

Novel Strecker Degradation Products of Tyrosine and Dihydroxyphenylalanine

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Abstract

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Tyrosine was oxidised with either potassium peroxodisulphate or glyoxal. Volatile reaction products were isolated and analysed by GC/FID and GC/MS, derivatised with diazomethane and analysed by the same methods. Eight reaction products were identified. The major products were the expected Strecker aldehyde (4-hydroxyphenylacetaldehyde) and its lower homologue 4-hydroxybenzaldehyde. They were followed by 1-(4-hydroxyphenyl)-3-propionaldehyde, phenylacetaldehyde, benzaldehyde, phenol, 4-hydroxybenzoic, and benzoic acid. Analogously, the oxidation of 3,4-dihydroxyphenylalanine yielded the corresponding Strecker aldehyde (3,4-dihydroxyphenylacetaldehyde), its lower homologue 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzoic, 3,4-dihydroxyphenylacetic, and caffeic acid. An identification of these oxidation products of tyrosine and 3,4-dihydroxyphenylalanine assumes homolytic cleavage of the Strecker aldehydes and a recombination of free radicals formed by this cleavage. As minor products, six *O*- and *N*-heterocyclic compounds arose in systems containing glyoxal (pyrazine, methyl- and ethylpyrazine, 3-furancarbaldehyde, 5-methyl-2-furancarbaldehyde, 2-pyrrolcarbaldehyde).

Key words: Strecker degradation; amino acids; glyoxal; sodium peroxodisulphate; radicals; tyrosine; 3,4-dihydroxyphenylalanine (DOPA); 4-hydroxyphenylacetaldehyde, 3,4-dihydroxyphenylacetaldehyde

The oxidative decarboxylation of α -amino acids to aldehydes with one less carbon atom is called Strecker degradation (SCHÖNBERG *et al.* 1948; SCHÖNBERG & MOUBACHER 1952). The reaction involves several steps and its mechanism depends *inter alia* on the nature of the degrading agent. The agents that bring about the Strecker degradation of amino acids are inorganic or organic. It is generally accepted that the oxidative decarboxylations of amino acids proceed as schematically indicated in Fig. 1. The reaction is a complicated process involving many steps, among others the formation of the corresponding imino acid (hypochlorite oxidation leads to the intermediate,

N-chloroamino acid – OGATA *et al.* 1981). The fate of the amino group in this reaction depends on the structure of the oxidising agent. It may be eliminated in the form of ammonia. This is the case when the amino acid is degraded by peroxodisulphates, hypochlorites, other inorganic agents and some organic reagents. The amino group may also become linked to the oxidising agent converting it into an amino derivative of a similar structure. Such reactions proceed when the oxidation reagents are sugars and α -dicarbonyl compounds such as glyoxal (Fig. 2).

The Strecker degradation of amino acids is important for the generation of flavour-active compounds in many

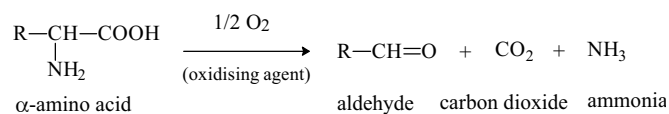


Fig. 1. Simplified mechanism of α -amino acid degradation

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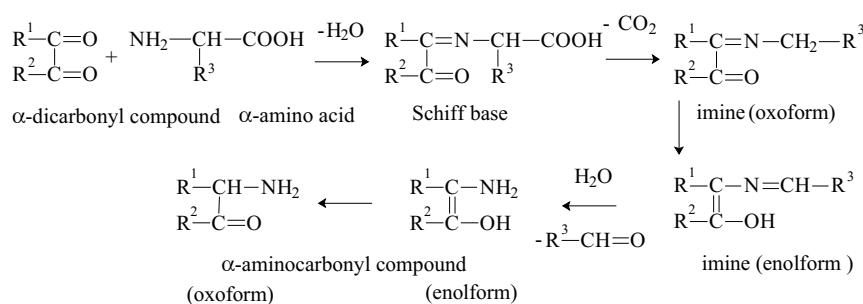


Fig. 2. Degradation of α -amino acids by α -dicarbonyl compounds

foods as it provides volatile aldehydes and subsequently alcohols, acetals, acids, esters and other compounds. The importance of Strecker degradation for biological processes such as its role in aging process and age-related diseases has been established for a long time (DEGENHARDT *et al.* 1998).

Tyrosine (Tyr) accompanies phenylalanine in the majority of proteins and its average content is about 3.5%. Enzymatic oxidation of Tyr in some food products such as beans and mushrooms, in the so called nonenzymatic browning reactions, leads to brown discoloration of these materials. Tyr is also readily oxidized to 3,4-dihydroxyphenylalanine (DOPA), which is a precursor of the so called melanins in animals.

The Strecker degradation of Tyr by hypochlorite yields 4-hydroxyphenylacetaldehyde (LANGELD 1909). In bamboo sprouts and sorghum grains, the same aldehyde arises from cyanogenic glycosides taxiphillin and dhuriin (VELÍŠEK 1999). The corresponding alcohol tyrosol (4-hydroxyphenyl-ethyl-alcohol) is a common constituent of alcoholic beverages (NYKANEN & SUOMALAINEN 1983). The Strecker aldehyde of DOPA, 3,4-dihydroxyphenylacetaldehyde, has been identified as a product of enzymatic oxidation of DOPA (HOLTZ *et al.* 1939). Together with tyrosol, 3,4-dihydroxyphenylethanol (hydroxytyrosol) occurs in significant quantities in unripe olives (*Olea europaea* L.) and virgin olive oils (SOLINAS & CICHELLI 1982).

This study was undertaken as a part of investigation of beer changes due to oxidation during pasteurization and storage which is followed by decomposition of amino acids, sugars and other compounds. The aim was to identify the minor compounds arising during the Strecker degradation of Tyr and DOPA and show the importance of radical reactions in the pathways leading to these minor products. The Strecker aldehydes derived from either Tyr or DOPA are nonvolatile compounds but they can influence taste and colour of foods as they readily become substrates of oxidoreductases in enzymatic browning reactions.

MATERIAL AND METHODS

Chemicals: Tyr, DOPA (Aldrich, Steinheim, Germany), and glyoxalhydrate trimer (Sigma Chemical Company, St.

Louis, USA) were commercial products. Potassium peroxodisulphate ($\text{K}_2\text{S}_2\text{O}_8$) and other compounds were obtained from Lachema (Brno, Czech Republic). Diazomethane solution in diethyl ether was prepared from *p*-toluenesulphonyl-*N*-methylnitrosamide (Aldrich, Steinheim, Germany). Solvent grade diethyl ether was purchased from Merck (Darmstadt, Germany).

Oxidative Decarboxylation of Amino Acids: Amino acid (5 mmol) and potassium peroxodisulphate or glyoxal (5 mmol) were dissolved in 500 ml water and the mixture was heated under reflux for 1 h. The reaction mixture was cooled to room temperature and extracted with two 50 ml and two 25 ml portions of diethyl ether. The combined extracts were dried over anhydrous sodium sulphate, concentrated to 500 μl using a Snyder column and a gentle stream of nitrogen and analysed by GC/FID and GC/MS method. An aliquot (usually 100 μl) of the concentrated extract was derivatised with diazomethane solution in diethyl ether, concentrated to 100 mL and analysed as described above.

Gas Chromatographic (GC/FID) and Gas Chromatographic/Mass Spectrometric (GC/MS) Analysis: A Hewlett-Packard (H/P) Model 4890A gas chromatograph equipped with a flame ionization detector and a fused capillary column (HP-Inowax, 30 m \times 0.25 mm i.d., film thickness: 0.25 μm) was used in this study. The GC oven was temperature programmed from 60 to 220°C at a rate of 5°C/min, the injector and detector temperatures were held at 220 and 250°C, respectively. The carrier gas (N_2) flow rate was 2 ml/min. The sample (1 μl) was injected using a split ratio of 1:10. Duplicate analyses of samples were done.

GC retention indices (relative retention index, R.I.) were determined internally with a series of *n*-alkanes (VAN DEN DOOL & KRATZ 1963). The GC conditions were the same as described above.

For GC/MS analysis, a H/P Model G1800A apparatus equipped with the same column operating under conditions described above were used. Carrier gas (He) flow rate was 0.7 ml/min. Mass spectra were obtained by EI ionization at 70 eV. The ion source temperature was maintained at 250°C. NIST/EPA/NIK 75k Mass Spectral Database (Hewlett-Packard) enabled tentative identification of analysed compounds.

RESULTS AND DISCUSSION

The oxidative decarboxylation of amino acids in this study was induced either by a free radical initiator potassium peroxodisulphate or by glyoxal (ADAMIEC *et al.* 2001). In the absence of these oxidation reagents, the spontaneous decomposition of amino acids was negligible.

Tyrosine: Under the reaction conditions employed the amount of Tyr decomposed by peroxodisulphate and glyoxal was 67 and 68%, respectively (Table 1).

Table 1. Decomposition of Tyr and DOPA by potassium peroxodisulphate or glyoxal

System	Final concentration [mmol/l] ^a				Decomposed amount [%]
	1	2	average	std dev	
Tyr/K ₂ S ₂ O ₈	3.4	3.2	3.3	0.14	67
Tyr/glyoxal	3.3	3.0	3.2	0.21	68
DOPA/K ₂ S ₂ O ₈	5.2	5.1	5.2	0.07	48
DOPA/glyoxal	5.7	5.6	5.7	0.07	43

^a The starting concentration was 10.0 mmol/l

As it is shown in Fig. 3, two major volatile compounds (4-hydroxyphenylacetaldehyde and its lower homologue 4-hydroxybenzaldehyde) were detected in the diethyl ether extract of Tyr solution oxidised by peroxodisulphate. Three additional compounds, i.e. benzaldehyde, phenylacetaldehyde, and phenol, were found in much lower quantities. The major compounds were tentatively identified comparing their mass spectra with mass spectral data contained by the mass spectral data base (Table 2). Other components were identified by comparison of their mass spectral data and retention time data with those of authentic compounds. The Strecker aldehyde, 4-hydroxyphenylacetaldehyde, arose from 0.57 mg of Tyr decomposed (0.063% of the starting amount), which corresponds to 0.13% of theory (Table 3).

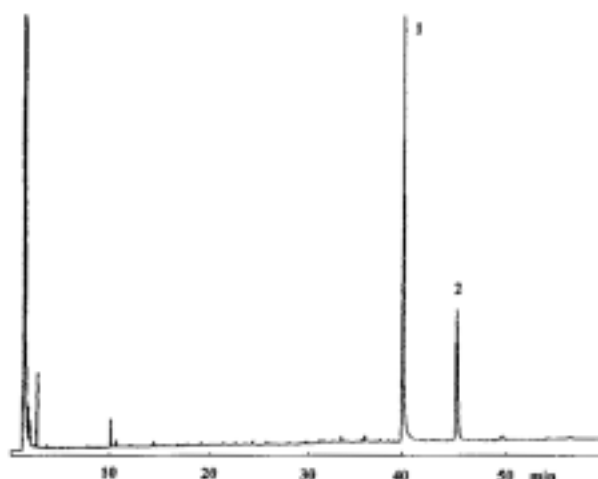


Fig. 3. Separation of Tyr/K₂S₂O₈ volatile reaction products (1 – 4-hydroxyphenylacetaldehyde, 2 – 4-hydroxybenzaldehyde)

Derivatisation with diazomethane and separation of the products arisen in the Tyr/K₂S₂O₈ system revealed the presence of a higher number of compounds. *N,N*-methylated and *N*-methylated methyl esters of Tyr were identified as the major volatile products (LIEBICH & FÖRST 1985). Due to its nucleophilic nature, diazomethane reacted with carboxylic groups of acids, hydroxyl groups of phenols, amino groups of amino acids and even with carbonyl groups of aldehydes (ČERNÝ *et al.* 1971). The latter reaction predominantly yielded the corresponding methylketones (Fig. 4). The major product was 1-(4-methoxyphenyl)-2-propanon, formed from the Strecker aldehyde. The methoxyderivative of the Strecker aldehyde (4-methoxyphenylacetaldehyde) was found in a lower amount. The same compound could also arise from 4-hydroxybenzaldehyde. The same precursor probably gave rise to 4-methoxyacetophenon and 4-methoxybenzaldehyde (anisaldehyde). The other identified compounds were

Table 2. Volatile products formed from Tyr oxidised by potassium peroxodisulphate or glyoxal

Peak No. (see Fig. 3)	Compound ^a	Identification ^b	RT (min) ^c	R.I. ^d	mg per starting amt of amino acid ^e	
					K ₂ S ₂ O ₈	Glyoxal
1	4-hydroxyphenylacetaldehyde 107(100),77(23),136M(17),108(9)	MS	39.7	2820	0.427	0.226
2	4-hydroxybenzaldehyde 121(100),122M(89),93(43),65(34)	MS	44.8	2958	0.233	0.002

^a Mass spectra (MS) are listed in parentheses with descending intensities of fragment ions, M = molecular peak

^b Mass spectra (MS) were consistent with published data (mass spectral database)

^c RT = retention time in min

^d R.I. = retention index

^e Expressed in mg of 4-hydroxyphenylacetaldehyde arising from 5 mmol (910.6 mg) of Tyr

Table 3. Amount of Strecker aldehyde formed from amino acid oxidised by potassium peroxodisulphide or glyoxal

Amino acid	Strecker aldehyde yield (% of theory) ^a	
	K ₂ S ₂ O ₈	Glyoxal
Tyr	0.13	0.07
DOPA	0.01	0.005

^a Mean values (2 parallel determinations, average relative standard deviation 7.3%)

benzylmethylketon which arose from benzaldehyde, 1-(4-methoxyphenyl)-3-butanon having its origin in 1-(4-hydroxyphenyl)-3-propionaldehyde derivatisation, methyl 4-methoxybenzoate, and methyl benzoate. Mass spectra of the identified methylketones are listed in Table 4. The occurrence of these and other minor compounds suggests a homolytic cleavage of 4-hydroxyphenylacetaldehyde or other compounds and a recombination of the resulting free radicals.

The amino acid solution oxidised by glyoxal contained the same two major aldehydes, i.e. 4-hydroxyphenylacetaldehyde and 4-hydroxybenzaldehyde. The Strecker aldehyde was formed from 0.30 mg of Tyr (0.033% of the starting amount) which corresponds to 0.07% of theory (Table 3). Some additional minor compounds that resulted from the reaction of the amino acid with glyoxal (py-

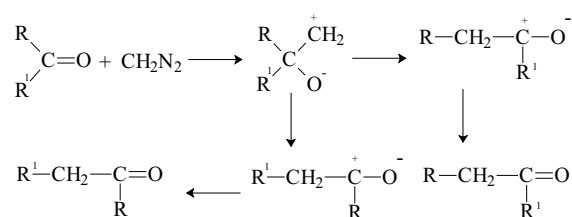


Fig. 4. Proposed reaction pathway leading to the formation of methylketones from aldehydes and diazomethane

razine, methyl- and ethylpyrazine, 3-furancarbaldehyde, 5-methyl-2-furancarbaldehyde, 2-pyrrolcarbaldehyde) were identified.

Glyoxal itself (present in diluted aqueous solutions as a dihydrate) yielded a small amount of glyoxylic acid by oxidation. The reaction can be initiated, for instance, by hydroxyl radicals (BUXTON *et al.* 1997) and proceeds *via* a peroxy radical that splits off HO₂• (Fig. 5).

Dihydroxyphenylalanine: The amount of DOPA decomposed by a reaction with peroxodisulphate and glyoxal was lower than the quantity of Tyr, being 48 and 47%, respectively (Table 1). The yield of the Strecker aldehyde was about ten times lower than of that arising from Tyr (Table 3).

The polar products obtained were either not extractable into diethyl ether or not sufficiently volatile to be amena-

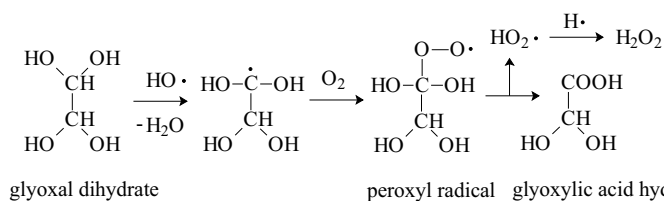


Fig. 5. Autoxidation of glyoxal to glyoxylic acid

Table 4. Volatile products arisen by reaction of diazomethane with phenolic aldehydes derived from Tyr and DOPA

Compound	Mass spectrum, m/z [%]	RT ^a	R.I. ^b
<i>Products from Tyr</i>			
benzylmethylketon	43(100), 91(66.4), 92(23.5), 134M(23.0), 65(15.9)	18.1	1710
4-methoxybenzaldehyde (anisaldehyde)	135(100), 136M(69.8), 77(25.4), 92(15.8), 107(11.6)	24.5	2055
4-methoxyphenylacetaldehyde ^c	121(100), 77(12.6), 150M(11.6), 91(9.3)	25.2	2090
4-methoxyacetophenon	135(100), 150M(32.6), 77(10.6), 92(6.3), 107(11.6)	26.7	2120
1-(4-methoxyphenyl)-2-propanon	121(100), 164M(12.6), 122(9.3), 77(7.9), 43(7.0), 91(4.8)	26.9	2185
1-(4-methoxyphenyl)-3-butanon	121(100), 178M(27.9), 43(15.6), 77(8.8), 122(8.1), 91(7.0)	29.3	2310
<i>Products from DOPA</i>			
3,4-dimethoxybenzaldehyde (veratraldehyde)	166M(100), 165(61.6), 95(22.0), 77(21.0), 151(10.8), 167(10.4)	31.5	2420
1-(3,4-dimethoxyphenyl)-2-propanon	151(100), 194M(15.6), 43(15.2), 152(13.0), 107(9.3)	32.9	2470

^a RT = retention time in min; ^b R.I. = retention index; ^c Consistent with mass spectrum published by BLUMENSTEIN *et al.* (1993)

ble to gas chromatographic separation. Therefore, an aliquot of the aqueous phase was evaporated to dryness and directly derivatised with diazomethane. Analogously to Tyr, the corresponding methoxyphenols, methylketons and carboxylic acids methyl esters were identified. Except the methyl ester of *N,N*-dimethylated DOPA, mass spectrum: 116(100), 208M-59(12), methyl 3,4-dimethoxycinnamate (derived from caffeic acid) was identified as the major product. The minor products found were 3,4-dimethoxybenzaldehyde (veratraldehyde) formed from 3,4-dihydroxybenzaldehyde, methyl 3,4-dimethoxybenzoate formed from 3,4-dihydroxybenzoic acid, 1-(3,4-dimethoxyphenyl)-2-propanone arising from the Strecker aldehyde, and methyl 3,4-dimethoxyphenylacetate derived from 3,4-dihydroxyphenylacetic acid. Mass spectra of the identified carbonyl compounds are summarized in Table 4.

CONCLUSIONS

Oxidation of Tyr with either peroxodisulphate or glyoxal yielded the expected Strecker aldehyde (4-hydroxyphenylacetaldehyde), its lower homologue 4-hydroxybenzaldehyde, its higher homologue 1-(4-hydroxyphenyl)-3-propionaldehyde, 4-hydroxybenzoic acid and several minor products. Analogously, the oxidation of DOPA predominantly yielded the expected Strecker aldehyde (3,4-dihydroxyphenylacetaldehyde), its lower homologue 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzoic, 3,4-dihydroxyphenylacetic, and caffeic acid. The identification of these oxidation products of Tyr and DOPA assumes homolytic cleavage of the Strecker aldehydes and a recombination of free radicals produced.

Abbreviations

Tyr	L-tyrosine, L-2-amino-3-(4-hydroxyphenyl)propionic acid
DOPA	L-3-(3,4-dihydroxyphenyl)alanine, L-2-amino-3-(3,4-dihydroxyphenyl)propionic acid

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Souhrn

ADAMIEC J., CEJPEK K., RÖSSNER J., VELÍŠEK J. (2001): **Nové produkty Streckerovy degradace tyrosinu a dihydroxyfenylalaninu.** *Czech. J. Food Sci.*, **19**: 13–18.

Tyrosin byl oxidován peroxodisulfátem draselným nebo glyoxalem. Těkávé produkty reakce byly izolovány a analyzovány metodou GC/FID a GC/MS, derivatizovány diazomethanem a znovu analyzovány stejnými metodami. Bylo identifikováno osm

reakčních produktů. Hlavními produkty reakce byly očekávaný Streckerův aldehyd (4-hydroxyfenylacetaldehyd) a jeho nižší homolog 4-hydroxybenzaldehyd, dále podle obsahu následovaly 4-hydroxypropionaldehyd, fenylacetaldehyd, benzaldehyd, fenol, kyselina 4-hydroxybenzoová a benzoová. Analogicky oxidace 3,4-dihydroxyfenylalaninu poskytovala příslušný Streckerův aldehyd, tzn. 3,4-dihydroxyfenylacetaldehyd a jeho nižší homolog 3,4-dihydroxybenzaldehyd, dále kyseliny 3,4-dihydroxybenzoovou, 3,4-dihydroxyfenylactovou a kávovou. Identifikace těchto oxidačních produktů tyrosinu a 3,4-dihydroxyfenylalaninu předpokládá homolytické štěpení molekuly Streckerových aldehydů a rekombinaci vzniklých volných radikálů. V systémech obsahujících glyoxal bylo prokázáno šest minoritních produktů – *O*- a *N*-heterocyklických sloučenin (pyrazin, methyl- a ethylpyrazin, 3-furankarbaldehyd, 5-methyl-2-furankarbaldehyd, 2-pyrrolkarbaldehyd).

Klíčová slova: Streckerova degradace; aminokyseliny; glyoxal; peroxodisulfát sodný; radikály; tyrosin; 3,4-dihydroxyfenylalanin (DOPA); 4-hydroxyfenylacetaldehyd, 3,4-dihydroxyfenylacetaldehyde

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