PREPARATION OF 5'-O-CARBOXYMETHYLCYTIDINE AND 5'-O-CARBOXYMETHYL-1-(β-D-ARABINOFURANOSYL)CYTOSINE*

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N^4-Benzoyl-2',3'-O-isopropylidenecytidine (*VI*) was prepared from N^4-benzoylcytidine (*V*) by reaction with 2,2-dimethoxypropane or by benzoylation of 2',3'-O-isopropylidenecytidine and selective O-debenzoylation. 5'-O-Carboxymethylcytidine (*VII*) was obtained by reaction of compound *VI* with sodium chloroacetate in the presence of sodium hydride, followed by alkaline and acidic removal of protecting groups. Epimerisation of the nucleoside *VII* with phosphorus oxychloride in ethyl acetate led to 5'-O-carboxymethyl-1-(β-D-arabinofuranosyl)cytosine (*VIII*). Benzoylation of 5'-O-trityl-1-(β-D-arabinofuranosyl)cytosine (*II*) and the acidic removal of the trityl group afforded an equimolar mixture of N^4,$O^{2',3'}$-tribenzoyl-1-(β-D-arabinofuranosyl)-cytosine (*III*) and N^3,$O^{2',3'}$-tribenzoyl-1-(β-D-arabinofuranosyl)cytosine (*IV*). Reaction of compound *III* with sodium chloroacetate in the presence of sodium hydride gave a mixture of 1-(β-D-arabinofuranosyl)cytosine O-carboxymethyl derivatives.

In connection with investigations on properties of protein conjugates with the nucleoside-type immunosuppressants, there has been in an earlier paper[1] reported the preparation of 6-azauridine-5'-uronic acid along with isomeric 6-azauridine O-carboxymethyl derivatives, and their binding to human serumalbumin and bovine gamma-globulin. Since the cytosine arabinoside, *i.e.*, 1-(β-D-arabinofuranosyl)cytosine[2-10] (*I*) is known to be a potent immunosuppressant, it was of interest to attempt the preparation of protein conjugates with compound *I* derivatives. In these derivatives, the structure of the immunosuppressant nucleoside should not be too modified with respect to stereochemistry or substitution. In view of the activity of the above mentioned 6-azauridine carboxylic acid derivatives[1], we have first attempted to prepare the 5'-uronic acid derived from compound *I*. The catalytic oxidation of cytosine nucleosides has been reported to be accompanied by a high degradation of the nucleoside bond and by deamination of the product[11,12]. Another method (successfully used in the 6-azauridine series[1]) consists in reaction with sodium periodate in the presence of the ruthenium trichloride catalyst[13]: the primary alcoholic function

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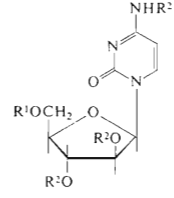
of sugars or nucleosides is smoothly converted to the carboxylic acid[1,13]. In the case of cytosine derivatives however, this reaction is accompanied by a high degradation even when the other hydroxylic groups of the sugar residue are protected (*e.g.*, 2',3'-O-isopropylidenecytidine). In view of these difficulties and low yields, the preparation of uronic-acid-type derivatives of compound *I* by the sodium periodate method was abandoned.

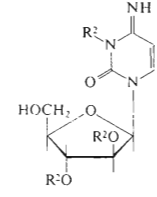
Another approach could consist in preparation of the 5'-O-carboxymethyl derivative *VIII*. In the 6-azauridine series, the analogous compounds with ethereally bound acetic acid residue proved to be equally good substrates for binding with proteins by the method of mixed anhydrides as the corresponding uronic acid[1]. The synthesis of nucleoside O-carboxymethyl derivatives is preferably performed by a direct reaction of sodium chloroacetate with the nucleoside alkoxide which is obtained on treatment with sodium hydride in an aprotic asolvent[1,14–17]. For this purpose, the other hydroxylic groups must be selectively protected. Since the secondary hydroxylic groups of compound *I* are in the *trans* configuration, the acidolabile dioxolane protecting groups cannot be used; the hydroxyls were consequently protected by benzoylation. The amino group at position 4 of the heterocyclic nucleus which is also susceptible to reaction with sodium chloroacetate, was benzoylated simultaneously. Reaction of compound *I* with trityl chloride in pyridine in the presence of pyridine hydrochloride[18] afforded the 5'-O-trityl derivative *II*. Compound *II* was benzoylated with benzoyl chloride in pyridine and the reaction mixture (after removal of pyridine) hydrolysed with 80% aqueous acetic acid. According to chromatography, the detritylated mixture contains an equimolar mixture of two compounds which were separated in pure state. As indicated by analysis and NMR spectrum, the mixture consists of two isomeric tribenzoyl derivatives of compound *I*. On the basis of the data mentioned and the course of alkali-catalysed methanolysis the derivatives were assigned structures *III* and *IV*. With compound *III*, the methanolysis affords the starting nucleoside *I*, while with compound *IV*, the corresponding uracil derivative is exclusively obtained. The behaviour of compound *IV* is in accordance with the expectation since the N^3-alkyl derivatives of cytosine afford uracil derivatives in alkaline media. The formation of the N^3-benzoyl derivative *IV* is noteworthy since compound *II* was benzoylated under mild conditions which with other cytosine derivatives favour the exclusive N^4-benzoylation. The formation of N^3-benzoyl derivatives has not been encountered even in detailed investigations on the benzoylation of compounds related to type *I* (*cf.*[18]); however, the presence of such a benzoyl derivative in those reaction mixtures[18] cannot be *a priori* excluded.

The formation of the N^3-benzoyl derivative *IV* from compound *I* or *II* substantially lowers the practical applicability of the method *via* the N^4-benzoyl derivative *III* in the synthesis of the O-carboxymethyl derivative. Half of the reaction mixture, compound *IV* is worthless for the reaction with sodium chloroacetate, since the removal of protecting groups leads always to a derivative of the uracil series. Notwith-

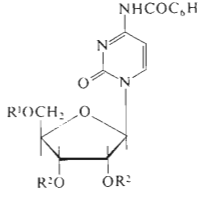
standing, we have attempted to prepare compound *VIII* from the tribenzoyl derivative *III* by reaction with sodium chloroacetate in dimethylformamide. The protecting groups were removed by methanolysis in the presence of sodium methoxide, the resulting mixture desalted by chromatography on an ion exchange resin, and then separated by chromatography on DEAE-cellulose and by paper chromatography. The reaction mixture contained roughly equal parts of compound *I* (arising from the unreacted compound *III*), the mono-O-carboxymethyl derivative *VIII* (or isomers), and the bis-O-carboxymethyl derivatives. Consequently, a partial O-debenzoylation takes place in the course of the reaction or, more probably, by the action of sodium hydride in dimethylformamide on compound *III*; the consequence is the multiple substitution of the sugar residue. It may be thus assumed that also the fraction of the monosubstituted O-carboxymethyl derivatives of compound *I* contains the derivative *VIII* along with the 2′- and/or 3′-isomers. The N^3-benzoyl derivative *IV* reacts with sodium chloroacetate analogously to afford after paper chromatography the uracil arabinoside, its mono- and bis-O-carboxymethyl derivatives. The deamination was quantitative since no cytosine derivative could be found. It may be inferred from the



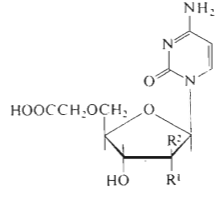
I, $R^1 = R^2 = H$
II, $R^1 = (C_6H_5)_3C$, $R^2 = H$
III, $R^1 = H$, $R^2 = C_6H_5CO$



IV, $R^2 = C_6H_5CO$



V, $R^1 = R^2 = H$,
VI, $R^1 = H$, $R^2 = (CH_3)_2C$



VII, $R^1 = OH$, $R^2 = H$
VIII, $R^1 = H$, $R^2 = OH$

above results that the use of benzoyl protecting groups in reactions in the presence of sodium hydride is limited to the N-blocking only; the ester-bound benzoyl groups are removed to a considerable extent.

The selective introduction of alkali-stable protecting groups into positions 2' and 3' of compound *I* is rather difficult and a multistep procedure would be required. In the preparation of compound *VIII*, an alternative route was therefore used consisting in the synthesis of 5'-O-carboxymethylcytidine (*VII*) from readily accessible cytidine derivatives and the subsequent epimerisation of compound *VII* at the $C_{(2')}$ carbon atom. Analogous reactions have been effected both with cytidine alone[19] and its O-alkyl derivatives[20,21] by the action of hydrolysed phosphorus oxychloride (*i.e.* dichlorophosphoric acid). Since the dioxolane protecting group may be applied in the case of cytidine, 2',3'-O-isopropylidene-N^4-benzoylcytidine (*VI*) was used as the starting material. Compound *VI* was prepared by two routes, either by isopropylidenation of N^4-benzoylcytidine (*V*; obtained in turn from cytidine[22]) on treatment with 2,2-dimethoxypropane, or more advantageously, by per-benzoylation of 2',3'--isopropylidenecytidine[23] with benzoyl chloride in pyridine and the subsequent selective O-debenzoylation[24] of the intermediary N^4,$O^{5'}$-dibenzoyl-2',3'-O-isopropylidenecytidine with sodium hydroxide in aqueous-methanolic medium. The latter route affords compound *VI* in fair yields. By the action of sodium chloroacetate in dimethylformamide followed by alkaline and acidic hydrolysis, compound *VI* was converted into the derivative *VII* which was isolated by the ion exchange resin chromatography as the free acid.

The preparation of 5'-O-carboxymethylcytidine has been also attempted by some other authors in connection with syntheses of related compounds. The results with cytidine alone were not probably encouraging since indirect routes were finally used, namely, nucleoside condensation of ribofuranose 5-O-carboxymethyl derivative[25] or treatment of 2',3'-O-isopropylidene-4--thiouridine with sodium chloroacetate followed by ammonolysis[26]. To our opinion, the present synthesis of 5'-O-carboxymethylcytidine (*VII*) is at least as convenient as the earlier routes.

The epimerisation of compound *VII* by reaction with hydrolysed phosphorus oxychloride in ethyl acetate[19] took place without any difficulty. The cytosine-containing components of the reaction mixture were isolated on a strongly acidic cation exchange resin and then subjected to chromatography on DEAE-cellulose in a borate cycle. Under such conditions[27], compound *VIII* lacking the *cis*-diol system is readily separated from a lesser amount of the unreacted *VII* which is eluted later due to the presence of the *cis*-diol grouping and complex formation with the borate. The thus-obtained triethylammonium salt of compound *VIII* was then converted to the free acid.

The structure of compounds *VII* and *VIII* was confirmed by analysis, chromatography, and electrophoresis. Furthermore, the NMR spectrum of compound *VIII* was also in accordance with the constitution proposed (with compound *VII*, the NMR spectrum cannot be measured because of the low solubility of the substance).

TABLE I
Chromatography (R_F Values) and Electrophoresis (Mobilities)

Compound	S_1	S_2	S_3	S_4	E_1[a]	E_2[b]
Uridine	0·45	0·41	—	—	0	1·00
I	0·50	0·49	—	—	−0·05	0
II	—	—	0·20	0·49	—	0
III	—	—	0·10	0·24	—	—
IV	—	—	0·07	0·14	—	—
V	—	—	—	0·13	—	—
VI	—	—	—	0·56	—	—
VII	0·31	0·35	—	—	0·43	1·72
VIII	0·36	0·39	—	—	0·43	1·12

[a] Referred to uridine 3'-phosphate; [b] referred to uridine.

Also the CD spectra in principle correspond to those of cytidine and compound *I* in positions of extrema as well as in signs and absolute values of the B_{2u} band of compound *VII* with respect to cytidine and compound *VIII* with respect to the arabinoside *I*. A similar accordance may be observed with the E_{1u} bands of both pairs. When compared with the parent nucleosides[28], the 5'-O-carboxymethyl derivatives *VII* and *VIII* exhibit shift of extrema to longer wavelengths by 4 nm with the B_{2u} band; this shift is in accordance with the change of the ultraviolet absorption maximum which in aqueous solutions of compounds *VII* and *VIII* corresponds to the spectrum of the protonated form of cytosine nucleosides (λ_{max} 280 nm) but not to that of the neutral form. This effect indicates (in addition to extraordinarily low solubility and crystallisation behaviour) the zwitter ion structure of compounds *VII* and *VIII*.

Unfortunately, the zwitter ion structure of compounds *VII* and *VIII* is also reflected in the reactivity of the carboxylic function which is lowered to such an extent that the mixed anhydride with ethoxycarbonyl chloride is not formed under standard conditions[1]. Consequently, the binding to proteins is not formed. This result is in accordance with the recent observations of other authors[29]: 1-carboxymethylcytosine, closely related to compounds *VII* and *VIII*, is also not bound to proteins by the method of mixed anhydrides. Accomplishment of such a reaction would require conditions which would exclude the occurence of the zwitter ion form. These problems will be dealt with elsewhere.

EXPERIMENTAL

Melting points were taken on a heated microscope stage (Kofler block) and are uncorrected. Unless stated otherwise, the solutions were taken down on a rotatory evaporator at 40°C/15 Torr and analytical samples were dried over phosphorus pentoxide at 0·1 Torr.

Methods

Paper chromatography was performed by the descending technique on paper Whatman No 1 in the solvent systems S_1, 2-propanol–conc. aqueous ammonia–water–water (7 : 1 : 2), and S_2, 1-butanol–glacial acetic acid–water (5 : 2 : 3). Thin-layer chromatography was performed on ready-for-use Silufol UV_{235} (Kavalier Glass-Works, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_3, chloroform, and S_4, chloroform–ethanol (97 : 3). Paper electrophoresis was carried out by the reported technique[30] at 20 V/cm (1 h) on paper Whatman No 3 MM in buffer solutions E_1, 0·1M triethylammonium hydrogen carbonate (pH 7·5), and E_2, 0·1M triethylammonium borate (pH 7·5) (Table I). The UV spectra were taken in aqueous solutions on a Zeiss Specord apparatus. The CD spectra were measured on a Jouan Dichrograph. The NMR spectra were recorded on a Varian 100 apparatus in deuteriochloroform or hexadeuteriodimethyl sulfoxide (hexamethyldisiloxane as internal standard). The chemical shift values are expressed in δ (p.p.m.) and the coupling constants in Hz.

1-(2,3-Di-O-benzoyl-β-D-arabinofuranosyl)-N^4-benzoylcytosine (*III*) and 1-(2,3-Di-O-benzoyl-β-D-arabinofuranosyl)-N^3-benzoylcytosine (*IV*)

To a solution of 5′-O-trityl-1-(β-D-arabinofuranosyl)cytosine[18] (*II*) (8·3 g; 17·1 mmol) in pyridine (50 ml) there was added dropwise with ice-cooling benzoyl chloride (8 ml; 9·66 g; 69 mmol) and the mixture was kept at room temperature for 3 days. Methanol (5 ml) was then added, the whole kept at room temperature for 2 h, poured into ice-cold water, the solid collected with suction and washed with water, refluxed in 80% aqueous acetic acid (100 ml) for 40 min, and kept at room temperature overnight. The crystalline triphenylmethanol was filtered off and washed with acetic acid. The filtrate and washings were combined and evaporated under diminished pressure. The residue was coevaporated with two 50 ml portions of ethanol and finally dissolved in chloroform (200 ml). The chloroform solution was washed with three 100 ml portions of saturated aqueous sodium hydrogen carbonate and one 50 ml portion of water, dried over anhydrous magnesium sulfate, filtered off, and the drying agent washed with chloroform. The filtrate and washings were combined and concentrated to a small volume. The concentrate was applied to a column of Pitra silica gel (particle size, 30—60 micron; 150 g) and the elution was performed with chloroform (50 ml fractions). The fractions containing compounds *III* and *IV* were combined, evaporated, and crystallised from ethanol to afford 3·4 g (36%) of the N^4,$O^{2',3'}$-tribenzoate *III*, m.p. 175—176°C. For $C_{30}H_{25}N_3O_8$ (555·5) calculated: 64·86% C, 4·53% H, 7·56% N; found: 64·06% C, 4·61% H, 6·91% N. NMR spectrum ($CDCl_3$): 4·05 (br d, 2 H, $J_{5',4'} = 4·0$) 2 $H_{5'}$; 4·37 (br q, 1 H, $J_{4',3'} = 3·5$, $J_{4',5'} = 4·0$) $H_{4'}$; 5·73 (q, 1 H, $J_{3',2'} = 1·8$, $J_{3',4'} = 3·6$) $H_{3'}$; 5·97 (q, 1 H, $J_{2',1'} = 4·0$, $J_{2',3'} = 1·8$) $H_{2'}$; 5·55 (d, 1 H, $J_{1',2'} = 4·0$) $H_{1'}$; 7·58 + 8·56 (2 d, 2 H, $J_{5,6} = 7·0$) H_5 + H_6; 9·95 (br s) NH; OH absent [in hexadeuteriodimethyl sulfoxide 4·25 (t, 1 H, $J_{OH,H_{5'}} = 7·5$)]; 7·25—8·15 (m, 15 H) aromatic protons. By the action of 0·1M methanolic sodium methoxide at 50°C for 2 h, the tribenzoate *III* was converted to compound *I*. In addition to the tribenzoate *III*, the crystallisation afforded 3·4 g (36%) of the N^3,$O^{2',3'}$-tribenzoate *IV*, m.p. 163—164°C. For $C_{30}H_{25}N_3O_8$ (555·5) calculated: 64·86% C, 4·53% H, 7·56% N; found: 64·57% C, 4·60% H, 7·14% N. NMR spectrum ($CDCl_3$): 3·85 (br s, 2 H) 2 $H_{5'}$; 4·23 (br q, 1 H, $J_{3',4'} = 4·0$, $J_{4',5'} = 4·0$) $H_{4'}$; 5·47 (br d, 1 H, $J_{5,6} = 7·0$, $J_{5,NH} = 1·0$) H_5;

5·65 (q, 1 H, $J_{3',2'} = 2·6$, $J_{3',4'} = 4·2$) $H_{3'}$; 5·70 (br s, 1 H) 5′-OH; 5·76 (q, 1 H, $J_{2',1'} = 4·0$, $J_{2',3'} = 2·6$) $H_{2'}$; 6·40 (d, 1 H, $J_{1',2'} = 4·0$) $H_{1'}$; 11·23 (br s, 1 H) NH; 7·20–8·10 aromatic protons. By the action of 0·1M methanolic sodium methoxide at 50 °C for 2 h, the tribenzoate *IV* is converted into 1-(β-D-arabinofuranosyl)uracil.

N^4-Benzoyl-2′,3′-O-isopropylidenecytidine (*VI*)

A. A mixture of N^4-benzoylcytidine[22] (*V*; 5·2 g; 15 mmol), 2,2-dimethoxypropane (45 ml), acetone (20 ml), and *p*-toluenesulfonic acid monohydrate (1·0 g) was stirred at room temperature for 90 min, diluted with acetone (20 ml), neutralised with triethylamine, evaporated under diminished pressure, and the residue dissolved in chloroform (100 ml). The solution was filtered through Celite, the filtrate applied to a column of Pitra silica gel (particle size, 30–60 micron; 100 g), and the column eluted with chloroform. The product-containing fractions were combined, evaporated, the residue dissolved in boiling ethanol (20 ml), the solution treated with light petroleum (200 ml), and the whole kept at −10°C overnight to deposit crystals which were collected with suction, washed with light petroleum, and dried. Yield, 3·3 g (57%) of compound *VI*, m.p. 182°C.

B. A mixture of cytidine (5·0 g; 20·6 mmol), acetone (25 ml), triethyl orthoformate (30 ml), and *p*-toluenesulfonic acid monohydrate (4·0 g) was stirred for 10 min and the resulting solution kept at room temperature overnight. Methanolic sodium methoxide (1M; 21 ml) was then added, the whole stirred for 30 min, diluted with chloroform (100 ml), filtered through Celite with suction, and the material on the filter washed with chloroform (100 ml). The filtrate and washings were combined (they contained pure 2′,3′-isopropylidene cytidine as indicated by thin-layer chromatography in the solvent system S_4), evaporated under diminished pressure, and the residue coevaporated with three 25 ml portions of pyridine. The final residue was dissolved in pyridine (20 ml) and the solution was treated dropwise under ice-cooling and stirring with benzoyl chloride (8 ml; 9·7 g; 69 mmol). The mixture was stirred at room temperature for 2 days, decomposed with water (20 ml), kept for 1 h, diluted with chloroform (300 ml), the chloroform solution washed with two 100 ml portions of saturated aqueous sodium hydrogen carbonate and one 50 ml portion of water, dried over anhydrous magnesium sulfate, and filtered. The drying agent was washed with chloroform, the filtrate and washings combined, evaporated to dryness, the residue dried under diminished pressure, and finally dissolved in 90% aqueous methanol (500 ml). The solution was cooled down to 0°C and treated dropwise under stirring with sodium hydroxide (2·4 g; 60 mmol) in water (30 ml). The mixture was stirred at 0°C for 10 min, neutralised with dry Dowex 50 X 8 (H^+) ion exchange resin, filtered, and the resin washed with methanol (50 ml). The filtrate and washings were combined, evaporated under diminished pressure, and the residue coevaporated with ethanol (50 ml). The thus-obtained crystals were dissolved in boiling ethanol (25 ml), the solution diluted with light petroleum (150 ml), and kept at −10°C overnight to deposit the product which was collected with suction, washed with light petroleum, and dried under diminished pressure. Yield, 4·0 g (50·0%) of compound *VI*, m.p. 181–182°C, homogeneous on thin-layer chromatography in solvent systems S_3 and S_4, and identical with the specimen obtained by procedure *A*. For $C_{19}H_{21}N_3O_3$ (387·4) calculated: 58·90% C, 5·46% H, 10·85% N; found: 58·12% C, 5·72% H, 10·36% N.

5′-O-Carboxymethylcytidine (*VII*)

To a mixture of compound *VI* (3·9 g; 10 mmol) and dimethylformamide (50 ml) there was added with stirring sodium hydride (0·50 g; 20·8 mmol), the whole stirred under exclusion of atmospheric moisture for 15 min, and treated successively with sodium chloroacetate (2·9 g; 25 mmol)

and dimethylformamide (10 mmol). The thus-obtained mixture was stirred at room temperature for 2 days, kept with 100 ml of 0·1M methanolic sodium methoxide overnight, neutralised with acetic acid, and evaporated under diminished pressure. The residue was coevaporated with three 25 ml portions of toluene at 50°C/0·1 Torr and the final residue refluxed in 80% aqueous acetic acid (100 ml) for 40 min. The mixture was evaporated, the residue diluted with water (50 ml), adjusted to pH 3 by the addition of Dowex 50 X 8 (H^+) ion exchange resin, the resulting suspension applied to a column (200 ml) of the same resin, the column washed with water to the drop of the UV-absorption and conductivity, and finally eluted with dilute (1 : 10) aqueous ammonia. The UV-absorbing NH_3-containing effluent was evaporated, and the residue in water (20 ml) applied to a column (100 ml) of Amberlite IR 4B (acetate cycle) ion exchange resin. The column was washed with water to the drop of the UV-absorption and conductivity, and then eluted with 2M acetic acid. The UV-absorbing fractions were pooled, evaporated, and the residue crystallised from water to afford 1·7 g (56·5%) of the freee acid *VII* which does not melt up to 260°C. For $C_{11}H_{15}N_3O_7$ (301·3) calculated: 43·85% C, 5·01% H, 13·94% N; found: 43·97% C, 5·03% H, 13·97% N. The UV spectrum (water): λ_{max} 276 nm (ε_{max} 10100), λ_{min} 244 nm. The NMR spectrum was not measured because of the insolubility of the substance in usual solvents. The CD spectrum (water): 274 (+11400), 239 (0), 217 (−10300), 205·5 (0).

1-(5-O-Carboxymethyl-β-D-arabinofuranosyl)cytosine (*VIII*)

To 18 ml of phosphorus oxychloride there was added dropwise with stirring and ice-cooling 3·6 ml of water, the mixture stirred at 0°C for 30 min, diluted with ethyl acetate (240 ml), and treated with compound *VII* (1·7 g; 5·65 ml). The whole was refluxed for 3 h, diluted with water (200 ml), and concentrated under diminished pressure to the volume of about 100 ml. The concentrate was applied to a column (400 ml) of Dowex 50 X 8 (H^+) ion exchange resin, the column washed with water to the drop of the UV-absorption and conductivity, and finally eluted with dilute (1 : 10) aqueous ammonia. The UV-absorbing NH_3-containing effluent was evaporated and the residue applied to a 80 × 4 cm column of DEAE-cellulose (Cellex D, standard capacity, borate form). The column was eluted with the use of a linear gradient of triethylammonium borate (pH 7·5), 2 l of the 0·02M buffer solution in the mixing chamber and 2 l of the 0·2M buffer solution in the reservoir. The product is contained in the 0·04—0·12M buffer fraction. The corresponding eluate was evaporated, the residue coevaporated with methanol in order to remove the volatile buffer, and the final residue applied to a column (100 ml) of Amberlite IR 4B (acetate cycle) ion exchange resin. The column was first washed with water to the drop of the UV-absorption and then eluted with 2M acetic acid. The UV-absorbing eluate was evaporated and the residue crystallised from water to afford 0·9 g (53%) of the acid *VIII*, homogeneous on paper chromatography (S_1) and electrophoresis (E_1 and E_2). For $C_{11}H_{15}N_3O_7$ (301·3) calculated: 43·85% C, 5·01% H, 13·94% N; found: 43·92% C, 5·08% H, 14·07% N. The UV spectrum (water): λ_{max} 276 nm (ε_{max} 10400), λ_{min} 246 nm. The CD spectrum (water): 273·5 (+20800), 242 s (+3600), 234·5 (0), 215 (−16100), 205 (0). The NMR spectrum (d_6-dimethyl sulfoxide): 3·65 (m, 2 H) 2 $H_{5'}$; 3·85 (m, 2 H) $H_{3'}$ + $H_{4'}$; 3·95 (m, 1 H) $H_{2'}$; 4·02 (s, 2 H) —CH_2O; 5·68 (d, 1 H), $J_{5,6}$ = 7·5) H_5; 6·04 (d, 1 H, $J_{1',2'}$ = 4·0) $H_{1'}$; 7·60 (d, 1 H, $J_{5,6}$ = 7·5) H_6; exchangeable protons: 5·10 (br, 4 H); 7·10 (br, 1 H).

Reaction of Compound *III* with Sodium Chloroacetate

A mixture of compound *III* (3·3 g; 6 mmol), sodium hydride (0·4 g; 16·7 mmol), and dimethylformamide (20 ml) was stirred at room temperature for 15 min, treated with sodium chloroacetate (18 mmol), and stirred for 2 days under exclusion of atmospheric moisture. Methanolic

sodium methoxide (0·2M; 100 ml) was then added, the whole heated at 50°C for 1 h, concentrated under diminished pressure, the concentrate diluted with water (100 ml), and washed with three 25 ml portions of ether. The aqueous phase was adjusted to pH 2·5—3·0 by the addition of Dowex 50 X 8 (H^+) ion exchange resin and the resulting suspension applied to a column (100 ml) of the same resin. The column was washed to the drop of the UV-absorption and conductivity, and then eluted with dilute (1 : 10) aqueous ammonia. The UV-absorbing NH_3-containing eluate was evaporated under diminished pressure and the residue applied to a column (80 × 4 cm) of DEAE--cellulose (Celex D, standard capacity, HCO_3^- form). The elution was performed with the use of a linear gradient of triethylammonium hydrogen carbonate (pH 7·5), 2 l of water in the mixing chamber, 2 l of the 0·2M buffer solution in the reservoir, elution rate 3 ml per min, and withdrawal of fractions in 10 min intervals. The 0·02—0·05M fraction yielded (as determined by spectrophotometry) 1·8 mmol fo compound *I*, homogeneous on chromatography (S_1 and S_2) and electrophoresis (E_2). The 0·08—0·12M fraction afforded 2·0 mmol of compound *VIII* (and further isomers). The 0·15—0·20M buffer fraction gave 1·6 mmol of a mixture of the bis(O-carboxymethyl) derivatives of compound *I* (R_F 0·10 in S_1; E_{Up} 1·25 in E_1).

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