DERIVATIVES OF 6-AMINO-6-N,N-BIS(CARBOXYMETHYL)-6-DEOXY--D-GALACTOSE

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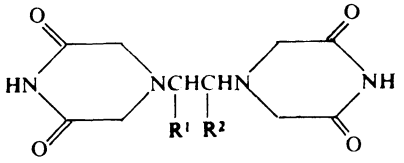
Received October 26th, 1983

On reaction of 6-amino-6-deoxy-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (*II*) with sodium chloroacetate 6-amino-6-N,N-bis(carboxymethyl)-6-deoxy-1,2;3,4-di-O-isopropylidene--α-D-galactopyranose (*III*) was obtained. Melting of compound *III* with urea or its heating with a mixture of formamide and urea causes cyclocondensation of the N,N-bis(carboxymethyl)group under formation of 6-deoxy-1,2;3,4-di-O-isopropylidene-6-C-(3,5-dioxopiperazin-1-yl)-α-D-galactopyranose (*IX*). Some derivatives of compound *III* are also described in the paper; the structures of all the products have been confirmed by NMR spectra.

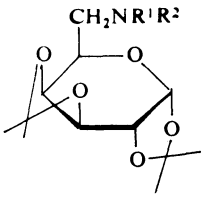
Creighton and coworkers[1] have described that reaction of ethylenediaminetetraacetic acid with formamide gives rise to 1,2-bis(3,5-dioxopiperazin-1-yl)ethane (*I*, $R^1 = R^2 = H$), a substance with a distinct antitumour activity. Its analogue, 1,2-bis(3,5-dioxopiperazin-1-yl)propane ($R^1 = CH_3$, $R^2 = H$), is used in clinical practice under the commercial name Razoxane[2]. Tests with the cyclopropane analogue of compound *I* (R^1 and R^2 form the methylene group), using a Syrian hamster lung adenocarcinoma have shown that the biological effect is closely connected with the spatial arrangement of the dioxopiperazine groups. While the *cis*-isomer inhibited lung metastases, the *trans*-isomer increased the number of metastatic nodules in the lung[3]. The dependence of the biological effect of compounds with 3,5-dioxopiperazine cycle on the steric arrangement is also described in a further paper[4] and therefore the idea of the preparation and the biological testing of substances containing 3,5-dioxopiperazine groups on the saccharide skeleton becomes obvious. In this case the presence of the hydroxyl groups should affect the solubility of these substances in water positively; the above mentioned substances lack this property.

In this paper we present the results of experiments aimed at the preparation of a saccharide with a 3,5-dioxopiperazine group on its exocyclic part. The same as in papers[1,3,4] our own procedure also required the construction of the 3,5-dioxopiperazine group by cyclocondensation of the bis(carboxymethyl)amino group

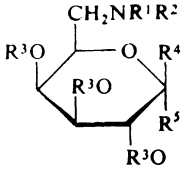
or bis-(methoxycarbonylmethyl)amino group. Substances with these groups are prepared only exceptionally[5] by substitution reactions in which the nucleophilic group is bis(carboxymethyl)amine. Experiments aimed at the introduction of the bis-(methoxycarbonylmethyl)amino group into the saccharide molecule by substitution of the tosyloxy group or by the cleavage of the oxirane ring with bis(methoxycarbonylmethyl)amine were unsuccessful[6]. Unlike this, procedures based on carboxymethylation of amines with sodium chloroacetate[7,8] or sodium cyanide and formaldehyde[9-11] generally afford satisfactory yields of bis(carboxymethyl)amino derivatives. Therefore we chose 6-amino-6-deoxy-1,2; 3,4-O-di-isopropylidene-α-D-galactopyranose[12] (*II*) as our starting material.



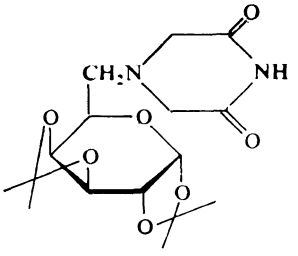
I



II, $R^1 = R^2 = H$
III, $R^1 = R^2 = -CH_2COOH$
IV, $R^1 = R^2 = -CH_2COOCH_3$



V, $R^1 = R^2 = -CH_2COOH$, $R^3 = H$, $R^4 = H(OH)$, $R^5 = OH(H)$
VI, $R^1 = R^2 = -CH_2COOCH_3$, $R^3 = R^4 = H$, $R^5 = OCH_3$
VII, $R^1 = R^2 = -CH_2COOCH_3$, $R^3 = CH_3CO-$, $R^4 = H$, $R^5 = OCH_3$
VIII, $R^1 = R^2 = -CH_2COOCH_3$, $R^3 = CH_3CO-$, $R^4 = OCH_3$, $R^5 = H$



IX

We prepared 6-amino-6-N,N-bis(carboxymethyl)-6-deoxy-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (*III*) in a 70% yield on reaction of aminogalactose *II* with sodium chloroacetate in aqueous alkaline medium. The presence of two carboxyl groups in compound *III* was confirmed by potentiometric titration. Its ^1H and ^{13}C NMR spectrum and the IR spectrum (bands at 1 740 cm^{-1} and 1 630 cm^{-1}) also confirm the structure proposed for compound *III*.

Among procedures from literature[1,13,14] for cyclocondensation of bis(carboxymethyl)amino group to 3,5-dioxopiperazine ring the procedure used in ref.[3] seems the mildest. According to it the carboxymethyl groups are first esterified and the bis(methoxycarbonylmethyl)amino group is condensed with formamide and sodium hydride. Since isopropylidene groups are present in compound *III* we tried such esterification methods for it which would not deacetalize them (esterification with diazomethane, dimethyl sulfate); only the reaction of the silver salt of acid *III* with iodomethane gave diester *IV* in low yield. The structure of compound *IV* follows from its ^1H NMR spectrum. The spin-spin coupling values of protons on the pyranose ring, $J_{1,2}$ = 4·9 Hz, $J_{2,3}$ = 2·4 Hz, $J_{3,4}$ = 7·8 Hz and $J_{4,5}$ = 2 Hz exclude the usual $^4C_1$ conformation and indicate that compound *IV* assumes the twist-boat conformation. Cone and Hough[15] came to an analogous conclusion in other derivatives of 1,2;3,4-di-O-isopropylidene-α-D-galactopyranose earlier, and it may be also applied to compound *III* and *IX*.

Di-isopropylidene derivative *III* was deacetalized with a cation exchanger. From the ^1H NMR and mainly ^{13}C NMR spectrum of the product it followed that it was a mixture of both anomers of 6-amino-6-N,N-bis(carboxymethyl)-6-deoxy-D-galactopyranose (*V*) in which the α-anomer predominated. We treated this product with boiling methanolic hydrogen chloride without further purification. According to chromatography on a thin layer two substances with close R_F values predominated in the reaction mixture. Chromatography on silica gel and crystallization of chromatographic fractions enriched in the more mobile component gave methyl 6-amino-6-deoxy-6-N,N-bis(methoxycarbonylmethyl)-α-D-galactopyranoside (*VI*). The structure of compound *VI* follows from its ^1H NMR spectrum, and its anomeric configuration from the coupling constant value $J_{1,2}$ = 2·5 Hz and the specific rotation. The mother liquors after crystallization of compound *VI* and the remaining chromatographic fractions were acetylated with acetic anhydride and pyridine. Chromatography on silica gel afforded a syrupy methyl 2,3,4-tri-O-acetyl-6-amino-6-deoxy-6-N,N-bis(methoxycarbonylmethyl)-α-D-galactopyranoside (*VII*) and its β-anomer *VIII*. The coupling constant values of the protons on the pyranose ring indicate that compounds *VII* and *VIII* assume the usual $^4C_1$ conformation, and the other parameters of the ^1H NMR spectrum confirm the proposed structure.

We tried to carry out the cyclocondensation of the bis(methoxycarbonylmethyl)-amino derivatives *IV* and *VII* according to ref.[3]. We were unable to obtain the required 3,5-dioxopiperazine derivative, probably because compounds *IV* and *VII* are

unstable and decompose on standing at room temperature. On the other hand, melting of bis(carboxymethyl)amino derivative *III* with urea or its heating with a mixture of formamide and urea cause the closure of the 3,5-dioxopiperazine ring. The reaction product which we isolated by chromatography displays in its IR spectrum a band at 3 380 cm^{-1} (N—H) and 1 730 cm^{-1} (C=O). Its ^1H NMR spectrum shows that it has the structure of 6-deoxy-1,2;3,4-di-O-isopropylidene-6-C-(3,5-dioxopiperazin-1-yl)-α-D-galactopyranose (*IX*) with a twist-boat conformation of the pyranose ring.

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. Optical rotation values were measured on an Opton polarimeter in a 2 dm cell, at *c* 1 ± 0·3 concentration and 20°C. Thin-layer chromatography was carried out on silica gel G according to Stahl (Merck, Darmstadt), 10—40 μm particle size; layer thickness 0·1 mm, plate dimensions 25 × 75 mm. Developing systems: chloroform–2-propanol–conc. NH_4OH–water 10 : 15 : 1·5 : 1·5 (system A), benzene–ethanol 100 : 5 (system B) and diethyl ether–light petroleum 3 : 1 (system C). Detection was carried out by spraying the plates with a 1% cerium-IV sulfate solution in 10% sulfuric acid and heating. Preparative chromatographies were carried out on silica gel columns (100—160 μm particle size) (Lachema, Brno). Samples for analysis were dried at room temperature and 10 Pa pressure for 8—10 h. The solvents were evaporated on a rotary evaporator in a vacuum (water pump) and at bath temperatures not exceeding 50°C. The potentiometric titration was carried out on a Radiometer RTS 622 (Copenhagen) instrument. The IR spectra were measured on a Perkin–Elmer 325 spectrometer. The ^1H NMR spectra were measured on a Varian XL-100 and Tesla BS 567 spectrometer at 31°C, using tetramethylsilane as a standard for deuteriochloroform and hexadeuteriodimethyl sulfoxide and 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) for deuterium oxide. The chemical shift values (in ppm) and coupling constants (in Hz) were obtained by first order analysis. The ^{13}C NMR spectra were measured at 15·04 MHz on a JEOL FX60 instrument in deuterium oxide at 25°C.

6-Amino-6-N,N-bis(carboxymethyl)-6-deoxy-1,2;3,4-di-O-isopropylidene--α-D-galactopyranose (*III*)

An aqueous NaOH solution (1M, 65 ml), 2 drops of a 1% phenolphthalein solution in ethanol and 7·4 g (28·5 mmol) of aminogalactose[12] *II* (syrup, $[\alpha]_D$ −43°, chloroform) dissolved in 25 ml of water were added to a solution of 6·0 g (63·5 mmol) of chloroacetic acid in 25 ml of water and the mixture was heated under stirring at 60°C. After decolorization 1M-NaOH was added in 1—2 ml doses. At the beginning the hydroxide was consumed rapidly (30 ml in 4 h), and after 9 h 48 ml of 1M-NaOH were consumed. Another 10 ml of the 1M-NaOH solution were then added to the mixture which was then allowed to stand at room temperature overnight. According to TLC (system A) the reaction mixture did not contain aminogalactose *II* (R_F 0·85), the dominant product was a substance with R_F 0·15 and the minor product had R_F 0·5. The mixture was neutralized to pH 7 with hydrochloric acid (consumption 8 ml of 1M-HCl) and filtered with charcoal. The filtrate was concentrated to 40 ml, the concentrate acidified with dilute hydrochloric acid (1 : 1) to pH 2. Substance *III* separated which was filtered off under suction after several hours' standing in a refrigerator and washed with water. The filtrate was neutralized immediately with dilute ammonia (1 : 1). Compound *III* was suspended in 40 ml of water and ammonium

hydroxide (1 : 1) was added to it under stirring until all compound *III* was dissolved. On acidification of this solution to pH 2 compound *III* precipitated again. Filtration under suction, washing with water and drying gave 5·6 g (52%) of compound *III*, m.p. 231–233°C (decomposition), $[\alpha]_D$ −70·0° (pyridine). For $C_{16}H_{25}NO_9$ (375·4) calculated: 51·18% C, 6·71% H, 3·75% N; found: 51·05% C, 6·70% H, 3·71% N. Titration equivalent: 19·00 mg (0·0561 mmol) of compound *III* consumed 0·1 mmol of sodium hydroxide. At pH 6 the titration curve had an inflexion point corresponding to a consumption of 1 ml of 0·05 mmol of hydroxide. IR spectrum (nujol): bands at 1 740 cm^{-1} and 1 630 cm^{-1} (—COOH). ^1H NMR spectrum (hexadeuteriodimethyl sulfoxide): 5·33 (1 H, d, $J_{1,2}$ = 5 Hz, H-1), 4·0–4·55 (3 H, m, H-2, H-3, H-4), 4·78 (1 H, m, H-5), 2·76 (2 H, d, J = 6·5 Hz, H-6), 3·38 (4 H, s, CH_2CO), 1·21 (6 H, s, CH_3), 1·28 (3 H, s, CH_3), 1·39 (3 H, s, CH_3). ^{13}C NMR spectrum (^2H_2O): 96·7 $C_{(1)}$, 65·4, 70·4, 70·8, 72·4 $C_{(2)}$, $C_{(3)}$, $C_{(4)}$, $C_{(5)}$, 54·8 $C_{(6)}$, 59·8 (2 C), CH_2CO), 174·4 (2 C) COOH, 109·2 and 109·4 —O—C—O, 24·7, 25·3 and 26·4 (2 C) CH_3.

The alkalized filtrates after suction off of compound *III* were combined and evaporated and the residue was chromatographed on a column of silica gel (50 g) in system A. In addition to the by-products 2·04 g of compound *III* were eluted. After addition of a small amount of water and hydrochloric acid (to pH 2) it crystallized out, m.p. 232–233°C (decomp.). The overall yield of compound *III* was 71·5%.

6-Amino-6-deoxy-1,2;3,4-di-O-isopropylidene-6-N,N-bis(methoxycarbonylmethyl)-α-D--galactopyranose (*IV*)

Acid *III* (3·4 g, 9 mmol) was stirred with 85 ml of N,N-dimethylformamide for 1 h, until all the substance passed into the solution. The flask was then wrapped in an aluminum foil and 2·7 g (11·6 mmol) of silver oxide were added to the solution. The mixture was stirred at room temperature for 10 h. The next day 3·5 ml (7·9 g, 56 mmol) of iodomethane were added and the mixture was stirred again at room temperature for another 10 h. The inorganic material was filtered off and the filtrate evaporated. The residue was extracted with acetone and acetone evaporated. The residue was syrupy and it was chromatographed on a column of silica gel (100 g). Elution with benzene–methanol 100 : 2 gave 1·01 g (2·5 mmol, 28%) of diester *IV* as a syrup, pure according to TLC in system B, $[\alpha]_D$ −50·5° (chloroform). For $C_{18}H_{29}NO_9$ (403·4) calculated: 53·59% C, 7·24% H, 3·47% N; found: 53·29% C, 7·04% H, 3·27% N. ^1H NMR spectrum (deuteriochloroform): 5·53 (1 H, d, $J_{1,2}$ = 4·9 Hz, H-1), 4·33 (1 H, dd, $J_{1,2}$ = 4·9 Hz, $J_{2,3}$ = 2·4 Hz, H-2), 4·62 (1 H, dd, $J_{2,3}$ = 2·4 Hz, $J_{3,4}$ = 7·8 Hz, H-3), 4·30 (1 H, dd, $J_{4,3}$ = 7·8, $J_{4,5}$ = 2 Hz, H-4), 3·95 (1 H, m, H-5), 3·03 (2 H, m, ^2J = 15 Hz, H-6), 3·65 (4 H, s, CH_2CO), 3·70 (6 H, s, CH_3O), 1·29 (6 H, s, CH_3), 1·39 (3 H, s, CH_3) and 1·48 (3 H, s, CH_3).

6-Amino-6-N,N-bis(carboxymethyl)-6-deoxy-D-galactose (*V*)

A solution of 4·2 g (11·2 mmol) of compound *III* in 30 ml of water was heated at 65°C and under stirring for 2 h in the presence of 15 ml of Dowex 50 (H^+). According to TLC (system A) the mixture did not contain compound *III* and the product remained on the start. The ion exchanger was filtered off and washed with water until 50 ml of filtrate no longer gave a weighable residue. The filtrate (300 ml) was evaporated to a syrup, 3·5 g (100%), $[\alpha]_D$ +42·0° (water). ^1H NMR spectrum (deuterium oxide): 4·05 (4 H, s, CH_2CO), other signals were in the 3·40–4·20 and 4·35 to 5·25 regions. ^{13}C NMR spectrum (deuterium oxide): 93·1, $^1J_{CH}$ = 171·6 Hz, $C_{(1\alpha)}$, 97·1, $^1J_{CH}$ = = 161·1 Hz, $C_{(1\beta)}$, 65·7, 68·7, 69·6, 70·5, 70·6, 70·9, 72·2, 73·1 $C_{(2)}$, $C_{(3)}$, $C_{(4)}$, $C_{(5)}$, 56·9 and 57·2 $C_{(6)}$, 58·0 (2 C) CH_2CO, 169·6 (2 C) COOH.

Methyl 6-Amino-6-deoxy-6-N,N-bis(methoxycarbonylmethyl)-α-D-galactopyranoside (*VI*)

A solution of 3·5 g (11·8 mmol) of compound *V* in 30 ml of 10% (w/w) methanolic hydrogen chloride was refluxed for 10 h and then allowed to stand overnight at room temperature. According to TLC (system A) the reaction mixture contained two compounds of R_F about 0·6 and 0·55 and a third substance near the start. The mixture was evaporated and then reevaporated with three 25 ml doses of toluene. Chromatography of the residue on a silica gel column (100 g) in system A gave two fractions: one weighing 1·0 g, containing a mixture of compounds of R_F 0·6 and 0·55 in which the compound of R_F 0·6 predominated, and another weighing 1·01 g in which the compound of R_F 0·55 predominated. Double crystallization of the first fraction from methanol gave a pure product, *VI*, R_F 0·6, yield 0·48 g (12%), m.p. 134–135°C, $[\alpha]_D$ +96·0° (water). For $C_{13}H_{23}NO_9$ (337·3) calculated: 46·29% C, 6·87% H, 4·15% N; found: 46·01% C, 7·01% H, 3·95% N. ^{1}H NMR (deuterium oxide): 4·80 (1 H, d, $J_{1,2}$ = 2·5 Hz, H-1), 3·65–4·05 (4 H, m, H-2, H-3, H-4, H-5), 2·97 (2 H, d, J = 6·5 Hz, H-6), 3·63 (4 H, s, CH_2CO), 3·73 (6 H, s, CH_3OOC) and 3·40 (3 H, s, CH_3O).

The mother liquor after crystallization of compound *VI* was combined with the fraction weighing 1·01 g (where the compound with R_F 0·55 prevailed), evaporated and the residue dissolved in 10 ml of pyridine and 2 ml of acetic anhydride. The mixture was allowed to stand overnight at room temperature. It was then decomposed by addition of 2 ml of water and then evaporated to dryness. The residue was evaporated twice with toluene (50 ml portions). The syrupy residue (1·5 g) contained according to TLC (system C) two substances with R_F 0·4 and 0·3. Its chromatography on a column of silica gel (70 g) gave 0·62 g (11%) of the α-anomer *VII*· 0·55 g (10%) of a mixture of *VII* and *VIII* and 0·3 g (6%) of pure β-anomer *VIII* (with R_F 0·3)·

Compound *VII*, syrup, $[\alpha]_D$ +87·4° (chloroform), for $C_{19}H_{29}NO_{12}$ (463·4) calculated: 49·24% C, 6·03% H, 3·02% N; found: 49·39% C, 6·23% H, 3·24% N. ^{1}H NMR spectrum (deuteriochloroform): 4·93–5·48 (4 H, m, H-1, H-2, H-3, H-4), 4·20 (1 H, m, H-5), 2·90 (2 H, m, ^2J = 15 Hz), 3·65 (4 H, broad singlet, CH_2CO), 3·75 (6 H, s, CH_3OOC), 3·47 (3 H, s, CH_3O), 2·0, 2·12 and 2·18 (9 H, s, CH_3CO).

Compound *VIII*, syrup, $[\alpha]_D$ −5·2° (chloroform). For $C_{19}H_{29}NO_{12}$ (463·4) calculated, 49·24% C, 6·03% H, 3·02% N; found: 49·40% C, 6·01% H, 3·00% N. ^{1}H NMR spectrum (deute: riochloroform): 4·43 (1 H, d, $J_{1,2}$ = 7 Hz, H-1), 5·22 (1 H, dd, $J_{2,1}$ = 7 Hz, $J_{2,3}$ = 10·5 Hz, H-2), 5·03 (1 H, dd, $J_{3,2}$ = 10·5, $J_{3,4}$ = 3·5 Hz, H-3), 5·42 (1 H, dd, $J_{4,3}$ = 3·5 Hz, $J_{4,5}$ = 1 Hz, H-4), 3·92 (1 H,. m, H-5), 2·94 (2 H, m, ^2J = 15 Hz, H-6), 3·64 (4 H, m, CH_2CO), 3·73 (6 H, s, CH_3OOC), 3·51 (3 H, s, CH_3O), 1·96, 2·05 and 2·13 (9 H, s, CH_3CO).

6-Deoxy-1,2;3,4-di-O-isopropylidene-6-C-(3,5-dioxopiperazin-1-yl)-α-D-galactopyranose (*IX*)

a) Compound *III* (3·75 g, 10 mmol) was ground with 1·0 g of urea and heated in a flask in a bath of 165°C temperature for 30 min; gases escaped from the reaction mixture. According to TLC (system B) the reaction mixture contained compound *IX* of R_F 0·45 and a small amount of a substance with a slightly lower R_F value, as well as substances on the start. According to TLC in system A compound *IX* had R_F about 0·9. Several additional substances with R_F values about 0·3 were also present, but none of them had an R_F value identical with that of compound *III*. The dark melt, only partly soluble in system B, was transferred onto a column of silica gel (50 g) and chromatographed with system B. A syrup was obtained (2·2 g) which according to TLC in system B contained compound *IX* contaminated with a substance of a slightly lower R_F value. Elution of the column with system A fractions were obtained, of which each contained several substances with R_F from 0·05 to 0·3; these fractions were not further analysed. The syrup (2·2 g) luted with system B was rechromatographed on 50 g of silica gel with benzene–ethanol 100 : 1.

Yield, 1·53 g (43%) of chromatographically pure compound *IX* (in system B) and 300 mg of this compound contaminated with a compound with a lower R_F value. On standing at room temperature compound *IX* decomposes (giving several substances with lower R_F values in system B). In a chromatographically pure state compound *IX* had specific rotation $[\alpha]_D$ — 50·5° (chloroform). For $C_{16}H_{24}N_2O_7$ (356·4) calculated: 53·92% C, 6·78% H, 7·86% N; found: 53·72% C, 6·66% H, 7·92% N. IR spectrum (chloroform): 3 380 cm^{-1} (N—H), 1 730 cm^{-1} (C=O). ^1H NMR spectrum (deuteriochloroform): 5·5 (1 H, d, $J_{1,2}$ = 5 Hz, H-1), 4·30 (1 H, dd, $J_{2,1}$ = 5 Hz, $J_{2,3}$ = 2·5 Hz, H-2). 4·56 (1 H, dd, $J_{3,2}$ = 2·5 Hz, $J_{3,4}$ = 8 Hz, H-3), 4·15 (1 H, dd, $J_{4,3}$ = 8 Hz, $J_{4,5}$ = 1·5 Hz, H-4), 3·90 (1 H, m, H-5), 2·80 (2 H, m, H-6), 3·47 (4 H, s, CH_2CO), 1·33 (6 H, s, CH_3), 1·44 (3 H, s, CH_3) and 1·54 (3 H, s, CH_3, 8·4 (1 H, s, NH).

b) A mixture of 2·0 g of acid *III*, 0·5 g of urea and 20 ml of formamide was heated at 165°C (bath temperature) for 1 h and volatile material was then evaporated at 8 Pa. The residue was chromatographed on a silica gel column (60 g). Yield, 0·61 g of compound *IX* and 0·4 g of compound *IX* contaminated with a substance with a slightly lower R_F value.

c) Sodium hydride (1·2 g; in the form of a 60% suspension in mineral oil) was added to 20 ml of anhydrous 1,2-dimethoxyethane and the mixture was heated at 90°C for 10 min. Then a mixture of 0·9 g of diester *IV*, 0·35 ml of formamide and 20 ml of 1,2-dimethoxyethane was added dropwise and the mixture refluxed for 2 h. TLC in system B showed the absence of compound *IX*. The mixture was evaporated, 70 ml of ether and 20 ml of icy water were added and the layers separated. Neither in the ethereal nor in the aqueous layer (acidified to pH 2) could compound *IX* be detected by TLC in system B.

The authors thank Dr P. Sedmera for the measurement and the interpretation of the ^{13}C NMR *spectra, Dr L. Helešic for potentiometric titration and Miss E. Kvapilová for chromatographic analyses.*

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Translated by Ž. Procházka.