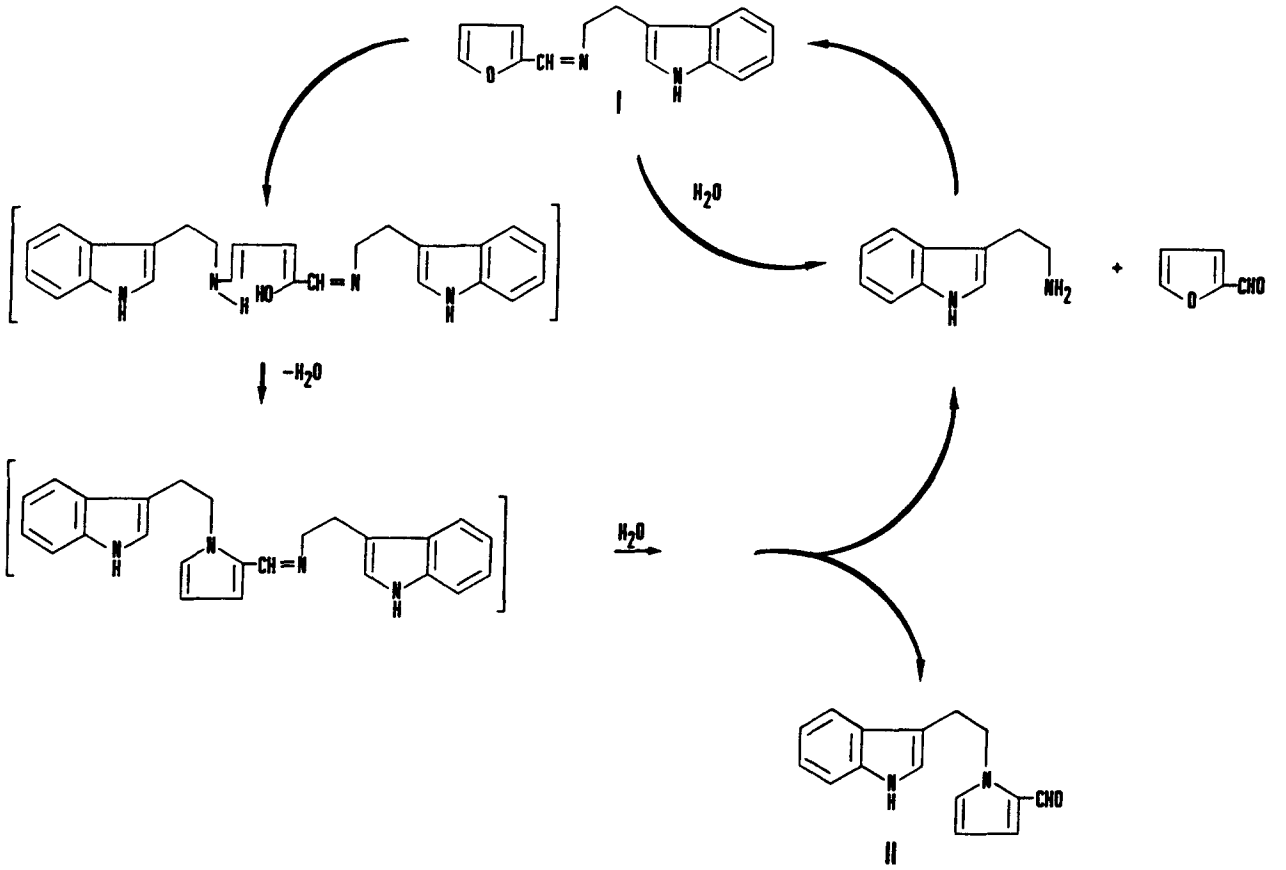


Fig. 3. Possible reaction pathway of (indolyl-3)-(2-formylpyrrolyl-1) ethane (**II**) via *N*-(furfurylidene)tryptamine (**I**).

Table 3. β-carbolines and β-carboline precursors from reaction of tryptophan with sugar and sugar degradation products or after roasting of fructose–tryptophan

Compound	*M*	RI[a]	*m/z* (relative intensity)	Ref.[b]
1-Methyl-β-carboline	182	<1967>	182(100) 181(27) 154(25) 91(18) 77(14) 183(14) 127(11) 63(10)	R
β-Carboline	168	<1975>	168(100) 140(22) 71(15) 84(13) 114(13) 169(13) 141(9) 113(9)	R
1-Acetyl-β-carboline	210	<2005>	168(100) 140(52) 210(51) 182(43) 43(42) 167(35) 114(20) 63(20)	S
1-Ethyl-β-carboline	196	<2032>	195(100) 196(81) 168(36) 98(33) 97(27) 140(15) 115(15) 71(11)	S
1-Propyl-β-carboline	210	<2100>	182(100) 91(39) 195(20) 210(19) 154(15) 183(14) 77(14) 181(12)	S
1-(Furyl-2)-1,2,3,4-tetrahydro-β-carboline	238	<2277>	180(100) 238(54) 209(42) 90(26) 237(26) 77(24) 181(19) 39(12)	S
N-(Furfuryl)tryptamine	240	<2284>	81(100) 131(61) 130(32) 110(16) 53(13) 77(9) 132(6) 103(5) 240(1)	S
N-(Furfurylidene)tryptamine	238	<2304>	130(100) 131(10) 109(9) 77(8) 81(6) 108(6) 103(5) 238(5)	S
1-(Furyl-2)-1,2 bzw. 3,4-dihydro-β-carboline	236	<2340>	236(100) 235(82) 207(42) 180(39) 77(29) 90(24) 206(21) 39(17)	P
1-(5-Methylfuryl-2)-1,2,3,4-tetrahydro-β-carboline	252	<2361>	180(100) 252(48) 251(27) 223(20) 209(19) 43(18) 181(16) 90(15)	S
N-(5-Methylfurfuryl) tryptamine	254	<2368>	95(100) 131(42) 130(28) 43(10) 96(6) 77(6) 41(6) 132(4) 254(1)	S
1-(Furyl-2)-β-carboline	234	<2390>	234(100) 205(78) 206(31) 117(31) 103(30) 102(18) 75(18) 76(17)	S
N-(5-Methylfurfurylidene) tryptamine	252	<2424>	130(100) 123(49) 122(20) 95(13) 131(10) 77(9) 252(7) 103(6)	S
1-(5-Methyl furyl-2)-1,2 bzw. 3,4-dihydro-β-carboline	250	<2439>	250(100) 207(87) 249(54) 206(47) 103(36) 43(30) 180(26)	P
1-(5-Methylfuryl-2)-β-carboline	248	<2482>	248(100) 205(54) 124(38) 219(35) 110(32) 233(28) 43(21) 140(20)	S

[a] RI, modified retention index on column 1; <RI>, modified retention index on column 2.
[b] P, proposed structure according to MS data; S, standard synthesized; R, standard available.

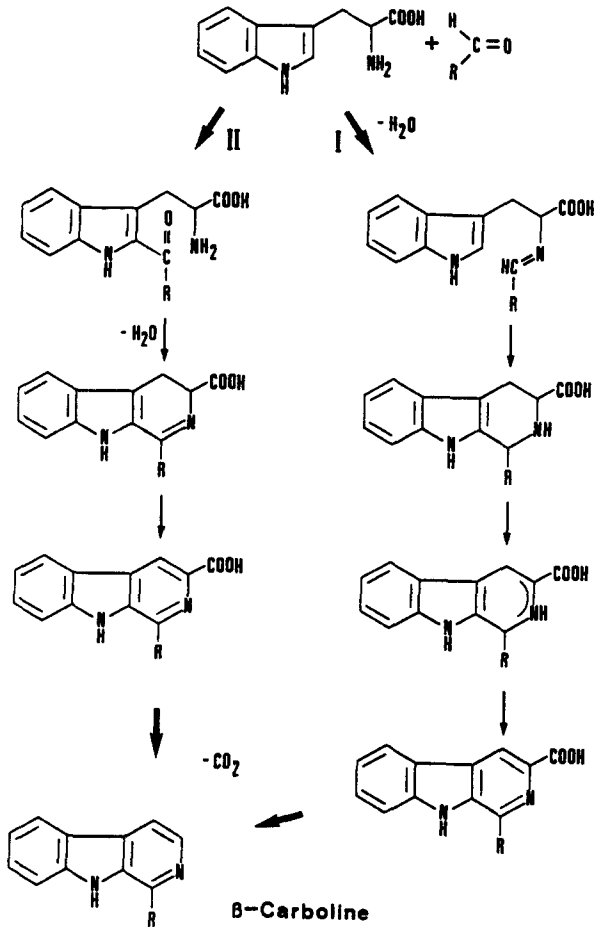


Fig. 4. Possible reaction pathway of β-carboline formation via azomethines or 2-acyl-tryptophan.

Roasting of tryptophan produced norharman and harman by reaction of the amino acid or tryptamine with formic or acetic aldehyde, which are pyrolytic degradation products. The formation of 1-acetyl, 1-ethyl and 1-propyl-β-carboline depend on the formation of methylglyoxal or propanoic or butanoic aldehyde from sugars. Reaction of tryptophan with furfuraldehydes directly, or after sugar decomposition, yields β-carboline derivatives with a furan ring. Among these compounds the structure of 1-(5-methylfuryl-2)-β-carboline is described in a roast aroma for the first time.

The detection of the azomethine- and tetrahydro-β-carboline structures as well as the furyl-β-carbolines after reaction of tryptophan with furfuraldehydes shows the importance of reaction pathway I of Fig. 4.

The absence of azomethines and di- and tetrahydro-β-carbolines, after roasting of fructose-tryptophan (Amadori product) as well as in tryptophan–sugar mixtures led us to suppose that the furyl-β-carbolines might have been formed directly by degradation of the Amadori product. The proposed formation pathway is shown schematically in Fig. 5. Table 3 shows the mass spectrometric data of the identified β-carbolines and their precursors.

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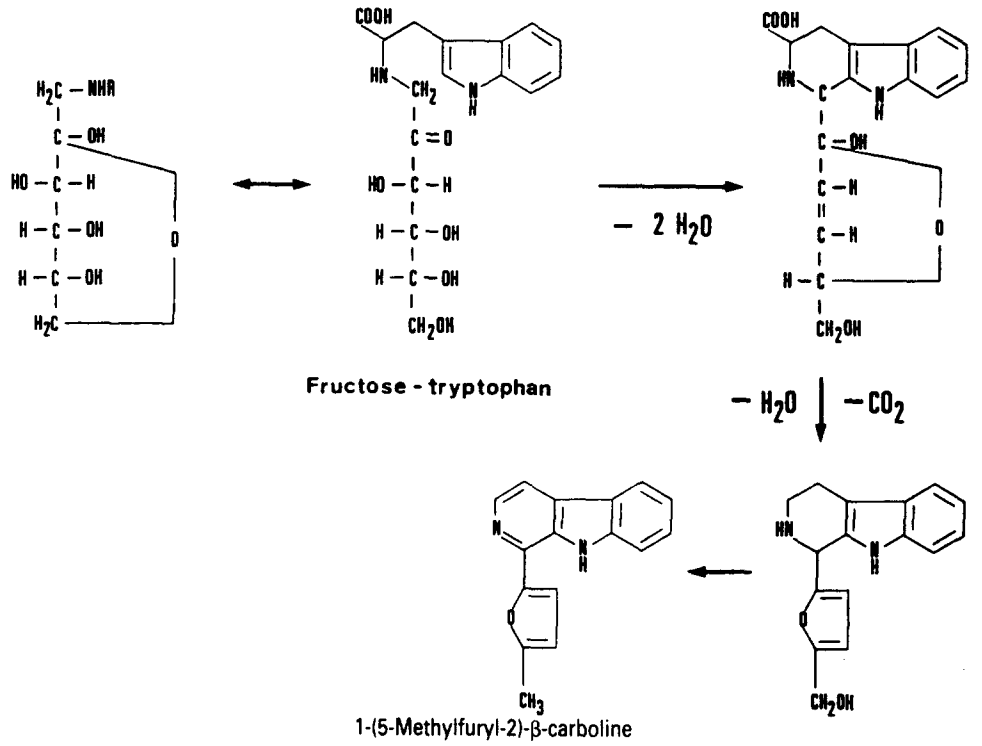

Fig. 5. Possible reaction pathway of 1-(5-methylfuryl-2)-β-carboline via the Amadori product.

REFERENCES

Akabori, S. & Saito, K. (1930). Synthetische Versuche in der Indolgruppe VIII: Synthese von Harman und Harmin, *Ber. Dtsch. Chem. Ges.*, **63**, 2245.

Baltes, W. & Bochmann, G. (1987*a*). Model reactions on roast aroma formation. I: Reaction of serine and threonine with sucrose under the conditions of coffee roasting and identification of new coffee aroma compounds. *J. agric. Food. Chem.*, **35**, 340–6.

Baltes, W. & Bochmann, G. (1987*b*). Model reactions on roast aroma formation. II: Mass spectrometric identification of furans and furanones from the reaction of serine and threonine under the conditions of coffee roasting. *Zeitschr. Lebensm.-unters. u.-forsch.*, **184**, 179–86.

Baltes, W. & Bochmann, G. (1987*c*). Model reactions on roast aroma formation. III: Mass spectrometric identification of pyrroles from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. *Zeitschr Lebensm.-unters. u.-forsch.*, **184**, 478–84.

Baltes, W. & Bochmann, G. (1987*d*). Model reactions of roast aroma formation. IV: Mass spectrometric identification of pyrazines from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. *Zeitschr. Lebensm.-unters. u.-forsch.*, **184**, 485–93.

Baltes, W. & Bochmann, G. (1987*e*). Model reactions of roast aroma formation. V: Mass spectrometric identification of pyridines, oxazoles and carboxylic compounds from the reaction of serine and threonine with sucrose under the conditions of coffee roasting. *Zeitschr. Lebensm.-unters. u.-forsch.*, **185**, 5–9.

Baltes, W. & Mevissen, L. (1988). Model reactions of roast aroma formation. VI: Volatile reaction products from the reaction of phenylalanine with glucose during cooking and roasting. *Zeitschr. Lebensm.-unters. u.-forsch.*, **187**, 209–14.

Brown, W. H. & Sawatzky, H. (1956). The condensation of furan and sylvan with some carbonyl compounds. *Can. J. Chem.*, **34**, 1147.

Brown, J. B., Henbest, H. B. & Jones, E. R. H. (1952). 3-Indolylacetaldehyde and 3-indolylacetone. *J. Chem. Soc.*, 3172.

Fitzpatrick, J. T. & Hiser, R. D. (1957). Non catalytic Fischer indole synthesis. *J. Org. Chem.*, **22**, 1703.

Heyns, K. & Noack, H. (1964). Die Umsetzung von L-Tryptophan und L-Histidin mit Hexosen. *Chem. Ber.*, **97**, 415.

Kari, I., Peura, P. & Airaksinen, M. M. (1980). Quantitative gas chromatographic–mass spectrometric determination of 1,2,3,4-tetrahydro-β-carboline in human plasma and platelets. *Biomed. Mass Spectr.*, **7**, 549.

Kunert-Kirchhoff, J. & Baltes, W. (1990*a*). Model reactions on roast aroma formation. VII: Specific products of phenylalanine after cooking phenylalanine with D-glucose in a laboratory autoclave. *Zeitschr. Lebensm.-unters. u.-forsch.*, **190**, 9.

Kunert-Kirchhoff, J. & Baltes, W. (1990*b*). Model reactions on roast aroma formation. VIII: Volatile reaction products from the reaction of phenylalanine with 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Puraneol) by cooking in a laboratory autoclave. *Zeitschr. Lebensm.-unters. u.-forsch.*, **190**, 14–16.

Leditschke, H. (1952). Über die Aufspaltung des Furankerns II: *N*-Aryl-pyrrolaldehyde und α-Cyan-β-(*N*-arylpyrryl)-acryl-saüreester aus Furfurol und α-Cyan-β-furyl-acryl-saüreestern. *Chem. Ber.*, **85**, 483.

Murahashi, S. I., Shimamura, T. & Moritani, I. (1974). Conversion of alcohols into unsymmetrical secondary or tertiary amines by a palladium catalyst. Synthesis of *N*-substituted pyrroles. *J.C.S. Chem. Commun*, 931.

Nagao, M. *et al.* (1977). Comutagenic action of norharmane and harmane. *Proc. Japan. Acad.*, **53**, 95.

Poindexter, E. H., Carpenter, I. and R. D. (1962). The isolation of harmane and norharmane from tobacco and cigarette smoke. *Phytochem.*, **1**, 215.

Severin, Th. & Heidenhain. B. (1966). Studien zur Maillard-reaktion. *Zeitschr. Lebensm.-unters. u.-forsch.*, **132**, 65.

Späth, E. & Lederer, E. (1930). Synthesen von 4-β-Carbolinen. *Ber. Dtsch. Chem. Ges.*, **63**, 2102.

Wilken, C. & Baltes, W. (1990). Model reactions on roast aroma formation. IX: Formation of pyrrole-2,5-diones by roasting of aspartic acid and asparagine. *Zeitschr. Lebensm.-unters. u.-forsch.*, **191**, 116.

Wittkowski, R., Kellert, M. & Baltes, W. (1983). Determination of the elemental composition of unknown aroma constituents by using a quadrupole mass spectrometer. *Int. J. Mass Spectrom. Ion Phys.*, **48**, 339.

Wittkowski, R., Kellert, M., Blaas, W. & Baltes, W. (1984). Ermittlung der Elementarzusammensetzung flüchtiger Verbindungen mit Hilfe der niederauflösenden Massenspektrometrie. *Lebensm. und gerichtliche Chemie*, **38**, 69–70.

Van den Dool, H. & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr.*, **11**, 463.