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CRYSTAL ENGINEERING OF CYTIDINE AND DEOXYCYTIDINE SULPHATES. I. PREPARATION, AND UNUSUAL PROPERTIES.

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This paper is dedicated to Dr Gabor Fodor on the occasion of his birthday.

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ABSTRACT: From three possible simple salts of Cyd (dCyd)[1] with H_2SO_4, we succeeded in preparation of only one type of a crystalline salt in which two monoprotonated cations: $2CydH^+$ ($2dCydH^+$) pair with one sulphate dianion (SO_4^{2-}) (Fig.3)[4]. Attempts to obtain crystalline sulphates with dimeric hemi-cations and/ or hydrogen sulphate anions completely failed. The presence or absence of 2'OH group drastically changes the thermochemical properties of Cyd and dCyd sulphates (Fig.4). Cyd sulphate undergoes rapid one-step thermal decomposition within the range 205-260°C with subsequent smoth further mass loss up to 340°C, whereas dCyd sulphate (Form A and B) undergoes a three stage thermal decomposition: I. 25-170°C, II. 170-230°C, III. 230-340°C. Clear differences in the DSC and TGA curves of both types of salt may reflect dissimilar mechanisms of their crystal structure decomposition. An extremely easy transformation of dCyd sulphates into the hydrochloride salt in the crystal phase was discovered. The reaction proceeds both in finely ground stoichiometric mixtures of $2dCydH^+ \cdot SO_4^{2-}$ + 2NaCl or KCl, and also in a nujol suspension of pure dCyd sulphate placed between NaCl plates. Similar transformation occurs also with KBr and leads to the crystalline dCyd hydrobromide which is isostructural to dCyd hydrochloride. Form A of dCyd sulphate, which contains methanol trapped in its crystal lattice, is particularly susceptible to the above transformation. Cyd sulphate undergoes transformation to hydrochloride and hydrobromide salts by a few orders of magnitude slower. These unidirectional ion exchange reactions proceed quantitatively in crystalline phase only, showing the significance of crystal structures of nucleosides and their salts. Unusual properties of sulphate counter anions may be exploited for a controlled modulation of reactivity of nucleosides.

INTRODUCTION

In the course of our systematic studies of cytidine, ε-cytidine and deoxycytidine salts[2] we have discovered some unexpected differences in the association mode between

isostructural Cyd and dCyd dihydrogenphosphates[3]. These encouraged us to prepare Cyd and dCyd sulphates and to investigate their physicochemical properties. First of all, we wanted to find out which of the theoretically possible salts could be easily obtained in the crystalline form from methanol/water systems, in expectation that the two hemisulphates with dimeric cations, $2(Cyd_2H)^+ \cdot SO_4^{2-}$ and $2(dCyd_2H)^+ \cdot SO_4^{2-}$, should crystallize well. However, irrespectively of the nucleoside : H_2SO_4 stoichiometry used (1:1, 2:1, and 4:1) we only succeeded in crystallizing salts with 2:1 composition of following formulas: $2CydH^+ \cdot SO_4^{2-}$ and $2dCydH^+ \cdot SO_4^{2-}$. During preparation of single crystals for X-ray analysis it emerged that dCyd sulphate crystallizes in two Forms, A and B, which give substantially different IR spectra. It also appeared that Form A which crystallizes from methanol/water, traps methanol in its crystal lattice which cannot be completely removed from the salt even on heating for several hours at 115°C. The crystals of Form B, crystallizing from ethanol/water, as well as the crystals of Cyd sulphates, which are obtained from the methanol/water or ethanol/water systems, do not contain either methanol or any other solvent molecules in their crystal lattices. Completely unexpectedly, when taking the infrared spectra of 2'-deoxycytidinium sulphate Form A, ($2dCydH^+ \cdot SO_4^{2-}$), as nujol suspensions placed between NaCl plates we noticed that they undergo gradual change with time and after about two hours become indistinguishable from those of crystalline 2'-deoxycytidine hydrochloride ($dCydH^+ \cdot Cl^-$). The transformation is much faster (30 min.) when the two crystalline salts: $2dCydH^+ \cdot SO_4^{2-}$ and NaCl are carefully ground in a mortar in 1:2 stoichiometry before forming a nujol suspension.

In this unusual solid-state transformation, a mixture of two different crystalline salts [$2dCydH^+ \cdot SO_4^{2-}$ + 2NaCl] undergoes a rapid exchange of ions, which are located in completely different crystal lattices.

The above observations triggered further studies aimed at answering the following questions.

1. Do other dCyd salts undergo similar transformations in the solid state on NaCl plates or in mixtures with stoichiometric amounts of NaCl?
2. Can other inorganic salts replace NaCl in the above transformation?
3. Do crystalline salts of other nucleosides undergo similar transformations?
4. What is the molecular mechanism of this transformation?
5. Why do sulphate dianions preclude the formation of dCyd hemications whereas other inorganic anions do not?

This report is the first of a series of papers dealing with these questions in which we discuss the following points concerning the sulphates of Cyd and dCyd : (a) preparation, recrystallization and characterization of Cyd and dCyd sulphates and (b) thermochemical data (DSC, TGA) of Cyd and dCyd sulphates and their abilities to transform into crystalline Cyd and dCyd hydrochlorides.

EXPERIMENTAL

Methods

Differential scanning calorimetric curves (DSC) and thermogravimetric data (TGA) were measured on a DuPont 10908 Thermal Analysis system. IR spectra were measured on Perkin-Elmer 180 and FT-IR Bomem 152 spectrophotometers using the suspension technique in fluorolub and/or nujol and with NaCl plates.

Solvents and reagents were purified according to standard procedures. Cytidine (Cyd) and deoxycytidine hydrochloride (dCyd·HCl) were from Pharma-Waldhof. Deoxycytidine hydrochloride was transformed into dCyd base with Dowex type 1x2 (OH^- form)(Serva), free from chloride ions and crystallized from aqueous methanol.

General procedure for the crystallization of Cyd and dCyd sulphates. Finely ground nucleosides were suspended in warm methanol (1g in ca.50 ml). Stoichiometric amounts of aqueous 2N H_2SO_4 (2:1) followed by small portions of warm water were added gradually, and clear solutions were left at room temperature for crystallization (24h). After collecting the first crop of crystals, the mother liquor was stored at 4°C and a second crop of crystals was collected after another 24 hours. Sometimes it was possible to collect a third fraction after dilution of the mother liquor with methanol. The final mother liquor was evaporated on a rotary evaporator to give a fine crystal residue. All crystal fractions were weighed and characterized by IR spectra. Those with identical IR spectra were combined and recrystallized from the H_2O/CH_3OH system (1:9 vol/vol). The recrystallized preparations were thoroughly characterized by physico-chemical methods.

Cyd sulphate ($2CydH^+ \cdot SO_4^{2-}$) crystallizes in 2:1 stoichiometry in a single form only. Its IR spectra and DSC and TGA curves are presented in Fig.1,4. The same form also obtained upon crystallization from ethanol/H_2O and acetone/H_2O systems in 1:1 and 4:1 stoichiometries. In the latter two cases, besides pure fractions of the formula $2CydH^+ \cdot SO_4^{2-}$, which are the main products of fractionated crystallization in both processes, there also appear microcrystalline fractions with blurred IR spectra, which have not been subjected to further studies. In 2:1 stoichiometry the yield of raw crystals is nearly 90%, and about 75% upon recrystallization.

dCyd sulphate ($2dCydH^+ \cdot SO_4^{2-}$) crystallizes in 2:1 stoichiometry with yields similar to Cyd sulphate (ca. 90% for raw crystal and ca. 75% upon recrystallization but in two different Forms. Form A is obtained under conditions described above and the other, Form B, crystallizes from ethanol/H_2O or acetone/H_2O. These two Forms have similar elemental analyses (A: C-38.67%; H-5.51%; N 15.09% B: C-38.53%; H-5.52%; N 15.05%), but crucial differences in their IR spectra and DSC and TGA data (see Figs. 1 and 4). ^1H NMR spectra of D_2O solutions differ only in one important detail: Form A, besides the expected signals of protons covalently bonded to the carbon atoms of the Cyt fragment (5C-H and 6C-H) and the sugar fragment (1'C-H; 2'CH_2; 3'CH; 4'CH and 5'CH_2) also shows the

presence of distinct signals from the CH_3 group of MeOH. According to preliminary results of X-ray analysis[4], methanol is trapped in some cavities of the crystal structure of Form A. The percentage of MeOH occluded in the crystals falls within 0.6-2.8% of that calculated for $2dCydH^+ \cdot SO_4^- \cdot 1/2\ CH_3OH$ and depends on the content of water in aqueous methanol used for crystallization, on particle size, temperature, and time of storage of crystalline Form A.

The lowest methanol content was found in a finely pulverized crystalline Form A heated for 30 min. at a temperature of 115°C, and the highest one in large-crystal preparations stored in desiccator at room temperature over P_2O_5. There are no differences in the IR spectra of the preparations of Form A with ca. 0.6 and 2.8% methanol content. We did not succeed in obtaining Form A without any methanol content, whereas Form B and all the preparations of Cyd sulphate crystallized from methanol do not contain any MeOH.

Figure 1
FT-IR spectra of crystalline samples of Cyd and dCyd sulphates (Form A and B) taken in nujol suspension between NaCl plates, immediately after preparation.

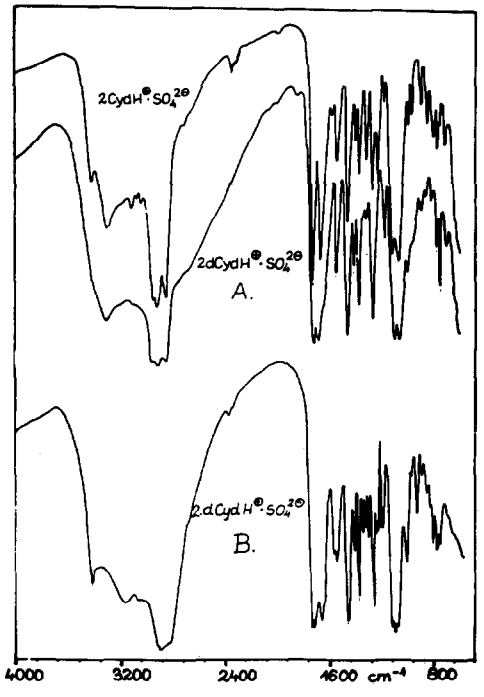
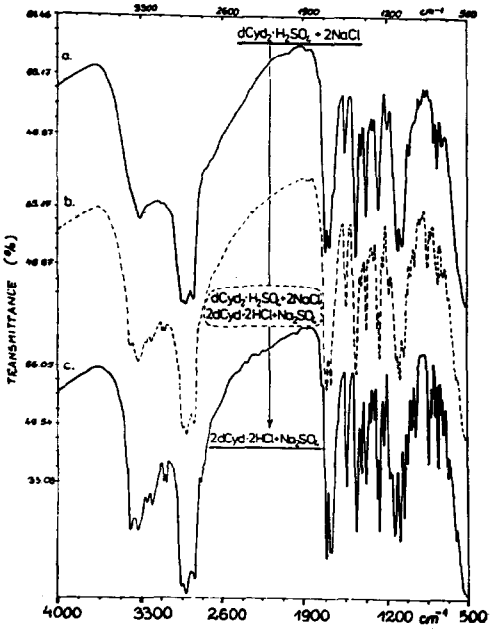


Figure 2
FT-IR spectra of stoichiometic mixtures of $2dCydH^+ \cdot SO_4^{2-}$ (Form A) with NaCl: a) just after the mixture has been ground, b) 15 min later, c) after 30 min, when the transformation is complete.



Conditions for transformation of Cyd and dCyd sulphates into hydrochloride salts in the solid phase.

a) between NaCl plates.

2-5 mg of a sulphate salt are ground with a steel spatula in 1 drop of nujol on an opaque glass plate. This suspension is transfered onto the centre of a NaCl plate and covered with a second one and gently pressed together until the suspension forms an even thin layer, and then inserted into the IR spectrometer to monitor the transformation process (see IR spectra Fig.2). The essential spectral changes indicate that the rate of transformation can be significantly influenced not only by the crystal structure of the sulphate salt, but also by such external factors as homogenity of the nujol suspension, degree of polish of the NaCl plate surfaces, and even traces of the solvent used for washing the plates, especially methanol, which is absorbed on those surfaces. To achieve reproducible results Method b (see below) is recommended.

b) within a stoichiometric mixture (1:2) of the crystalline preparations of Cyd (or dCyd) sulphate and sodium chloride.

The two salts are separately ground in an agate mortar, mixed in 1:2 molar ratio, and ground again (2 x 2 min. with a 5 min. interval). From this mixture, samples of 2-5 mg were taken at time intervals, and their IR spectra in nujol were recorded (see Fig. 2). The method is reproducible and therefore was used for determination of relative rates of anion exchange in crystalline sulphates of Cyd and dCyd. For the conversion of Cyd and dCyd sulphates into the corresponding hydrobromides, NaCl was replaced by KBr. The some procedure was also used during an attempted transformation of Cyd and dCyd phosphates into hydrochloride salts.

RESULTS AND DISCUSSION

Preparation of crystalline Cyd and dCyd sulphates and their characterization

When attempting the preparation of Cyd and dCyd sulphates we expected to obtain four salts depending on the ratio of Cyd(dCyd) to H_2SO_4 from combinations of (i) monoprotonated cations, $2CydH^+$ or $2dCydH^+$ (ii) dimeric hemications, $2(Cyd_2H)^+ \cdot SO_4^{2-}$ or $2(dCyd_2H)^+ \cdot SO_4^{2-}$; (iii) hydrogensulphate monoanions (HSO_4^-), and (iv) sulphate dianions (SO_4^{2-}). We readily obtained crystalline preparations with the first and last ion types. We have been unable to obtain crystalline Cyd and dCyd sulphates with either dimeric hemications or hydrogensulphate anions (HSO_4^-).

2'-Deoxycytidinium sulphate

Although dCyd forms hemisalts very easily (Table 1), especially dihydrogenphosphates[3], for 2'-deoxycytidine sulphates the case is very different. From methanol/water systems, the salt $2dCyd^+ \cdot SO_4^{2-}$ crystallizes in very good yield from 2:1 molar eqv. ratio of $dCyd:H_2SO_4$, while the yields are still quite good from 1:1 and even 4:1 stoichiometry. The "2:1 salt" is the first and main product of those crystallization processes. Crystallizations of dCyd sulphates conducted under 1:1 and 4:1

stoichiometries do not lead to $dCydH^+ \cdot HSO_4^-$ and $2(dCyd_2H)^+ \cdot SO_4^{2-}$ salts, although in the starting solutions the hemication ion is undoubtedly present. There are various possible reasons for the difficult in obtaining such salts in crystalline forms.

The poor crystallization ability of dCyd hemisulphate can be a result of considerably diminished hetero-association properties of sulphate dianions with dimeric dCyd hemications ($dCyd_2H^+$) in comparison with the easy association of SO_4^{2-} anion with 2 monomeric cations of $dCydH^+$ (Fig. 3)[4].

TABLE 1. Crystallization abilities (from methanol/water) of Cyd and dCyd salts with HCl, HNO_3, H_3PO_4, $HClO_4$ and H_2SO_4 acids.

Anions	Cations			
	monocations		hemications	
	$CydH^+$	$dCydH^+$	$(CydHCyd)^+$	$(dCydHdCyd)^+$
Cl^-	+++ x-ray[5]	++ x-ray[6]	-	++
NO_3^-	++ x-ray[7]	++	-	++
$H_2PO_4^-$	+++ x-ray[8]	+ x-ray[9]	-	+++ x-ray[10]
ClO_3^-	++ x-ray[11]	++ x-ray[11]	++	++ x-ray[10]
SO_4^{2-}	+++ x-ray[10]	+++ x-ray[10]	-	-
HSO_4^-	-	-	-	-

Legend:
- \- crystalline preparation could not be obtained
- \+ can be crystallized only under specific conditions
- ++ crystallizes from solutions of correct stoichiometry
- +++ crystallizes preferentially even when stoichiometry was unfavorable and from various solvents
- x-ray - the molecular and crystal structure of the salt was solved by X-ray

Our observations are evidence of drastic differences in an association properties of phosphate and sulphate anions. We hope the present paper will be a step towards understanding them, and in effect will enable their application to the crystal engineering of nucleosides.

$2dCydH^+ \cdot SO_4^{2-}$ can be easily recrystallized with good yield from three systems: (i) methanol/water, (ii) ethanol/water, and (iii) acetone/water. At first, we thought that all three routes led to identical crystal forms. It turned out, however, that the crystals obtained by route (i), which we named Forms A, are different from those obtained by routes (ii) and (iii) (Forms B). Elemental analysis gives comparable results for both forms but they are characterized by markedly different IR spectra (Fig. 1 and experimental). The crystals obtained from methanol/water (Form A) are transparent, shiny and well-defined single crystals which X-ray analysis is almost finished[4]. Crystals from the ethanol/water or acetone/water systems (Form B) do not differ one from the other. They are fine crystals, which upon filtering and washing, become opaque. We have only lately succeeded in obtaining single crystals suitable for X-ray analysis. Each form can

be purified or transformed into the other one by crystallization, using appropriate solvent mixtures.

$2dCydH^{+}\cdot SO_4^{2-}$ (polymorph A)

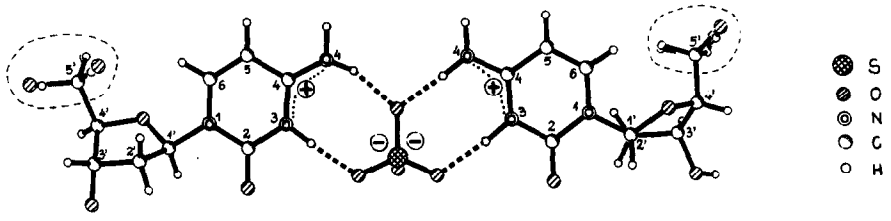


Figure 3. Anion-cation interaction in the crystal structure of $2dCydH^{+}\cdot SO_4^{2-}$ (Form A)[4].

Cytidinium sulphate

In our experiments with cytidine sulphates we have not yet fully utilized the method of fractional crystallization at various molar ratios of Cyd and H_2SO_4. In this case, $2CydH^{+}\cdot SO_4^{2-}$ is the easiest to crystallize in good yields also from stoichiometries other than 2:1. However, in contrast to $2dCydH^{+}\cdot SO_4^{2-}$, it crystallizes only in one form.

Interpretation of FT-IR and NMR spectra

In Fig.1 the IR spectra of the three crystalline monosulphates are shown. Essential similarities in three crucial regions: νNH_2 (ca. 3300 cm^{-1}), $\nu 2C{=}O$ (ca. 1725 cm^{-1}), νSO_2 (ca. 1100-1117 and ca. 1060-1073 cm^{-1}) are visible in the spectra of $2CydH^{+}\cdot SO_4^{2-}$ and $2dCydH^{+}\cdot SO_4^{2-}$ Form A. The IR spectra of the two polymorphic dCyd sulphates (A and B) also show numerous similarities (e.g. in the region 1500-1750 cm^{-1}), but important differences are also evident, e.g. in the regions 3500-3000 cm^{-1} (νN-H and νO-H), 1150-900 cm^{-1} (νSO_4^{-}) and 700-900 cm^{-1}, which are signs of different crystal structures and, perhaps, even of conformational differences of the sugar fragments.

From preliminary crystallographic investigations of $2dCydH^{+}\cdot SO_4^{2-}$ Form A, it has been postulated that the crystals are solvated and contain, in addition to $dCydH^{+}$ and SO_4^{2-} ions, methanol, probably in non stoichiometric proportion[4].

It is interesting to note that the presence of methanol could not be ascertained from our elemental analyses: because the experimental data for C and H content are consistent with the data calculated for both formulas: $2dCydH^{+}\cdot SO_4^{2-}$ (C 39.13% H 5.11%) and $2dCydH^{+}\cdot SO_4^{2-}\cdot 1/2CH_3OH$ (C 39.08% H 5.32%), but they slightly differ for N contents (15.11% and 14.79% respectively).

A series of ^1H NMR spectra in D_2O of the three crystalline sulphates of Cyd and dCyd (Forms A and B) showed the presence of methanol only in the samples of Form A of dCyd sulphate. The methanol content in a preparation stored at room temperature for several months did not change. The methanol content corresponds to ca. one CH_3OH

molecule per two $2dCydH^+ \cdot SO_4^{2-}$ units estimated by signal integration for ^1H NMR spectra recorded for dissolved Form A, When the solvated crystals are heated for 30 min. at a temperature of 115°C, they lose ca. 2/3 of the methanol, but heating for 4 h does not lead to further loss of methanol. Grinding the crystalline preparation prior to heating makes the process of methanol evaporation at 115°C more efficient and after 30 min. of heating only 17% of the initial content is left. It seems that at this temperature this is the maximum loss of methanol.It is interesting to note that the IR spectra of the original and 80% "demethanolated" crystals of polymorph A are identical. This is an evidence of the conservation of the crystal structure of polymorph A upon 80% methanol removal indicating that the 80% of trapped methanol molecules do not participate in significant manner in the formation of the crystal lattice of polymorph A.

Thermochemical data of the Cyd and dCyd sulphates and their abilities to transform into crystalline Cyd and dCyd hydrochlorides

The thermochemical properties of $2CydH^+ \cdot SO_4^{2-}$ and $2dCydH^+ \cdot SO_4^{2-}$ (Form A and B) are markedly different, as illustrated by the DSC and TGA curves in Figs. 4 and 5.

The isostructural dihydrogenphosphates of Cyd and dCyd have also significantly different profiles and temperatures of transition on the DSC and TGA curves[3]. The interpretation of their DSC and TGA curves was relatively easy since it could be based on precisely solved molecular and crystal structures of both salts [8,9], which showed that thermochemical differences between the two phosphates were mainly caused by the presence or absence of the 2'OH group. When absent, i.e. in dCyd dihydrogenphosphate, the endo- and exothermic effects are more numerous and take place in temperatures lower by ca. 50°.

For Cyd sulphate no thermochemical effect is observed up to 205°C. The first endothermic effect with a minimum at 229°C is immediately followed by an exothermic effect with a maximum at 240°C, which is accompanied by a sudden loss of 20% mass (Fig. 4).

For $2dCydH^+ \cdot SO_4^{2-}$ polymorph A, the DSC curve shows two stages of decomposition of the crystal and molecular structure (Figs. 4 and 5). The first stage proceeds in the temperature range 100-160° and is manifested by 2-3 overlaped endothermic minima accompanied by a ca. 4% mass loss, which can be assigned to the liberation of 1.5 mole (4.8%) of constitutive water or 1/2 mole of H_2O + 1/2 mole of CH_3OH (4.3%). The next stage of thermolysis takes place in the temperature range 160-205°C and is manifested by a strong endothermic effect (ca. 140 kJ/M) with two minima (190° and 201°), accompanied by substantial mass loss (respectively 22 and 14%), attributed to the dehydration and carbonization of the 2'-deoxyribose fragments.

The differences observed in the courses of the DSC and TGA curves of Cyd and dCyd sulphates were further checked using IR spectroscopy. The crystalline preparations were thoroughly ground and then heated for 30 min. at 170° and 205°C. After cooling, nujol suspensions of the four heated samples were prepared, and their IR spectra recorded. As expected, the IR spectrum of the Cyd sulphate sample heated for 30 min. at 170°C was

fully identical with the spectrum of the initial salt, while the IR spectrum of dCyd sulphate Form A heated for 30 min. at the same temperature differs significantly from the spectrum of the initial preparation. We assume that during heating, the glycosidic bonds of the $dCydH^+$ cations must be broken since in the IR spectrum of the heated product the bands of cytosinium cations ($CytH^+$) are easily recognized.

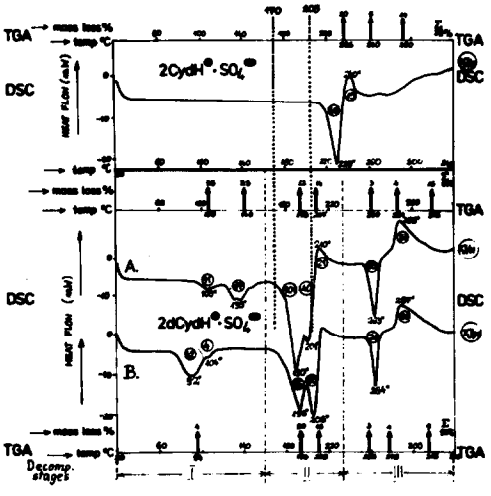


Figure 4. Simple schematic representation of DSC curves and TGA data of the three crystalline sulphate preparations of Cyd and dCyd, (scanning rate 10°C/min).

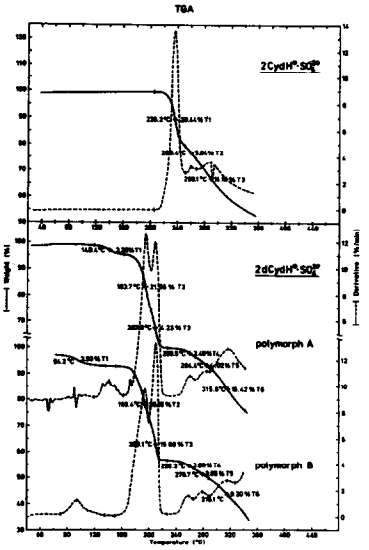


Figure 5. Comparison of TGA and DTG profiles and data of three crystalline sulphate preparations of Cyd and dCyd (scanning rate 10°C/min).

The IR spectra of samples of sulphates of Cyd and dCyd (Form A) heated for 30 min. at 205°C differ significantly from the spectra of both initial preparations. In the case of Cyd sulphate it was still possible to isolate ~60% of crystalline substrate from the product heating 30 min. at 205°C. No traces of the dCyd sulphate Form A could be detected in the products of the heating process carried out at identical conditions on Form A. Here, the crystalline residue contained mostly cytosinium sulphate. The process of thermolysis of the glycosidic bond must be accompanied by the dehydration of the sugar residue with a ca. 19% loss of mass (ca. 6 moles of H_2O).

Major differences in the course of the DSC and TGA curves of Cyd and dCyd sulphates which are clearly visible (Figs. 4 and 5) and are most likely caused by completely different mechanisms of a crystal structure decomposition. In the case of dCyd sulphate, the three-stage controlled thermal decomposition is determined by the irreversible endothermic effects of the first stage, connected with small mass losses which, taken together, are probably the reason of a major reorganization of the crystal lattice of this salt so that it is well adjusted to the next ordered thermal transformation that

occurs in the second stage.

The DSC and TGA data for polymorph B of dCyd sulphate (Fig. 4) seem to support this hypothesis. The DSC and TGA data of polymorphs A and B are similar, and can be compared in terms of the three-stage process as follows:

- stage III of decomposition (230-340°C) - almost identical DSC and TGA profiles with similar values
- stage II (170-230°C) - more similarities than differences
- stage I (25-170°C) - major differences

Such a course of the DSC and TGA curves of both dCyd sulphates shows that it is unlikely to induce an A → B or B → A transformation in the solid phase solely by thermal factors.

Relative abilities for transformation of crystalline sulphate of Cyd and dCyd (Forms A and B) into respective crystalline hydrochlorides

Contrary to our initial view, the ability for transformation into crystalline hydrochlorides is not limited to polymorph A of dCyd sulphate. Polymorph B and Cyd sulphate can be transformed into hydrochloride salts using the same procedure as for the polymorph A. They differ however in the relative rates of transformation (see experimental). We describe in this paper only the experiments conducted for molar 1:2 mixtures of Cyd or dCyd sulphate with sodium chloride, according to the standard procedure described in the experimental section. FT-IR spectra have been recorded for each mixture at different times of incubation allowing study of the relative rate of transformation.

We demonstrate (Fig. 2) three FT-IR spectra of a stoichiometric mixture of $dCyd_2 \cdot H_2SO_4$ (Form A) + 2NaCl. Spectrum "a" corresponds to a physical mixture of both initial components, while spectrum "c" shows the same mixture after completed transformation into a new crystalline mixture: 2(dCyd·HCl) + Na_2SO_4. Spectrum "b" represents some advanced stage of that transformation.

In the case of the Form A, the transformation begins within 10 min. and the process is over in 30 min. Form B is less active. The transformation becomes visible after 20 min. and ends after 1 hour. Cyd sulphate reacts more slowly yet, as the first distinct signs of the process can be observed only after 1 hour, and it is over in about 4 hours.

Other salts of Cyd and dCyd (nitrates, perchlorates and phosphates, see Table 1) do not show any signs of transformation even after 48 hours of incubation with carefully ground stoichiometric mixture with NaCl. Thus, at the present stage of our studies those salts are classified as showing no transformational abilities.

Potassium chloride also reacts easily with crystalline dCyd and Cyd sulphates indicating that the differences in ionic radii, Na^+ (0.97 Å) and K^+(1.33 Å), have no significant effect on the process of transformation of dCyd and Cyd sulphates into hydrochlorides. In a similar way, crystalline Cyd and dCyd sulphates react also with potassium bromide yielding Cyd and dCyd hydrobromides at similar rates. This shows that the differences in the ionic radii of Cl^- (1.81 Å) and Br^-(1.96 Å) also have no

substantial influence on the rate of transformation of Cyd and dCyd sulphates into their hydrobromide or hydrochloride salts in the crystalline phase.

It is very interesting to note that the FT-IR spectra of crystalline hydrochloride and hydrobromide salts of dCyd are very similar, almost overlapping in the region 4000 - 900 cm^{-1}, whereas the IR spectra of analogous salts of Cyd differ drastically. Our prediction that the first pair of salts should be isomorphous, whereas the second not, are fully confirmed by X-ray analysis data[10]. We believe that the precise knowledge of the crystal structures of two pairs of hydrochloride and hydrobromide salts of Cyd and dCyd will help us to solve the molecular mechanism of these peculiar transformations.

Conclusions

The unique association and thermochemical properties of the sulphates in comparison with other Cyd and dCyd salts are the result of the electronic structure of the SO_4^{2-} dianion and its ability to participate in proton-transfer.

Available experimental data are not yet sufficient to present a detailed mechanism for the postulated processes. Nevertheless, on the basis of a partially solved crystal structure of polymorph A of dCyd sulphate (see Fig. 3) and on its thermochemical data (Fig. 4) we can reasonably speculate on some mechanistic aspects. From X-ray analysis it is evident (Fig. 3)[4] that each sulphate dianion bridges two $dCydH^+$ cations acting as four proton acceptor center symetrically distributed on the three oxygen atoms. Such three ionic association units linked by four hydrogen bonds (in which H-donating roles play: $3N^+$-H and $4N^+$-H groups) must be strong enough at room temperature to prevent the transprotonation process leading to neutral Cyd molecules and HSO_4^- anions.

Assuming that the process of transprotonation between sulphate anions and $dCydH^+$ cations, similarly to that observed in dCyd dihydrogenphosphate[3], triggers reorganization of the crystal structures of these salts, we should at the same time notice significant differences between these processes in both salts. In dCyd dihydrogenphosphate the transprotonation process is connected with the dominating tendency for transformation of the monophosphate into hemiphosphate which leads to the dimeric $(dCydHdCyd)^+$ cations (easily formed even during normal recrystallization procedure[3,12]), whereas in dCyd sulphate such tendency does not exist at all. Most likely, the lack of tendency of dCyd sulphates to transform into hemisulphate with dimeric dCyd cations is closely connected with their very pronounced ability to transformation into hydrochloride in solid phase.

These ions exchange reactions take place both in finely ground stoichiometric mixtures of $2dCydH^+ \cdot SO_4^{2-}$ and 2NaCl, and in nujol suspensions of crystalline dCyd sulphate placed between NaCl plates, but not during co-crystallization of the above mixtures from water/methanol systems, indicating on the significance of information encoded into the crystal structures of nucleosides and their salts.

From what has been said above it can be concluded that this study (being far from completion), resulted in a number of important and unexpected observations of major significance for the further development of the crystal engineering of nucleosides[13]. We

believe that continuation of these studies will contribute to better understanding of the conformational and functional dynamism of nucleic acids. Growing number of reports on the hypothetical significance and biological function of ionic base pairs [14] and of protonated DNA fragments [15] (leading to the formation of four stranded helical structures), strongly support this point of view.

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