Strecker Degradation Products of Aspartic and Glutamic Acids and their Amides

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Abstract

RÖSSNER J., VELÍŠEK J., PUDIL F., DAVÍDEK J. (2001): **Strecker degradation products of aspartic and glutamic acids and their amides**. Czech J. Food Sci., **19**: 41–45.

Aspartic and glutamic acids, asparagine and glutamine were oxidised with either potassium peroxodisulphate or glyoxal. Non-volatile products were derivatised and analysed by GC/FID and GC/MS. Volatile reaction products were isolated and analysed by the same methods. It was found that the degradation reactions of amino acids are complex. Amino acids are principally degraded via the corresponding α-keto acids to Strecker aldehydes (aspartic acid to oxalacetic and 3-oxopropionic acids and glutamic acid to α-ketoglutaric and 4-oxobutyric acids), which are unstable and decomposed by decarboxylation to the corresponding aldehydes. Aspartic acid also eliminates ammonia and yields fumaric acid whereas glutamic acid gives rise to an imine, pyroglutamic acid. A recombination of free radicals leads to dicarboxylic acids (succinic acid from aspartic acid, succinic, glutaric and adipic acids from glutamic acid). The major volatile products (besides the aldehydes) are lower carboxylic acids (acetic acid from aspartic acid and propionic acid acid from glutamic acid) that can at least partly arise by radical reactions. In both quality and quantity terms, a higher amount of degradation products arises by oxidation of amino acids by peroxodisulphate.

Keywords: Strecker degradation; Strecker aldehydes; amino acids; glyoxal; sodium peroxodisulphate; aspartic acid; glutamic acid; asparagine; glutamine; radicals

The reaction of an α-amino acid with an oxidation reagent to give carbon dioxide and an aldehyde containing one carbon atom less is known as Strecker degradation (SCHÖNBERG *et al*. 1948). A number of chemical reagents have been recognised as having the power to cause such oxidative decarboxylation of amino acids. The reactions between amino acids and α-dicarbonyl compounds are of special importance. They involve transaminations and yield carbon dioxide, aldehyde and aminocarbonyls. The aldehydes formed, often called Strecker aldehydes, can act as food odourants *per se*. The aminocarbonyls formed can yield pyrazine derivatives known as important flavour-active constituents of many processed foods. Mechanisms of these reactions have been recently described (BELITZ & GROSCH 1999; ADAMIEC *et al*. 2001a, b).

When amino acids with functional groups in the side chain are involved in the Strecker degradation, even more complex reactions are possible. Such amino acids are aspartic (Asp) and glutamic (Glu) acids and their amides asparagine (Asn) and glutamine (Gln). The Strecker degradation of Asp theoretically leads to 3-oxopropionic acid (malonic semialdehyde). This Strecker aldehyde has been identified as a product of Asp oxidation with *N*-bromoacetamide (BISHNOI & BANERJI 1985; REDDY *et al*. 1990), chloramine T (GOWDA & RAO 1987) and potassium peroxodisulphate (SRIVASTAVA & MATHUR 1982). On the other hand, the action of methylglyoxal on Asp resulted in the formation of acetaldehyde, which arises by decarboxylation of 3-oxopropionic acid (SCHÖNBERG & MOUBACHER 1952). Analogously, the Strecker degradation of Glu leads to 4-oxobutyric acid (succinic semialdehyde), which was found to be a product of Glu oxidation by isatin (indole-2,3-dione) (SCHÖNBERG & MOUBACHER 1952), sodium hypochlorite (FRIEDMAN & MORGULIS 1936) or sodium hypobromite (FOX & BULLOCK 1951), *N*-bromoacetamide (REDDY *et al*. 1990) and potassium peroxodisulphate (SRIVASTAVA & MATHUR 1982).

It is well known that Asp also behaves as a β-amino acid. It can eliminate ammonia and yield fumaric acid (BELITZ & GROSCH 1999). Similarly, Glu behaves as a γ-amino acid as it readily forms 5-oxopyrrolidine carboxylic (pyroglutamic) acid upon heating. Under roasting conditions where radical reaction mechanisms can predominate, Asp and Asn yield various pyrrole-2,5-diones (WILKEN & BALTES 1990). Asp gives rise to maleimide (pyrrole-2,5-dione) as the major component. A pathway for the formation of this compound may be maleic acid formed from Asp by the loss of ammonia, which subsequently reacts with carboxyl groups of dicarboxylic acid, yielding the imide. Substituted pyrrole-2,5-diones (3-methyl and 3,4-dimethyl) were minor products. Asn yields predominantly 3-methyl- and 3,4-dimethyl-pyrrole-2,5-diones, whereas the concentration of succinimide (pyrrolidine-2,5-dione) is low. It is suggested that pyrrole-2,5-diones arise by a radical methyl group transfer to either maleimide or succinimide.

This study was undertaken as a part of the investigation of beer changes due to oxidation during pasteurization and storage which is followed by a decomposition of amino acids and other compounds. Glutamin is a very labile amino acid. It was even used as an indicator of beer staling (HILL *et al.* 1998). The aim was to identify the compounds arising during Strecker degradation of Asp and Glu, their amides Asn and Gln, and to show the importance of radical reactions in the pathways leading to these products.

MATERIAL AND METHODS

Chemicals: Aspartic acid (L-isomer), glyoxalhydrate trimer and 4-oxobutanoic acid (Sigma Chemical Company, St. Louis, USA), L-asparagine monohydrate, L-glutamine (Aldrich, Steinheim, Germany), L-glutamic, acetic, propionic, butanoic, 2-methylbutanoic, pentanoic (Lachema, Brno, Czech Republic), 2-oxobutyric acid and oxalacetic acid (Merck, Darmstadt, Germany) were commercial products. Potassium peroxodisulphate ($K_2S_2O_8$), hydroxylamine.HCl 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, pyridine, hexamethyldisilazane and trimethylchlorosilane were 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 in the Likens-Nickerson apparatus and extracted with 100 ml of diethyl ether for 1 h. The solvent was 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 meth-od. The aqueous phase was re-extracted with one 50 ml a two 25 ml portions of diethyl ether and the combined extracts were treated as described above. An aliquot (usually 1 ml) of the aqueous phase was evaporated in a vacuum evaporator, the residue was derivatised with diazomethane solution in diethyl ether, concentrated to 100 μl and analysed by GC/FID and GC/MS.

Oxoacids: Using another aliquot of the evaporated aqueous phase, 10 mg of hydroxylamine.HCl and 1 ml of dry pyridine were added and the mixture was let to stand for 10 min at room temperature. Then 0.1 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane were added and 1 μl of the solution was analysed by GC/FID and GC/MS after 5 min of standing at room temperature.

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 × 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 1 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.

A H/P Model G1800A apparatus equipped with the same column operating under conditions described above was used for GC/MS analysis. 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. The NIST/EPA/NIK 75k Mass Spectral Database (Hewlett-Packard) enabled the tentative identification of analysed compounds.

RESULTS AND DISCUSSION

The oxidative decarboxylation of amino acids in this study was induced by a free radical initiator, potassium peroxodisulphate, or achieved by glyoxal, a representative of α-dicarbonyl compounds (ADAMIEC *et al.* 2001a, b). Under the reaction conditions employed, the decomposed amount of Asp ranged from 53 to 64% and that of Asn from 40 to 46%. Similar results were achieved with Glu (52–67%) and its amide Gln (37–62%) (Table 1).

Major pathways of Asp transformation are outlined in Fig. 1. The intermediate oxalacetic acid can yield either pyruvic or 3-oxopropionic acid (the Strecker aldehyde) by decarboxylation and fumaric acid by the elimination of ammonia. Decarboxylation of pyruvic acid gives acetaldehyde. Acetaldehyde was identified as a product of

Table 1. Decomposition of Asp, Asn, Glu and Gln by potassium peroxodisulphate or glyoxal

System	Concentration [mmol/l][a]				
	1	2	Average	Std. dev.	Decomposed amount [%]
Asp/$K_2S_2O_8$	4.7	4.6	4.7	0.1	53
Asp/glyoxal	3.6	3.5	3.6	0.1	64
Asn/$K_2S_2O_8$	6.0	6.0	6.0	0.0	40
Asn/glyoxal	5.4	5.3	5.4	0.1	46
Glu/$K_2S_2O_8$	4.8	4.7	4.8	0.1	52
Glu/glyoxal	3.2	3.3	3.3	0.3	67
Gln/$K_2S_2O_8$	3.8	3.7	3.8	0.1	62
Gln/glyoxal	6.3	6.2	6.3	0.1	37

[a] The starting concentration of all amino acids was 10.0 mmol/l

Asp, oxalacetic and pyruvic acids but not quantified. Analogous reactions catalysed by enzymes occur during transamination of Asp. Analysis of the keto acids arising by the oxidation of Asp with peroxodisulphate revealed the presence of only 0.006 mg of pyruvic acid and 0.010 mg of 3-oxopropionic acid (malonic semialdehyde) and no oxalacetic acid. The absence of oxalacetic acid in the reaction mixture is not surprising as it was found in another experiment that this acid is totally decomposed under the reaction conditions employed. The oxidation of Asp by glyoxal yielded 0.70 mg of pyruvic acid and 0.100 mg of 3-oxopropionic acid. Again, oxalacetic acid was not identified. In addition to these acids, trace amounts (< 0.005 mg) of glyoxylic acid and ethylene glycol were found. These compounds arise from glyoxal by oxidation and reduction reactions, respectively. Analogously, the only products identified in the reaction mixture Glu/$K_2S_2O_8$ were the Strecker aldehyde 4-oxobutyric acid (succinic semialdehyde) and 2-oxobutyric acid (Fig. 2). The former acid was found in the concentration of 1 mg, the latter was present in a trace amount not exceeding 0.005 mg. The same two acids were also found in Glu/glyoxal mixture (8.9 mg and

traces, resp.) and in mixtures comprising Asn and Gln in levels similar to those given above. Propionaldehyde was another product identified but the intermediate α-ketoglutaric acid was not identified.

Dicarboxylic and other nonvolatile acids were analysed as methyl esters and the results obtained are summarized in Table 2. As can be seen, fumaric acid arises as the major product from Asp in both systems studied. This acid was accompanied by two other dicarboxylic acids, i.e. maleic acid (an isomer of fumaric acid) and succinic acid. The occurrence of succinic acid suggests a homolytic cleavage of Asp (or intermediates) and subsequent recombination of so formed free radicals, as is schematically shown in Fig. 3. Only traces of fumaric acid were detected in systems comprising Asn. Other nonvolatile products are probably formed from Asn as this compound is in equilibrium with two other forms, i.e. isoasparagine and 3-aminosuccinimide (SHU & LAWRENCE 1995). As expected, Glu yielded its lactame, pyroglutamic acid as the major product and the same compound also arised from Gln. Succinic and adipic acids were found in relatively high levels. Again, this finding suggests a radical
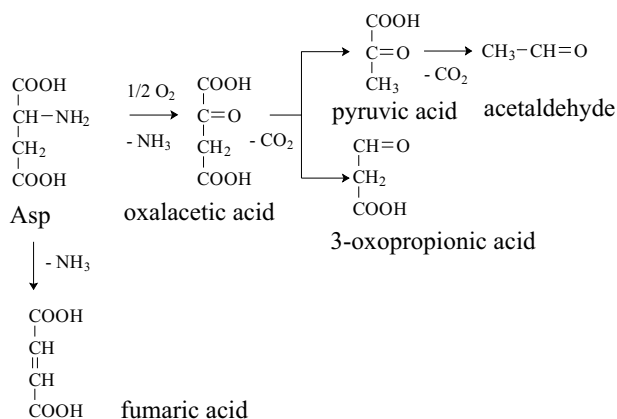

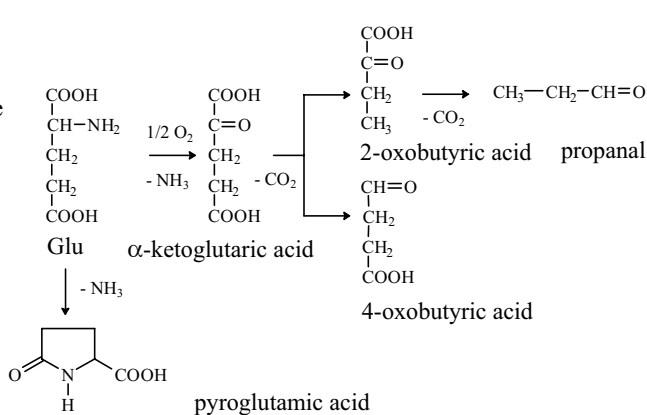Fig. 1. Major pathways of Asp transformation

Fig. 2. Major pathways of Glu transformation

Table 2. Nonvolatile acids arising from Asp, Asn, Glu and Gln oxidised by potassium peroxodisulphate or glyoxal

Concen-tration[a]	Acid[b]					
	Fumaric	Maleic	Succinic	Glutaric	Adipic	Pyro-glutamic
Asp/$K_2S_2O_8$	2.93	0.86	0.53	n.d.	n.d.	n.d.
Asp/glyoxal	0.67	0.27	0.13	n.d.	n.d.	n.d.
Asn/$K_2S_2O_8$	traces	n.d.	n.d.	n.d.	n.d.	n.d.
Asn/$K_2S_2O_8$	traces	n.d.	n.d.	n.d.	n.d.	n.d.
Glu/$K_2S_2O_8$	n.d.	n.d.	9.78	0.22	2.13	4.48
Glu/glyoxal	n.d.	n.d.	0.66	n.d.	0.22	23.15
Gln/$K_2S_2O_8$	n.d.	n.d.	3.29	n.d.	1.62	20.00
Gln/glyoxal	n.d.	n.d.	6.86	n.d.	1.91	19.78

[a] in mg arising from the amino acids present (5 mmol each), [b]n.d. – not detected, traces < 0.005 mg

Table 3. Volatile products arising from Asp, Asn, Glu and Gln oxidised by potassium peroxodisulphate or glyoxal

Concen-tration[a]	Acid			
	Acetic	Propionic	Butyric	Valeric
Asp/$K_2S_2O_8$	0.495	0.015	< 0.010	n.d.
Asp/glyoxal	0.013	n.d.	n.d.	n.d.
Asn/$K_2S_2O_8$	0.021	n.d.	n.d.	n.d.
Asn/$K_2S_2O_8$	0.010	n.d.	n.d.	n.d.
Glu/$K_2S_2O_8$	0.032	0.550	0.013	0,018
Glu/glyoxal	0.032	n.d.	n.d.	n.d.
Gln/$K_2S_2O_8$	0.035	1.341	0.015	0.100
Gln/glyoxal	0.010	n.d.	n.d.	n.d.

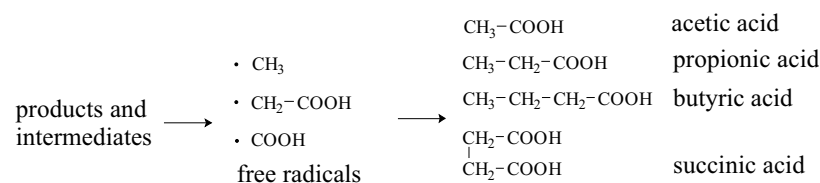[a] in mg arising from the amino acids present (5 mmol each), n.d. – not detected

Fig. 3. Products arising from Asp by radical reactions

mechanism by which these acids arise, similar to that given in Fig. 3.

In all cases the corresponding methyl esters of *N*-methyl and *N,N*-dimethylamino acids were the major constituents of the derivatised aqueous phase that arose by methylation of the residual amino acids with diazomethane (LIEBICH & FÖRST 1985).

The volatile fraction obtained by simultaneous steam distillation/solvent extraction of Asp/$K_2S_2O_8$ reaction mixture contained acetic acid as the major volatile product and smaller amounts of propionic and butyric acid (Table 3). The major portion of acetic acid arose by oxidation of Asp by $K_2S_2O_8$, nevertheless only 0.50 mg (0.008 mmol) were formed from 5 mmol of Asp. Acetic acid was the only volatile product found in the Asp/glyoxal reaction mixture and in systems comprising Asn. The same volatiles (acetic, propionic and butyric acids) were isolated from Glu/$K_2S_2O_8$. Propionic acid was the major volatile acid. Most of it arose from Gln oxidised by $K_2S_2O_8$. Only 1.34 mg (0.018 mmol) of this acid arose from 5 mmol of Gln. Valeric and 2-methylbutyric acids were the newly identified compounds. The latter acid was only formed by reactions of Glu and Gln with $K_2S_2O_8$ at about the same concentrations as valeric acid. Acetic acid was the only volatile product found in the Glu/glyoxal and Gln/glyoxal reaction mixtures. In addition to the compounds listed in Table 3, pyrazin, 3-furancarbaldehyde and 5-methyl-2-furancarbaldehyde, which undoubtedly arose from

glyoxal (ADAMIEC *et al.* 2001a, b), were the minor constituents of the volatile fraction.

However, the amount of acids arising as products of decomposition of the amino acids studied was relatively low. For example, the amount of volatile acids arising in the Asp/$K_2S_2O_8$ system (from 5 mmol of Asp, 665 mg) totals only 0.5 mg and the amount of the nonvolatile acids 4.3 mg, which corresponds to 0.7 % of Asp decomposed. Similarly, the total amount of volatile acids arising in the Glu/$K_2S_2O_8$ system (from 5 mmol of Glu, 735 mg) is 0.6 mg and the total amount of the nonvolatile acids is 16.6 mg, which corresponds to 2.3% of Asp decomposed.

CONCLUSIONS

It was found that dicarboxylic amino acids Asp, Glu and their amides decompose by reactions with peroxodisulphate or glyoxal. They are not only decomposed by Strecker degradation but also by other pathways. The major reaction products are keto acids including Strecker aldehydes, dicarboxylic acids and lower fatty acids. Some of these products may arise by reactions involving recombination of free radicals.

Abbreviations

Asp   L-aspartic acid
Glu   L-glutamic acid
Asn   L-asparagine
Gln   L-glutamine

References

ADAMIEC J.; RÖSSNER J.; VELÍŠEK J.; CEJPEK K.; ŠAVEL J. (2001a): Minor Strecker degradation products of phenylalanine and phenylglycine. Eur. Food Technol., **212**: 135–140.

ADAMIEC J., CEJPEK K., RÖSSNER J., VELÍŠEK J. (2001b): Novel Strecker degradation products of tyrosine and dihydroxyphenylalanine. Czech. J. Food Sci., **19**: 13–18.

BELITZ H.-D., GROSCH W. (1999): Food Chemistry. 2nd Ed. Springer, Berlin.

BISHNOI M.L., BANERJI K.K. (1985): Kinetics and mechanism of the oxidation of α-amino acids by *N*-bromoacetamide. Tetrahedron, **41**: 6047–6050.

DOOL VAN DEN H., KRATZ P.D. (1963): A generalization of the retention index system including linear temperature programmed gas-liquid portition chromatography. J. Chromatogr., **11**: 463–471.

FOX S.W., BULLOCK M.W. (1951): Synthesis of indoleacetic acid from glutamic acid and a proposed mechanism for the conversion. J. Amer. Chem. Soc., **73**: 2754–2755.

FRIEDMAN A.H., MORGULIS S. (1936): The oxidation of amino acids with sodium hypobromite. J. Amer. Chem. Soc., **58**: 909–913.

GOWDA B.T., RAO R.V. (1987): Kinetics and mechanism of uncatalyzed and chloride-catalyzed oxidative decarboxylation of glycine and aspartic acid by chloramine-T in perchloric acid medium. J. Indian Chem. Soc., **64**: 467–473.

HILL P., LUSTIG S., SAWATZKI V. (1998): Die Aminosaure Glutamin als Parameter zur Bestimmung des Alterungszustandes von Bieren. Mschr. Brauwissensch., **3/4**: 1–3.

LIEBICH H.M., FÖRST C. (1985): *N*-methylation and *N*,*N*-dimethylation of amino acids. An artifact production in the analysis of organic acids using diazomethane as derivatizing agent. J. Chromatogr., **338**: 33–40.

REDDY M.K., SRIBABU, C., SUNDARAM E.V. (1990): Kinetics and mechanism of oxidation of serine, threonine, arginine, aspartic acid and glutamic acid by *N*-bromoacetamide in alkaline medium. Indian J. Chem., Sect A., **29**: 61–62.

SCHÖNBERG A., MOUBACHER R. (1952): The Strecker degradation of α-amino acids. Chem. Rev. , **50**: 261–277.

SCHÖNBERG, A., MOUBACHER, R., MOSTAFA A. (1948): Degradation of α-amino acids to aldehydes and ketones by interaction with carbonyl compounds. J. Chem. Soc., **XX**: 176–182.

SHU C.-K., LAWRENCE B.M. (1995): 3-methyl-2(1H)-pyrazinones, the asparagine-specific Maillard products formed from asparagine and monosacharides. J. Agr. Food Chem., **43**: 779–781.

SRIVASTAVA S.P., MATHUR B.B.L. (1982): Oxidation of amino acids with persulphate and linearity correlations of free energies. Zh. Obshch. Khim.: **52**: 1740–1746.

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. Z. Lebensm. Unters. Forsch., **191**: 116–118.

Souhrn

RÖSSNER J., VELÍŠEK J., PUDIL F., DAVÍDEK J. (2001): **Produkty Streckerovy degradace asparagové a glutamové kyseliny a jejich amidů**. Czech. J. Food Sci., **19**: 41–45.

Asparagová a glutamová kyselina, asparagin a glutamin byly oxidovány peroxodisulfátem nebo glyoxalem. Netěkavé reakční produkty po derivatizaci a těkavé produkty po izolaci byly analyzovány metodou GC/FID a GC/MS. Bylo prokázáno, že degradační reakce studovaných aminokyselin jsou značně komplexní. V zásadě se aminokyseliny rozkládají přes odpovídající α-ketokyseliny na příslušné Streckerovy aldehydy (asparagová kyselina přes oxaloctovou na 3-oxopropionovou kyselinu, glutamová kyselina přes α-ketoglutarovou na 4-oxomáselnou kyselinu), které jsou nestálé a dekarboxylují na příslušné aldehydy. Asparagová kyselina rovněž eliminuje amoniak a poskytuje fumarovou kyselinu, zatímco glutamová kyselina cyklizuje na imin, pyroglutamovou kyselinu. Rekombinace při reakci vzniklých volných radikálů vede k dikarboxylovým kyselinám (ke kyselině jantarové z asparagové a ke kyselině jantarové, glutarové a adipové z kyseliny glutamové). Hlavními těkavými produkty rozkladu aminokyselin jsou vedle aldehydů nižší karboxylové kyseliny (octová z asparagové a propionová z glutamové kyseliny), které mohou částečně vznikat rovněž radikálovými reakcemi. Kvalitativně i kvantitativně poskytují více reakačních produktů oxidace aminokyselin peroxodisulfátem.

Klíčová slova: Streckerova degradace; Streckerovy aldehydy; aminokyseliny; glyoxal; peroxodisulfát sodný; asparagová kyselina; glutamová kyselina; asparagin; glutamin; radikály

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