New 3-Chloro-1,2-propanediol Derived Dihydroxypropylamines in Hydrolyzed Vegetable Proteins

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Reaction products of 3-chloro-1,2-propanediol (the main contaminant of protein hydrolysates produced by hydrochloric acid hydrolysis; used as food seasonings) with ammonia in model aqueous solutions were analyzed, and except for the already known compounds (glycerol, 3-amino-1,2-propanediol, 2-amino-1,3-propanediol) bis(2,3-dihydroxypropyl)amine and tris(2,3-dihydroxypropyl)amine were newly identified. Commercial seasonings, a soybean meal hydrolysate containing more than 400 mg kg^{-1} of 3-chloro-1,2-propanediol, and an alkaline-treated hydrolysate containing less than 1 mg kg^{-1} 3-chloro-1,2-propanediol were analyzed, and bis(2,3-dihydroxypropyl)amine (in concentrations of 6 and 3 mg kg^{-1}, respectively) and tris(2,3-dihydroxypropyl)amine (2 and 4 mg kg^{-1}, respectively) were newly identified as protein hydrolysates chemical constituents. Mass spectra of their silyl derivatives and ^{1}H and ^{13}C NMR spectra of these newly found compounds in foods are presented.

3-Chloro-1,2-propanediol (glycerol α-monochlorohydrin) has been identified as the main endogenous contaminant of protein hydrolysates (Davídek et al., 1981) that are commonly produced by hydrochloric acid hydrolysis of various proteinaceous vegetable materials (Prendergast, 1974; Weir, 1984). It has been shown that 3-chloro-1,2-propanediol levels in classical hydrolysates currently reach the amounts of several hundreds of milligrams per kilogram (Velíšek et al., 1991; Van Bergen et al., 1991), whereas the nowadays produced hydrolysates accepted by the supervisory bodies in many countries contain less than 10 or 1 mg kg^{-1} of 3-chloro-1,2-propanediol (Van Bergen et al., 1991). These relatively low levels of 3-chloro-1,2-propanediol can be achieved by certain special treatments of hydrolysates.

Protein hydrolysates contain as one of their main chemical constituents ammonia (ammonium salts), which represents approximately 10% of their total nitrogen content. It has been demonstrated that 3-chloro-1,2-propanediol reacts with ammonia under the formation of 3-amino-1,2-propanediol (Smith and Nilsson, 1943; Maeda et al., 1982) in aqueous solutions. 3-Amino-1,2-propanediol was also identified in commercially available hydrolyzed vegetable proteins and was present in concentrations amounting to about 30 mg kg^{-1} (Velíšek et al., 1991). In the industrial production of 3-amino-1,2-propanediol from 3-chloro-1,2-propanediol and ammonia, bis(2,3-dihydroxypropyl)amine was formed as a byproduct. This compound was separated into a mixture of optically active compounds and a mesoform by chromatography of its pentakis(trifluoroacetyl) derivatives on a silica gel column (Brynildsen et al., 1985). It can be used to introduce hydrophilic function in X-ray contrast media (for synthesis of iodinated 3-amino- and 3,5-diaminobenzylamine for X-ray diagnosis) (Hebký et al., 1976). It also reacts with epoxide-containing polymers, e.g., glycidyl methacrylate–methyl methacrylate copolymer under the formation of polymers useful in the building industry (Kita and Nakanishi, 1973). Gas chromatographic analysis of ammonia hydroxypropylation products also revealed the presence of tris(2,3-dihydroxypropyl)amine (Mokeeva et al., 1986).

The objective of our study was the evaluation of the reaction of 3-chloro-1,2-propanediol with ammonia in model solutions in more detail and identification of bis-(2,3-dihydroxypropyl)amine and tris(2,3-dihydroxypropyl)amine as new constituents of protein hydrolysates that are widely used as food seasonings and savory flavors.

MATERIALS AND METHODS

Chemicals. 3-Chloro-1,2-propanediol (Böeseken and Hermans, 1923) and glycidol (Rider and Hill, 1930) were synthesized. 3-Amino-1,2-propanediol and 2-amino-1,3-propanediol (serinol) were purchased from Aldrich-Chemie, D. All other chemicals were of analytical grade (purchased from Lachema, Brno, Czechoslovakia).

The protein hydrolysate samples were obtained from Vitana, Byšice, Czechoslovakia (soybean meal hydrolysate) and Haco AG, Gümligen, CH (alkaline treated hydrolysates).

Gas–Liquid Chromatography. GC analyses were performed on a Hewlett-Packard 5890A instrument equipped with a flame ionization detector and a SPB-1 fused silica capillary column (15 m × 0.53 mm, film thickness 1.5 μm). Split injection (1:10) was performed with the injector heated at 250 °C; the temperature was programmed from 80 to 250 °C at 5 °C min^{-1}, and the detector temperature was held at 300 °C. Nitrogen carrier gas flow rate was 5 mL min^{-1}.

Gas Chromatography–Mass Spectrometry. For the measurements of mass spectra a Jeol DX 303 mass spectrometer/data station 5000 was used. The gas chromatograph was fitted with a splitless injection port (vent opened after 1 min) and equipped with a SPB-5 fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The injector was held at 240 °C, and the column temperature was programmed from 65 to 290 °C at a rate of 8 °C min^{-1}. Helium carrier gas flow rate was 1.5 mL min^{-1}. Ionization was achieved by electron impact, the beam energy was set to 70 eV, and the source temperature was 280 °C.

Detection and quantitative analysis of 3-amino-1,2-propanediol, bis(2,3-dihydroxypropyl)amine, and tris(2,3-dihydroxypropyl)amine in hydrolysates were performed by selective-ion recording on a Hewlett-Packard 5971A quadrupole mass spec-

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trometer linked to a Hewlett-Packard Model 5890A gas chromatograph. The gas chromatograph was fitted with a splitless injection port (vent opened after 1.5 min) and equipped with a HP-5 fused silica capillary column (25 m × 0.2 mm, film thickness 0.33 μm). The injector was held at 240 °C, and the column temperature was programmed from 60 to 260 °C at a rate of 8 °C min^{-1}. Helium carrier gas flow rate was 1.5 mL min^{-1}. Ionization was achieved by electron impact, the beam energy was set to 70 eV, and the source temperature was adjusted to 290 °C. The following ions were monitored: m/z 228 for 3-amino-1,2-propanediol (6–15 min), m/z 248 for bis(2,3-dihydroxypropyl)amine (15–22 min), and m/z 466 for tris(2,3-dihydroxypropyl)amine (22–40 min), respectively.

NMR Spectrometry. The ^1H and ^{13}C NMR spectra were measured on a Bruker AM400 spectrometer (400.13 MHz for ^1H, 100.62 MHz for ^{13}C) with internal deuterium stabilization at 27 °C. Chemical shifts were referred to 2,2-dimethyl-2-silapentane-5-sulfonic acid (sodium salt) and transformed to TMS scale.

Model System. To 10-mL volumetric flasks containing 1.1 g (0.01 mol) of 3-chloro-1,2-propanediol was added aqueous ammonia (25% w/w) to make the molar ratio 3-chloro-1,2-propanediol/ammonia 1:1 and 1:10. The flasks were filled with water, stored at 20 and 85 °C for 8 h, and cooled to room temperature. An aliquot of 0.1 mL was then transferred to a 10-mL flask. To this was added 1 mL of methanolic solution of 1,4-butanediol (internal standard) containing 5 mg of the substance, and the resulting solution was evaporated to dryness under reduced pressure. The residue was derivatized by adding 0.5 mL of a reagent consisting of pyridine/hexamethyldisilazane/trifluoroacetic acid (10:9:1 v/v/v) for 15 min at 40 °C; 1-μL aliquots were analyzed by GC and GC–MS.

Twenty-five milliliters of the 1:1 85 °C reaction mixture was evaporated to dryness, and the individual amines were isolated by ion-exchange chromatography according to the procedure described below. The isolated compounds were analyzed by GC–MS and NMR spectrometry and used as standards for quantitative measurements.

Analysis of Amines in Hydrolysates. The weighed amount (about 10 g) of hydrolysate was evaporated to dryness under reduced pressure. The residue was extracted with two 5-mL portions and one 2-mL portion of methanol, the combined extracts were evaporated, and the resulting residue was dissolved in 2 mL of water. The solution was transferred onto the top of a 25 × 1 cm Dowex 50WX4 (0.037–0.074 mm, H^+) ion-exchanger column. The stepwise elution was performed with 100 mL of water and 500 mL of 0.1 M hydrochloric acid. The last 300 mL of the eluate was collected and evaporated to dryness, and the residue was silylated and analyzed by GC and GC–MS.

RESULTS AND DISCUSSION

The reaction of 3-chloro-1,2-propanediol with ammonia was used for the synthesis of 3-amino-1,2-propanediol (Smith and Nilsson, 1943; Maeda et al., 1982) and bis(2,3-dihydroxypropyl)amine (Brynildsen et al., 1985) under conditions (temperature, pH) that significantly differ from those encountered during the production and storage of protein hydrolysates. It was also demonstrated that 3-amino-1,2-propanediol can arise in solutions of pH 7 stored at 20 °C and even in protein hydrolysates with a pH range 5–7 (Velišek et al., 1991b).

Reactions of 3-chloro-1,2-propanediol with ammonia in model solutions have been carried out to identify all of the main products of 3-chloro-1,2-propanediol/ammonia reactions and evaluate food protein hydrolysates for the presence of these products.

Reactions in Model Systems. Reactions of 3-chloro-1,2-propanediol with ammonia were carried out at either 20 or 85 °C. The temperature of 20 °C simulated the temperature under which hydrolysates are ordinarily stored; the temperature of 85 °C is used during the alkaline treatment of hydrolysates during which 3-chloro-1,2-propanediol is decomposed. In hydrolysates the molar ratio of 3-chloro-1,2-propanediol to ammonia (ammonia salts) is about 1:10.

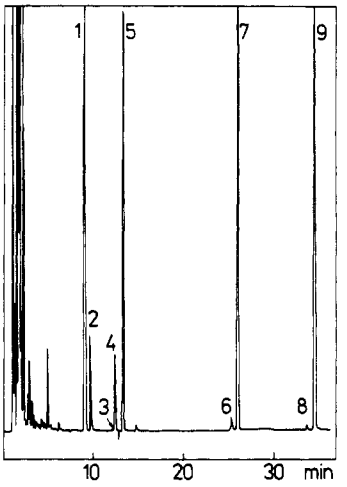
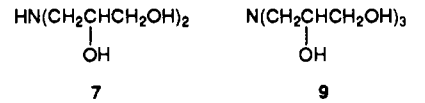


Figure 1. Separation of reaction products of 3-chloro-1,2-propanediol with ammonia: molar ratio 1:1, 85 °C, 8 h. 1, 1,4-Butanediol (internal standard); 2, 3-chloro-1,2-propanediol; 3, 2-amino-1,3-propanediol; 4, glycerol; 5, 3-amino-1,2-propanediol; 6, isomer of 7 (see text); 7, bis(2,3-dihydroxypropyl)amine; 8, isomer of 9 (see text); 9, tris(2,3-dihydroxypropyl)amine.

3-Amino-1,2-propanediol and glycerol were already identified as the main reaction products of 3-chloro-1,2-propanediol decomposition in ammonia buffers (Velišek et al., 1991b). 2-Amino-1,3-propanediol (serinol) was identified as a minor product present in concentrations amounting to about $^1/_{40}$ of those of 3-amino-1,2-propanediol. Reaction of 3-chloro-1,2-propanediol with ammonia at 85 °C (molar ratio 1:1) is illustrated in Figure 1. As can be seen, the amount of 3-amino-1,2-propanediol is about 5 times higher than that of 3-chloro-1,2-propanediol; the amount of glycerol is relatively low but much higher than that of 2-amino-1,3-propanediol. Apart from these already known compounds, four other unknown peaks can be seen in the chromatogram. Compounds corresponding to peaks 6–9 were separated from the reaction mixture on a cation-exchanger column and the isolated pure compounds subjected to MS and NMR spectrometric analysis. Mass spectra of the individual silylated compounds together with their retention indices are summarized in Table I. Only the most prominent and diagnostically important peaks are included. NMR spectral data of compounds 7 and 9 are summarized in Table II.

On the basis of the above presented spectral data, the compounds corresponding to peaks 7 and 9 (Figure 1) were identified as bis(2,3-dihydroxypropyl)amine and tris(2,3-dihydroxypropyl)amine, respectively:



7 9

Due to the presence of two asymmetric carbon atoms in the molecule of bis(2,3-dihydroxypropyl)amine, two optically active stereoisomers (*R,R* and *S,S*) and a mesoform (*R,S* = *S,R*) in a ratio of 1:1 are present. These two groups of compounds were separated into two peaks by gas chromatography using a capillary column with a HP-5 phenylmethylsiloxane stationary phase (Δt = 0.04 min). Symmetrical tris(2,3-dihydroxypropyl)amine is a mixture of four stereoisomers (*R,R,R*; *S,S,S*; *R,R,S*; and *S,S,R*). A mixture of the *R,R,R* and *S,S,S* isomers eluted first followed by the mixture of the other two isomers (Δt = 0.07 min). The ratio of these two peaks was 1:3 in accordance with

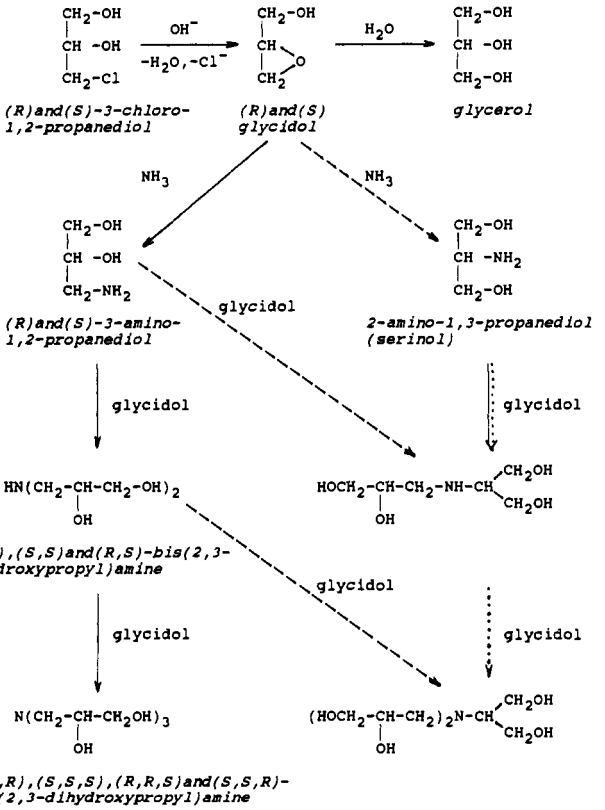
Table I. Mass Spectra of Silylated Compounds 6–9

compound (see Figure 1)	retention index (SPB-1)	mass spectrum, m/z (%)	
6 (tetrakis-TMS)	1810	$[M - CH_3]^+$	438 (8.2)
		$[M - CH_2OTMS]^+$	350 (90.1)
		$[M - CH_3 - HOTMS]^+$	348 (<5)
		$[M - CH_2OTMS - CHOTMS]^+$	248 (33.3)
		$[M - CH_2OTMS - CHOTMS - OTMS]^+$	146 (73.4)
7 (tetrakis-TMS)	1840	$[M - CH_3]^+$	438 (27.8)
		$[M - HOTMS]^+$	363 (<5)
		$[M - CH_2OTMS]^+$	350 (8.8)
		$[M - CH_3 - HOTMS]^+$	348 (5.9)
		$[M - CH_2OTMS - CHOTMS]^+$	248 (100.0)
		$[M - CH_2OTMS - CHOTMS - OTMS]^+$	146 (6.7)
8 (hexakis-TMS)	2230	$[M - CH_3]^+$	656 (12.5)
		$[M - CH_2OTMS]^+$	568 (35.5)
		$[M - CH_2OTMS - CHOTMS]^+$	466 (100.0)
		$[M - CH_2OTMS - (CHOTMS)_3]^+$	262 (67.8)
9 (hexakis-TMS)	2275	$[M - CH_3]^+$	656 (16.8)
		$[M - CH_2OTMS]^+$	568 (<5)
		$[M - CH_2OTMS - CHOTMS]^+$	466 (100.0)
		$[M - CH_2OTMS - (CHOTMS)_3]^+$	262 (37.4)

Table II. ^1H and ^{13}C NMR Spectra of Compounds 7 and 9

compound	position	chemical shift, ppm: ^1H NMR	chemical shift, ppm: ^{13}C NMR
1	CH_2	2.8	51.7
	CH_2OH	3.6	64.8
	CH(OH)	3.9	70.6
9	CH_2	3.3–3.75	57.57–59.62
	CH_2OH		64.08–64.53
	CH(OH)	4.1–4.28	66.09–67.54

Scheme I. Reaction of 3-Chloro-1,2-propanediol with Ammonia



the theory. Because of this phenomenon it was not possible to identify the individual signals in NMR spectra of compounds 9 (Table II).

The influence of reaction conditions on the yields of the individual main reaction products of 3-chloro-1,2-propanediol with ammonia is documented in Figure 2.

It was not possible to identify the minor compounds

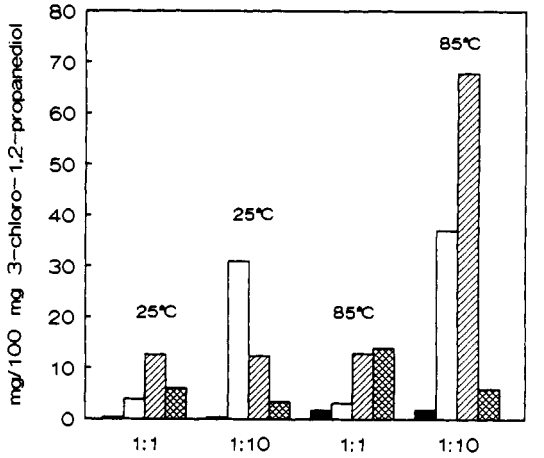
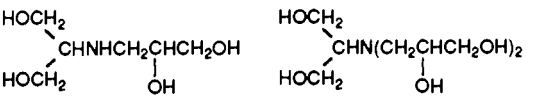


Figure 2. Formation of glycerol and amines in 3-chloro-1,2-propanediol/ammonia model solutions. (Solid bars) glycerol; (open bars) 3-amino-1,2-propanediol; (slashed bars) bis(2,3-dihydroxypropyl)amine; (cross-hatched bars) tris(2,3-dihydroxypropyl)amine.

corresponding to peaks 6 and 8 in Figure 1 by NMR spectrometry. The area of peaks 6 and 8 was about 40 and 110 times smaller than that of peaks 7 and 9, respectively. In the chromatogram of reaction mixture of glycidol with serinol the same two single prominent peaks appeared at retention times of peaks 6 and 8. Due to their mass spectra, chromatographic behavior, and amount, compounds 6 and 8 were tentatively identified as isomers of bis(2,3-dihydroxypropyl)amine and tris(2,3-dihydroxypropyl)amine, respectively, being derived either from 2-amino-1,3-propanediol (serinol) or from 3-amino-1,2-propanediol and glycidol. The most probable structures of these compounds are



On the basis of the above results as well as previously published data, we can conclude that the main reactions of 3-chloro-1,2-propanediol taking place in aqueous solutions of ammonia can be schematically outlined as indicated in Scheme I.

A compound similar in structure to our bis(2,3-dihydroxypropyl)amine (an analogue of tetraglycerol) was formerly identified as the reaction product of 1,3-dichloro-

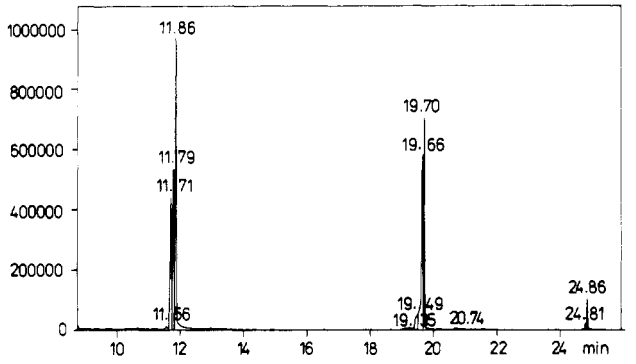
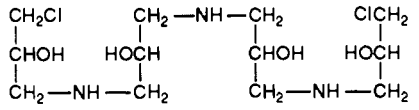
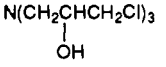


Figure 3. Analysis of 3-amino-1,2-propanediol, bis(2,3-dihydroxypropyl)amine, and tris(2,3-dihydroxypropyl)amine in soybean meal hydrolysate by single ion recording GC–MS.

2-propanol with ammonia (Claus, 1873). A compound similar in structure to our tris(2,3-dihydroxypropyl)amine is a known reaction product of epichlorohydrin with ammonia [tris(3-chloro-2-hydroxypropyl)amine] (McKelvey et al., 1960):



1,3-dichloro-2-propanol/ammonia product



epichlorohydrin/ammonia product

With the exception of bis(2,3-dihydroxypropyl)amine (an N-containing analogue of diglycerol), no other linear or cyclic nitrogen-containing analogue of polyglycerols was found in our model systems. We cannot exclude, however, the presence of small amounts of either polyglycerols (retention times of cyclic diglycerols lie in the region of 16.8–17.7 min, those of linear diglycerols are 22.6–23.9 min, and those of linear triglycerols are 31.2–33.0 min) or their amino analogues that could arise in very small quantities as well (Figure 1).

New Amines in Hydrolysates. To identify the reaction products of 3-chloro-1,2-propanediol with ammonia in protein hydrolysates, fractions of the basic constituents of two different samples of hydrolysates were separated, derivatized, and analyzed by GC and GC–MS under the conditions used for the analysis of model solutions of 3-chloro-1,2-propanediol with ammonia. For detection and quantitative analysis of bis(2,3-dihydroxypropyl)amine and tris(2,3-dihydroxypropyl)amine, the major characteristic $[M-(CH_2-OTMS+CH-OTMS)]^+$ ions were monitored in the electron impact by single ion recording (Figure 3). A similar record was obtained by analyzing the alkaline-treated hydrolysate. As can be seen, only 3-amino-1,2-propanediol (retention time 11.8 min), bis(2,3-dihydroxypropyl)amine (19.7 min), and tris(2,3-dihydroxypropyl)amine (24.8 min) were detected. The results of quantitative measurements are summarized in Table III. Both of the newly identified amines (amino alcohols) occurred in concentrations comparable with that

Table III. Content of 3-Amino-1,2-propanediol, Bis(2,3-dihydroxypropyl)amine, and Tris(2,3-dihydroxypropyl)amine in Protein Hydrolysates

compound	content, mg kg^{-1} soybean meal hydrolysate	content, mg kg^{-1} alkaline-treated hydrolysate
3-amino-1,2-propanediol	5.6	11.3
bis(2,3-dihydroxypropyl)amine	6.0	2.9
tris(2,3-dihydroxypropyl)amine	1.8	3.9

of 3-amino-1,2-propanediol. It seems that there is almost no difference between the levels of the amines that occur in classical hydrolysates and in new chemically treated hydrolysates. 3-Chloro-1,2-propanediol occurs in hydrolysates in much higher concentration (several hundreds of milligrams per kilogram), and it is almost totally destroyed during the alkaline treatment. We suppose, therefore, that its reaction with ammonia is not the only reaction taking place in hydrolysates. Many other reaction products of the destroyed 3-chloro-1,2-propanediol (or glycidol) with chemical constituents of protein hydrolysates might arise in these commodities.

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Registry No. (*R,R*)-7, 141901-42-0; (*S,S*)-7, 141901-44-2; *meso*-7, 104411-92-9; (*R,R,R*)-9, 141901-43-1; (*S,S,S*)-9, 141901-45-3; (*S,S,R*)-9, 141901-46-4; (*R,R,S*)-9, 141901-47-5; (*R*)-$OHCH_2CH(OH)CH_2Cl$, 57090-45-6; (*S*)-$OHCH_2CH(OH)CH_2Cl$, 60827-45-4; (*R*)-$OHCH_2CH(OH)CH_2NH_2$, 66211-46-9; (*S*)-$OHCH_2CH(OH)CH_2NH_2$, 61278-21-5; NH_4OH, 1336-21-6; $OHCH_2CH(NH_2)CH_2OH$, 534-03-2.