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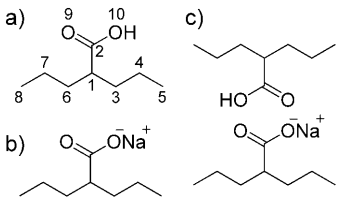
Solid-State Forms of Sodium Valproate, Active Component of the Anticonvulsant Drug Epilim

Gjorgi Petruševski,[b] Panče Naumov,*[a, b] Gligor Jovanovski,[b, c] Gordana Bogoeva-Gaceva,[d] and Seik Weng Ng[e]

The results of the first detailed and systematic investigation of the solid-state forms of sodium valproate, one of the most potent and widely used anticonvulsant medicines, are presented. By using wet and dry methods, eight solid forms of varying stability in air were obtained and characterized. Three extremely hygroscopic polycrystalline hydrates, $Na(C_8H_{15}O_2)\cdot H_2O$ (form A), $Na(C_8H_{15}O_2)\cdot x\,H_2O$ (form B), and $Na(C_8H_{15}O_2)\cdot y\,H_2O$ (form D), three acid-stabilized stoichiometric solvates, $Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot H_2O$ (form C), $Na(C_8H_{15}O_2)(C_8H_{16}O_2)$ (form E), and $Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot 2\,H_2O$ (form F), the pure anhydrous salt $Na(C_8H_{15}O_2)$ (form H), and an additional unstable thermal intermediate $Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)_{0.5}$ (form G) were prepared. Under ambient conditions, forms A and B as well as the commercially available compound appear as very hygroscopic white powders. Form C is less hygroscopic, while forms E and F are stable and are not hygroscopic. Partial stabilization of forms A and B can be achieved by evacuation and pressing, which results in a lower hydrate D, or after a heating–cooling cycle, resulting in crystallization of the anhydrous salt H. Addition of one molecule of valproic acid and saturation with one molecule of water of forms A and B results in the less hygroscopic form C. Addition to form C of a second water molecule affords form F, which is not hygroscopic and is indefinitely stable. The symmetric structure and medium alkyl chain length of the valproate ion are some of the probable reasons for the presence of a number of solid solvates: in its most stable conformation, the valproate ion cannot simultaneously pack efficiently and interact strongly through the negatively charged carboxylate group without leaving voids in the crystalline lattice. The conformational flexibility of the aliphatic chains probably aids the penetration of water molecules, which results in a strong affinity for the absorption of water.

1. Introduction

2-Propylvaleric (valproic) acid and its sodium salts (Scheme 1) have been extensively clinically used as anticonvulsants and mood-stabilizing drugs to treat cases of epilepsy and bipolar disorders, diseases which affect approximately 1% of the human population. The valproate drugs are commonly available under the commercial names Epilim, Depakon, Depakene, and Orlept. The valproates have been used for treatment of migraine headaches and schizophrenia. Recent studies[1] showed that valproic acid also acts as inhibitor of histone deacetylase 1, an enzyme required to retain the human immunodeficiency virus (HIV) within the infected cells; patients treated with valproic acid combined with highly active antiretroviral therapy show a 75% decrease in the latent HIV infection. According to several research groups, including the US National Institutes of Health, valproic acid exhibits significant effects in the treatment of various cancers,[2] including multiple myeloma (bone marrow cancer),[3] glioma (an aggressive type of brain tumor),[4] and melanoma (rare but very lethal skin cancer).[5] The anticancer activity of valproic acid has been prescribed to its cytotoxic activity caused by the inhibition of the histone deacetylase. It is surprising that despite being used in clinical



Scheme 1. Structural formulas of a) valproic acid, b) sodium valproate, and c) sodium hydrogen divalproate.

[a] *Prof. P. Naumov*
Frontier Research Base for Global Young Researchers
Graduate School of Engineering, Osaka University
2-1 Yamadaoka, Suita 565-0871, Osaka (Japan)
Fax: (+81) 6-6879-4726
E-mail: npance@wakate.frc.eng.osaka-u.ac.jp

[b] *G. Petruševski, Prof. P. Naumov, Prof. G. Jovanovski*
Institute of Chemistry, Faculty of Science
Ss. Cyril and Methodius University
P.O. Box 162, MK-1001 Skopje (Macedonia)

[c] *Prof. G. Jovanovski*
Macedonian Academy of Sciences and Arts
P.O. Box 428, MK-1001 Skopje (Macedonia)

[d] *Prof. G. Bogoeva-Gaceva*
Institute of Organic Technology, Faculty of Technology and Metallurgy
Ss. Cyril and Methodius University
Ruger Boškoviḱ 16, MK-1001 Skopje (Macedonia)

[e] *Prof. S. W. Ng*
Department of Chemistry, University of Malaya, 50603 Kuala Lumpur (Malaysia)

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practice as anticonvulsants for decades, the exact mechanism of the physiological action of valproic acid and sodium valproate have not been entirely understood yet. The most often used theory invoked to explain their action on the central nervous system is the one based on various actions, involving inhibition of the enzyme control of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and/or inhibition of voltage-gated sodium channels in the cell membrane.[6,7]

The synthesis of valproic acid was described as early as in 1882.[8] At room and physiological temperatures it appears as a clear, viscous, colorless liquid. The acid is only slightly soluble in water, but it is highly soluble in organic solvents. For about eight decades the compound has been considered metabolically inert, and it was used as a convenient solvent for testing anticonvulsant activity of other organic compounds. In 1962, Eynard and co-workers were the first to notice that the acid itself exhibits anti-seizure effects.[9,10] A year later, the initial results on the use of valproic acid for treatment of epilepsy became available.[11] Sodium valproate can be conveniently prepared by treating the acid with sodium methoxide in methanol or heptane, or by neutralization in water.[12] A very brief report on the characterization of the salt[13] indicated the presence of several solid forms, but none of them has been chemically identified.

The two most serious obstacles to the widespread use of the valproate are the liquid state and the low solubility of the acid, and the very pronounced hygroscopicity of the sodium salt at ambient conditions. These properties largely burden processing of the two medicines in the course of tablet preparation of oral formulations, and also cause practical problems with their storage. In the late 1970's the problem was occasionally solved by mixing sodium valproate with valproic acid in 1:1 molar ratio, resulting in a non-hygroscopic solid which is advantageous for pharmaceutical preparations. The compound is easily prepared from hot acetone[14,15] and it is now commercially available as "valproate semisodium", "divalproex sodium" or Depakote. Although several studies[9] have pointed out the advantageous anticonvulsant properties of the mixed compound relative to the pure salt, there are also counterarguments to this.[16] Partial stabilization may be also achieved by mixing with citric, succinic, tartaric[17] and diethyl acetic acid.[14] The attempts to use other salt-forming cations for stabilization of sodium valproate have failed due to the poor reactivity or instability of the products in air.

In view of the great importance of sodium valproate and sodium hydrogen valproate,[18] and the stability of the latter in air, it is surprising that except one crystal structure recently reported by our group,[19] no detailed results on their solid-state properties and crystal structures have been reported to date.[20] In general, screening for polymorphs and/or solvates of compounds that are used as medicines is an important research area because different solid forms of chemically identical compounds can occasionally exhibit drastically different chemical, physical and physiological properties.[21] The energy differences between various polymorphs of the same compound are often very low, which may result in some of the metastable forms appearing as stable at ambient conditions, which is important for practical applications in the pharmaceutical industry. The most essential prerequisites for successful preparation of a particular solid-state form of a pharmaceutical compound are detailed knowledge of the conditions for its formation, and finding means for actual control of conditions of the phase transition process, such as crystallization.

Here we present the results of the first detailed study and characterization of the solid-state forms of sodium valproate. As many as eight solid forms were prepared and identified by employing several physicochemical methods, including Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). The geometrical preferences of the valproate ion were also investigated and its spectra were interpreted based on extensive density functional theory (DFT) calculations.

2. Results and Discussion

2.1. Compositional characterization

Owing to the pronounced affinity towards moisture, precise determination of the chemical composition of forms A–D proved extremely difficult and special care had to be taken with handling and storage of the samples in the course of their physicochemical characterization. The results of the elemental analysis of forms A, C, E, H and the native (commercial) sample, and of the sodium content of forms E and F determined by atomic absorption spectrometry are deposited as tables S1 and S2, respectively (Supporting Information). According to the elemental analysis, the composition of form A corresponds to a monohydrated sodium valproate of formula $Na(C_8H_{15}O_2)\cdot H_2O$, with experimentally determined water content of ~1.03 moles per mole of the salt. Along with the composition determined by the crystal structure analysis,[19] the elemental analysis proved that form C is a monohydrated 3:1 solvate of sodium valproate with valproic acid, $Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot H_2O$. The elemental analysis and thermal data (vide infra) showed that form E is an anhydrous 1:1 solvate of sodium valproate with valproic acid, $Na(C_8H_{15}O_2)(C_8H_{16}O_2)$. Form F corresponds to a dihydrated 3:1 solvate, $Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot 2H_2O$ with determined water content of ~1.8. Based on the IR spectra (see Figure 6 below), form H was identified as anhydrous sodium valproate, $Na(C_8H_{15}O_2)$. Determination of the elemental composition of forms B and D in pure form was not possible, but based on the solid-state conversion reactions to the other forms and the IR results, their molecular formulas can be best described as $Na(C_8H_{15}O_2)\cdot x H_2O$ and $Na(C_8H_{15}O_2)\cdot y H_2O$, respectively, for which $y < x$,1.

2.2. Crystal structure of form C

Form C, identified as monohydrated 3:1 solvate, trisodium hydrogen tetravalproate monohydrate (Figure 1), is the only form of sodium valproate whose structure has been determined by X-ray diffraction analysis. The basic structural characteristics of the crystal of form C were described in a communication preceding this article;[19] to correlate the structure with the

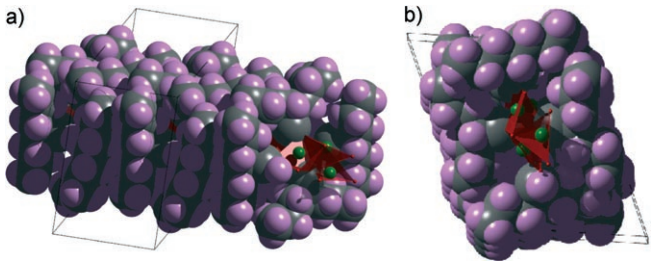


Figure 1. Space filling representation, in two orientations (a and b), of the molecular packing in the crystal of form C, $Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot H_2O$. The green and red balls represent sodium and oxygen atoms, respectively, surrounded with alkyl residues with carbon and hydrogen atoms represented respectively as gray and violet spheres.

physicochemical data in the course of making structural predictions on the other forms, a brief description of the main structural features is presented here. The structure is organized as sodium–oxygen clusters, which are wrapped up by the alkyl residues (Figure 1). The inner core of the clusters is formed by two monodentate oxygen atoms from two acid carboxyl groups and two bidentate oxygen atoms from two carboxylate groups connected to four pentacoordinated sodium ions (Figures 1 and 2).

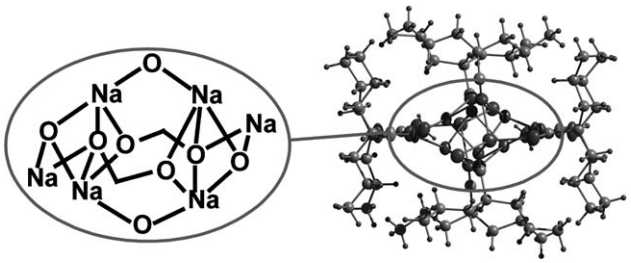


Figure 2. Schematic representation and three-dimensional structure of the sodium–oxygen clusters in the crystal of form C, $Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot H_2O$.

The water molecule is bonded to one of the pentacoordinated sodium ions. The periphery of the cluster is formed by bridging two pairs of tetracoordinated sodium ions with oxygen atoms of the tetradentate valproate ions. A remarkable characteristic of this structural assembly, which is unique among the known simple organic salts (as concluded by a search of the Cambridge Structure Database[19]), is that the sodium–oxygen clusters are wrapped up by valproate alkyl residues in a "vvv" conformation that form a hydrocarbon case of approximately rectangular shape. The structure demonstrates that due to the symmetric shape of the alkyl groups, the negatively charged, polar, hydrophilic valproate ions can self-assemble in stable, large, effectively lipophilic and thus hydrophobic oligomeric formations.

2.3. FTIR spectroscopy

The FTIR spectra of forms A, B, C, E, F, and H were recorded from Nujol mulls and KBr pellets (Figures 3–6). The spectrum

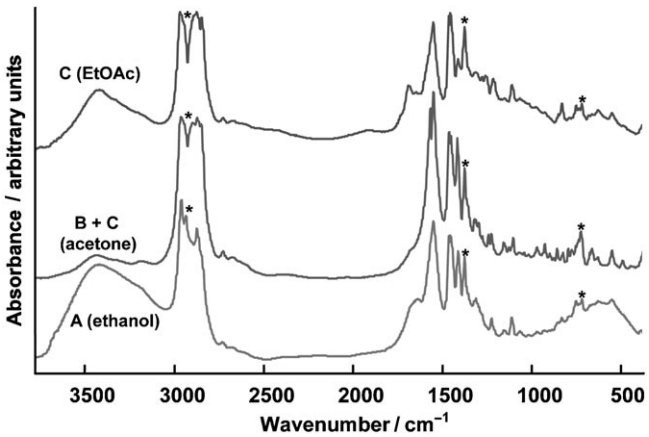


Figure 3. IR spectra of form C, a mixture of forms B and C, and form A of sodium valproate recorded from Nujol-mulled samples (the asterisks denote bands from the mulling agent, and the solvent used for recrystallization is in parentheses).

of form H (Figure 6) was obtained from a mulled moisture-protected sample of form A submitted to heating and subsequent cooling under dry nitrogen atmosphere during the DSC analysis. The spectra recorded from KBr pellets (Figure 4) clearly in-

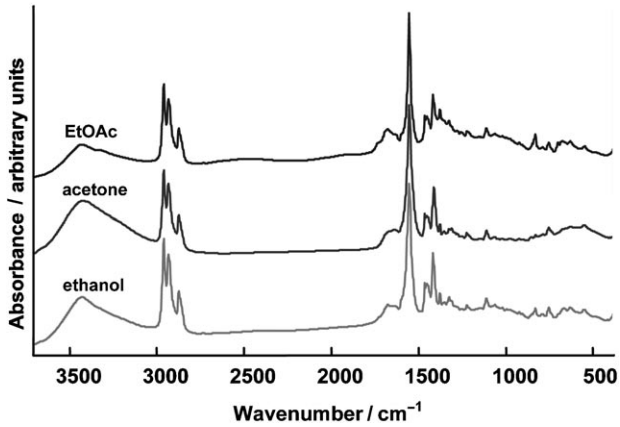


Figure 4. IR spectra of form D obtained by pressing and evacuation with KBr of sodium valproate recrystallized from ethyl acetate (EtOAc), acetone, and ethanol.

dicate that upon evacuation and pressing with KBr forms A, B and C are transformed into the same hydrate form. This new form, which appears to be thermodynamically more stable at ambient conditions, will be referred to as form D. Contrary to the pure salts and form C, the FTIR spectra of the hydrogen salts E and F recorded using both methods (Figure 5) show that they are stable towards evacuation by oil pump.

The IR band frequencies of the different forms are deposited as table S3 (Supporting Information). The spectra recorded from mulled samples (Figure 3) which are characteristic and discriminative towards the various sodium valproate forms, consist of three band regions where the characteristic valproate and water vibrations occur. The most useful for identifi-

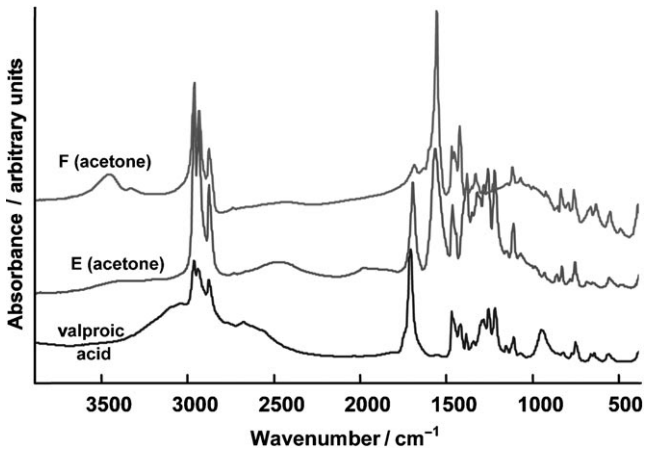


Figure 5. IR spectra of sodium valproate (forms F and E) and valproic acid, recorded from KBr pallets. The solvent used for recrystallization is in parentheses.

cation purposes are the OH stretching region (~3500 cm^{-1}) and the carbonyl stretching region (1550–1690 cm^{-1}). Due to the band overlap and low band intensity, the low-frequency spectral region (<1500 cm^{-1}), containing bands originating from the CH_2 deformations (~1450 cm^{-1}), symmetric CO stretch (~1415 cm^{-1}), CH_3 deformations (~1370 cm^{-1}), water vibrations (<1350 cm^{-1}) and bands from the mulling agent (~1375 cm^{-1}, 730 cm^{-1}), has comparably lower discriminating power. The valproate CH stretches around 3000 cm^{-1} do not represent well different forms both because of their weak intensity and the presence of bands from the IR medium.

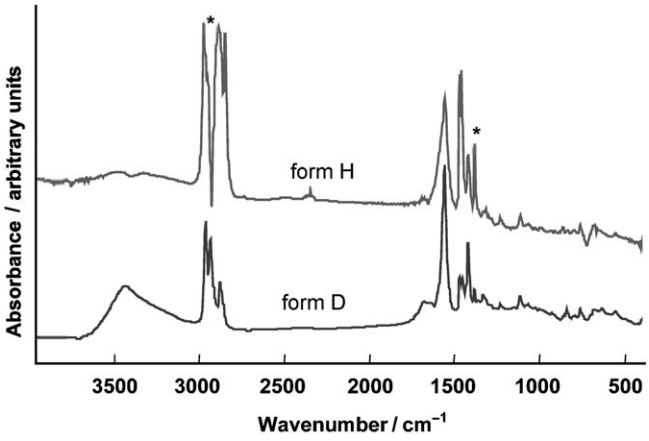


Figure 6. IR spectra of form H (in Nujol) obtained by heating and cooling of form A, and of form D (in KBr pellet) obtained by evacuation of form A. The asterisks denote bands from the mulling agent.

Except the anhydrous forms E and H, all forms of sodium valproate exhibit broad bands around 3400 cm^{-1} which are due to the presence of water in their structures; due to the pronounced hygroscopicity of the studied samples, distinction between the stoichiometric water and eventual physisorbed water was not possible. The IR spectrum of valproic acid contains the OH vibrations of the carboxyl group in the 3400–2400 cm^{-1} region (submaxima at 3340, 3043, 2762, 2674 and 2564 cm^{-1}), typical for strongly hydrogen bonded OH groups.

The antisymmetric carbonyl stretching mode of the valproate ion appears as characteristic strong band around 1550 cm^{-1}. Being a good group vibration, this mode can be employed for distinction among different forms of sodium valproate. The ν(CO) mode of form A results in a strong band at 1553 cm^{-1} (Figure 3 and table S3). Additionally, a shoulder appears at 1655 cm^{-1}, which is also present in the spectrum of form D (in KBr) and in the spectrum of the native sample (before recrystallization). The disappearance of the shoulder upon heating of the native sample at 383 K for 48 h (see the inset in Figure 7) evidences that it is due to the δ(HOH) vibration of water molecules.

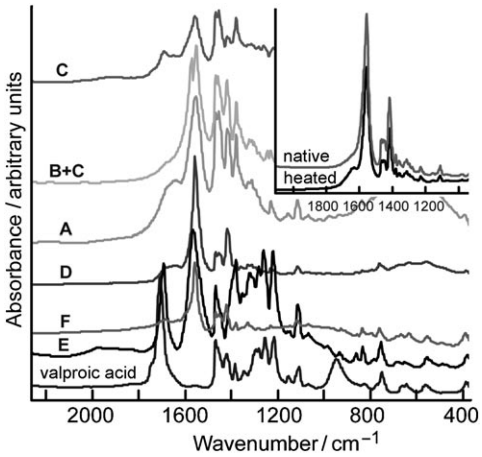
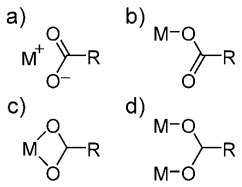


Figure 7. Carbonyl stretching region in the IR spectra of various solid forms of sodium valproate and valproic acid; inset: change in the IR spectra of native (commercial) sodium valproate induced by heating for 48 h at 293 K.

As expected for a mechanical mixture of two forms (B and C), the IR spectrum of the sample obtained from acetone is characterized by a larger number and doubling of many bands. Accordingly, two strong ν(CO) bands (1570 cm^{-1} and 1551 cm^{-1}) appear. Spectral comparison with pure form C obtained from ethyl acetate shows that the band at 1551 cm^{-1} is due to form C (the corresponding band in the IR spectrum of pure form C appears at 1554 cm^{-1}, Figure 3). The band at 1570 cm^{-1} is assigned to form B. Form E exhibits well separated carbonyl stretching bands at 1562 and 1692 cm^{-1} due to the presence in its structure of valproate ions and acid molecules, respectively (Figure 5). The red shift of the latter band with respect to pure valproic acid (1706 cm^{-1}) probably reflects differences in hydrogen bonding strength. As expected from the identical 3:1 ratio of the salt and the acid, the spectrum of form F (dihydrate) is very similar to that of form C (monohydrate), but the frequencies of the ν(CO) mode of the acid are considerably different (Figure 5 and Figure 7), indicating that their structures are different. The IR spectrum of form H (Figure 6) is clear evidence that this form is anhydrous. The car-



Scheme 2. Different types of bonding between metal ions (M) and carboxylate groups: a) ionic, b) monodentate, c) bidentate chelating, and d) bidentate bridging.

bonyl stretching of this form appears at 1552 cm^{-1}. The pronounced similarity of the IR spectra (Figure 6) and the small positional difference of the ν(CO) mode (table S3) indicates that the ionic arrangement in the structure of form H is very similar to that of form D.

In solid metal salts and complexes, the carboxylate ions can be present either as ionic species, or as monodentate, bidentate chelating or bidentate bridging ligands (Scheme 2). The corresponding bonding modes have been often correlated with the carbonyl stretching frequencies.[22] In return, the frequencies of the respective bands can be employed for predictions related to the bonding mode of the carboxylate group in crystals of unknown structure. In particular, the frequency split between the antisymmetric carbonyl stretch ν_{as}(CO), usually appearing in the region 1650–1550 cm^{-1}, and the symmetric carbonyl stretch ν_s(CO), observed in the region 1450–1360 cm^{-1}, is a convenient semi-quantitative indicator for distinction between these binding modes.[23] For ionic carboxylates, the frequency difference $\Delta\tilde{\nu} = \tilde{\nu}[\nu_{as}(CO)] - \tilde{\nu}[\nu_s(CO)]$ is ~164–171 cm^{-1}. When the carboxylate group acts as monodentate ligand, the difference is larger, up to ~565 cm^{-1}, while in the case of bidentate chelating carboxyl ligands, the difference is smaller with respect to the ionic carboxylates and it can be as small as 42 cm^{-1}.[23] For bidentate bridging carboxylate ligands the difference is usually within the range for ionic type. A practical burden to these spectra–structure correlations represents the band assignment of the symmetric stretch, which is inherently weak and occasionally obscured by bands from CH_3 deformations.[24] In the case of the solid forms of sodium valproate, the difference between the two carbonyl stretches ranges from 121 cm^{-1} up to 154 cm^{-1} (Table 1). Application of the above criterion indicates that all forms contain bidentate chelating carboxylate ligands, which is supported by the structure of form C.[19]

Table 1. Frequency splits between the antisymmetric and symmetric carbonyl stretch in the FTIR spectra of the solid forms of sodium valproate.

Form	$\tilde{\nu}[\nu_{as}(CO)]$ [cm^{-1}]	$\tilde{\nu}[\nu_s(CO)]$ [cm^{-1}]	$\Delta\tilde{\nu}$ [cm^{-1}][a]
A	1553	1413	140
B	1570	1416	154
C	1554 (1690)[b]	1415	139
D	1555	1413	142
E	1562 (1692)[b]	1441	121
F	1554 (1682)[b]	1420	134
G	N/A[c]	N/A[c]	N/A[c]
H	1552	1417	135

[a] $\Delta\tilde{\nu} = \tilde{\nu}[\nu_{as}(CO)] - \tilde{\nu}[\nu_s(CO)]$. [b] ν_{as}(CO) mode of valproic acid. [c] These values cannot be determined due to the instability of the compound which appears as intermediate during the thermal decomposition.

2.4. Differential scanning calorimetry

The cyclic (heating–cooling) DSC traces in Figure 8, forms A, B and C exhibit different thermal behavior, although the thermal events appear in similar temperature ranges. Two regions can

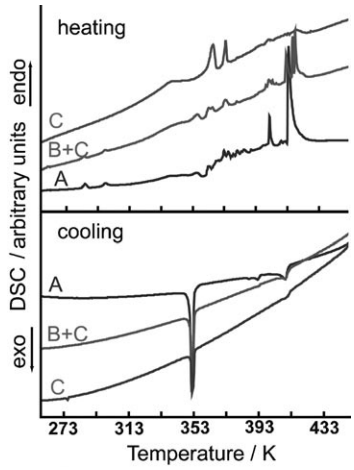


Figure 8. DSC traces recorded on heating (first heating run) and cooling (first cooling run) of pure form A, a mixture of forms B and C, and pure form C.

be discerned, low-temperature (LT) region below ~297 K and high-temperature (HT) region above that point. In the LT region form A shows two weak endothermic peaks (284 and 297 K), while pure form C (from ethyl acetate) does not undergo any thermal events. As the mixture of forms B and C (obtained from acetone) also exhibits the two peaks (at nearly identical temperatures with form A), they are assigned to form B. The small intensity and the temperature of appearance of these peaks indicate that they are related to evaporation of the adsorbed water or to enthalpy relaxation processes. This result is supported by the observed relative stability of forms A, B and C. When exposed to air at ambient conditions, forms A and B adsorb moisture and turn into a syrupy mass after several minutes, while form C is stable for at least several hours. The lesser hygroscopicity (higher stability) in air of form C and the smaller amount of adsorbed water may be the reason for the absence of thermal effects in this region. In the second heating run of the mixture of forms B and C (Figure 9) these effects are absent in the LT region. Therefore, it seems more probable that the effects are due to evaporation of the absorbed water than to relaxation processes.

The HT region of all forms is very complex. To aid the assignments of the DSC peaks, the DSC effects of form C, which is of known and well-defined composition, were correlated with its decomposition profile studied by TG/DTA (figure S1). Detailed interpretation of the TG/DTA profile of form C was intricate due to poor separation of the initial thermal processes. Heating up to 523 K causes evaporation of the water and thermal decomposition of the clusters. In the first stage, the salt is dehydrated (DSC peak at 339 K, Figure 8). At higher temperatures, the DSC and DTA curves exhibit a pair of peaks (365 and

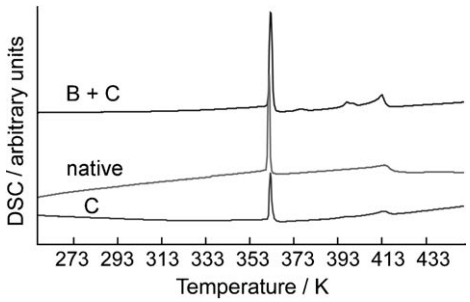


Figure 9. Second heating run (DSC) on the samples of form C (from ethyl acetate), mixture of forms B and C (from acetone) and the native (commercial) sample of sodium valproate, which is identical to form A.

373 K by DSC, 367 and 371 K by DTA) corresponding to the onset of evaporation of half a molecule of the acid. The process is completed around 413 K with formation of the metastable semi-solvate $Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)_{0.5}$, denoted form G, which decomposes at higher temperatures. Based on the calculated and observed weight losses, the thermal behavior of form C can be represented as:

I stage (331–339 K):

$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot H_2O$ (form C)→
$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2) + H_2O$
weight loss: exptl 3%, calcd 2.7%

II stage (344–412 K):

$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)$→
$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)_{0.5} + 0.5\,C_8H_{16}O_2$
weight loss: exptl 9%, calcd 11.2%

In the HT region the DSC curve of form A undergoes several events (363–385 K, 401 K, 413 K). Because form A does not include acidic molecules in its structure all events are related to evaporation of water and/or thermal processes related to sodium valproate. In the HT region, the mixture of forms B and C exhibits several endothermic events which are prescribed to form C. Of these, the shoulder at 339 K probably corresponds to evaporation of water, while the peaks around 365 K and 373 K are due to partial evaporation of the acid. Similarly to form A, the effects at higher temperatures are related to thermal changes of sodium valproate. On cooling, forms A, B and C have identical DSC profiles, which means that they are transformed into the same form, denoted form H, of composition $Na(C_8H_{15}O_2)$ established by elemental analysis. The FTIR spectrum of form H confirms the absence of water. Actually, the absence of water vibrations is the only significant spectral difference with form D (obtained by evacuation of forms A, B and C), indicating that except for the presence of water, they have similar molecular arrangements. The exothermic peak (in the cooling run) at 351–352 K can be prescribed to crystallization of the anhydrous salt. Therefore, it appears that after the partial decomposition of the salts on heating, all residues melt and on cooling they are converted into the same, more stable form of sodium valproate—form H. The DSC curves of the second heating runs following cooling to 253 K are identical for the mixture of B and C, pure C and the native compound, showing that form H can be obtained by heating and subsequent cooling of forms A, B, C and the native compound (Figure 9).

Being stable adducts of sodium valproate and valproic acid, forms E and F exhibit different thermal behavior (Figure 10). Dehydration of form F, a dihydrated 3:1 adduct of valproic

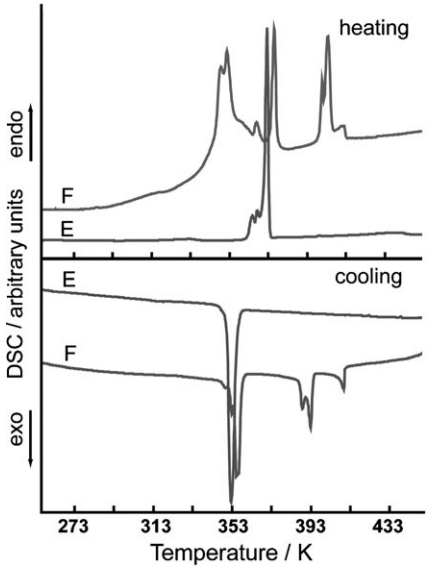


Figure 10. DSC traces recorded on heating (first heating run) and cooling (first cooling run) of the 1:1 (form E) and 3:1 (form F) adducts of sodium valproate with valproic acid.

acid, is a two-step process. The first step represents evaporation of 0.5 molar equivalents of water at 352 K, corresponding to two endothermic peaks (348 K and 351 K) on the DSC curve. This process is followed by immediate evaporation of the remaining water, a process which is completed at 407 K (DSC peaks at 367 K, 375 K, 400 K and 403 K). The thermal decomposition of the anhydrous salt starts around 410–411 K and is completed at 440 K, by formation of the metastable semi-solvate, the form G. According to the TG/DTA analysis (figure S2), the thermal decomposition of form F can be represented as:

I stage (351–407 K):

first step:
$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot 2\,H_2O$→
$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot 1.5\,H_2O + 0.5\,H_2O$
weight loss: exptl 1.4%, calcd 1.3%

second step:
$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)\cdot 1.5\,H_2O$→
$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2) + 1.5\,H_2O$
weight loss: exptl 3.6%, calcd 4%

II stage (410–439 K):

$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)$→
$Na_3(C_8H_{15}O_2)_3(C_8H_{16}O_2)_{0.5} + 0.5\,C_8H_{16}O_2$
weight loss: exptl 10%, calcd 11.2%

On heating, form E exhibits three endothermic peaks (Figure 10). According to the IR spectra, this form is anhydrous, so that the two weak LT peaks (364 K and 367 K) correspond to decomposition of the solvate. The HT endothermic peak at 372 K represents evaporation of the valproic acid, because on cooling form E exhibits identical thermal behavior as forms A, B and C (single strong exothermic peak at 352 K owing to the crystallization to the same stable form), which do not contain acid molecules, corresponding to the form H. It is interesting that only form F exhibits different DSC pattern on cooling compared with the other forms. This is probably because of the different structural preferences of the product obtained by cooling of form F (Figure 10).

2.5. Theoretical calculations

2.5.1. Optimized structures

To assign the vibrational spectrum of the valproate ion and to make spectra–structure correlations, its molecular conformations were investigated on an in vacuo single molecular model. A set of eight (including two mirror isomers) optimized molecular structures was obtained (Figure 11).

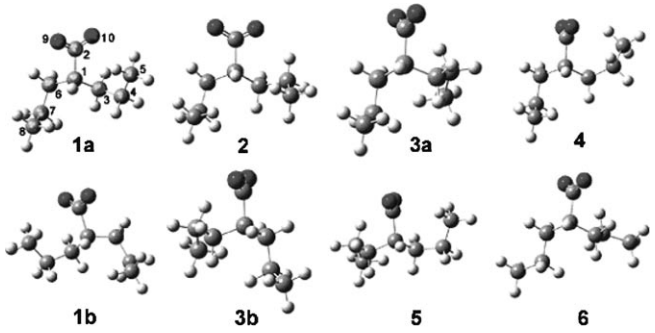


Figure 11. DFT-optimized molecular structures of the valproate ion. The pairs of mirror isomers are denoted **1a**/**1b** and **3a**/**3b**.

Molecular structures **1a** and **2** were obtained by rotation around C4–C5, while structures **1b**, **3a** and **4** were obtained by rotation around C1–C3. In the case of structures **3b** and **5**, the alkyl substituents were rotated around C1–C3 and C1–C6, respectively. The molecular structure **6** was obtained during the attempts to optimize a structure with the characteristic "vvv" shape observed in the crystal of form C. The relevant bond lengths, angles and dihedral angles are deposited as table S4. The optimized structures generally differ in the position of carboxylate group and in the dihedral angles formed by the alkyl chains and the carbonyl groups, while the differences in the bond lengths and angles are insignificant.

2.5.2. Vibrational spectra and spectra–structure correlations

The IR spectra of the valproate ions calculated on the optimized structures at the B3LYP/6-31G(d,p) level are presented in Figure 12. Although the overall appearance of the theoretical spectra of the eight structures is similar, there are minor differences in the position, number and shape of the bands. Al-

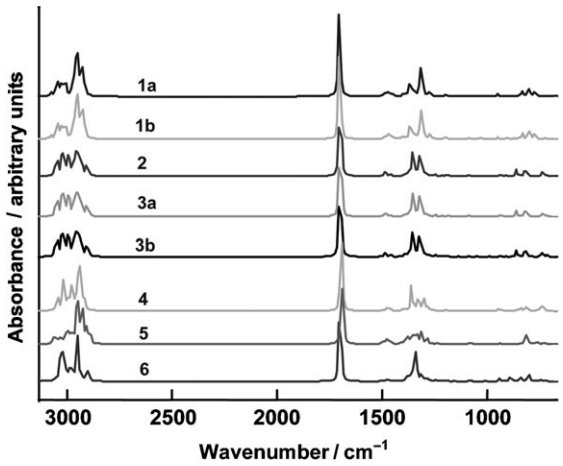


Figure 12. Scaled theoretical IR spectra of the eight optimized structures of the valproate ion.

though structure **2** has slightly different structural features compared with structures **3a** and **3b**, their spectra are very similar. To distinguish between different conformations of the alkyl chains of the valproate ion, very small spectral differences need to be considered.

The eight optimized molecular structures afforded five different theoretical spectra (Figure 12). Inspection of the structures that have identical spectra (structures **1a** and **1b** and structures **3a** and **3b**) shows that these structures represent mirror isomers (e.g., the absolute value of the dihedral angle C5–C4–C3–C1 in structure **1a** is identical with the dihedral angle C8–C7–C6–C1 in structure **1b**). Although the alkyl conformation in structure **2** is only slightly different from structures **3a** and **3b**, (for **2**, C1–C2 = 1.593 Å and C3–C1–C6 = 112.7°; for **3a**/**3b**, C1–C2 = 1.583 Å and C3–C1–C6 = 115.6°), their spectra are nearly identical.

The theoretical spectrum of the valproate ion consists of three major regions (Figure 12). The high-frequency region (3076–2904 cm^{-1}) is characterized by the appearance of four to six weak bands originating from the CH stretches of the CH, CH_2, and CH_3 groups. The experimental spectra of the sodium valproate forms did not exhibit any bands due to the CH stretches above 3000 cm^{-1}. Owing to the band overlap and the inherently low intensity, this spectral region does not have sufficient discriminative potential for making structural predictions. The most dominant band in the second region, between 1693 cm^{-1} (**5**) and 1705 cm^{-1} (**1a** and **1b**), is the antisymmetric carbonyl stretch of the carboxylate group. The very small frequency difference in the calculated spectra is expected from the similarity of the predicted C–O bond lengths.

The measured spectra of forms A and B each exhibit one carbonyl stretching band (1553 and 1570 cm^{-1}, respectively), indicating that there is a single crystallographic type of carbox-

ylate ions in their structure or that all carbonyl groups are structurally similar. In accordance with the crystal structure, the measured IR spectrum of form C shows two different CO stretches, arising from the valproate ions and the corresponding acid (1554 cm^{-1} and 1690 cm^{-1}, respectively) (Figure 3). Similarly, the solvates E and F give rise to CO multiplets (Table 1, Figure 5, and Figure 7). Each of the native (un-recrystallized) compound, form D and the anhydrous form H exhibit single carbonyl stretching bands. The number of CO bands can be therefore employed for distinction between the pure sodium valproate salts and their solvates with valproic acid.

The low-frequency region ($<1500\ cm^{-1}$) in the predicted spectra contains weak bands originating from the deformation modes of the CH_2 and CH_3 groups, and of the symmetric carbonyl stretch of the carboxylate group. Structures **1a** and **1b** exhibit a triplet in this spectral region: the band at 1470 cm^{-1} originates from CH_2 deformation, the one at 1368 cm^{-1} is due to the symmetric carbonyl stretch, and the weak band at 1314 cm^{-1} corresponds to the CH_3 deformation mode. In the case of structures **2**, **3a** and **3b** the band from the CH_2 deformation is positioned at 1486 cm^{-1}, the symmetric CO stretch at 1353 cm^{-1} and the CH_3 deformation appears at 1320 cm^{-1}. Calculated relative ratio of band intensity of the symmetric CO stretch and the CH_3 deformation in the spectra of structures **2**, **3a** and **3b** is close to one, while in the case of structures **1a** and **1b** the band arising from the deformation CH_3 vibration is twice as strong. Structure **4** exhibits four bands in this region, with an additional band at 1303 cm^{-1}. Structure **5** shows weak and overlapped quintet of bands, and structure **6** shows only one strong band at 1342 cm^{-1}. The experimental spectra of sodium valproate forms have three bands in this region, but contrary to the theoretical spectra, the CH_2 deformation is a poorly resolved doublet (Figures 3–7). There is a pronounced similarity between the theoretical and experimental spectra in the cases of structures **1a**, **1b**, **2**, **3a** and **3b.**

The orientation of the carboxylate group relative to the alkyl backbone was also studied. The calculations showed that the (gas phase) energy differences among the various conformations of the alkyl chains of the valproate ion are of order of 0–1 kcal mol^{-1} only. At the global minimum the examined angle C3–C1–C2–O10 amounts to 78.8° (Figure 13) and represents the most stable orientation of the carboxylate group relative to the alkyl backbone.

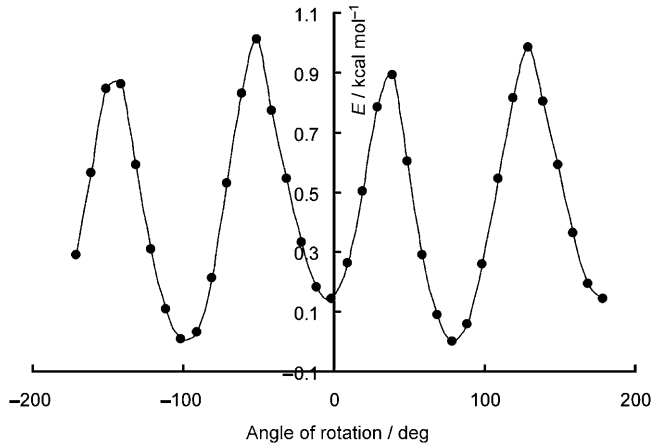
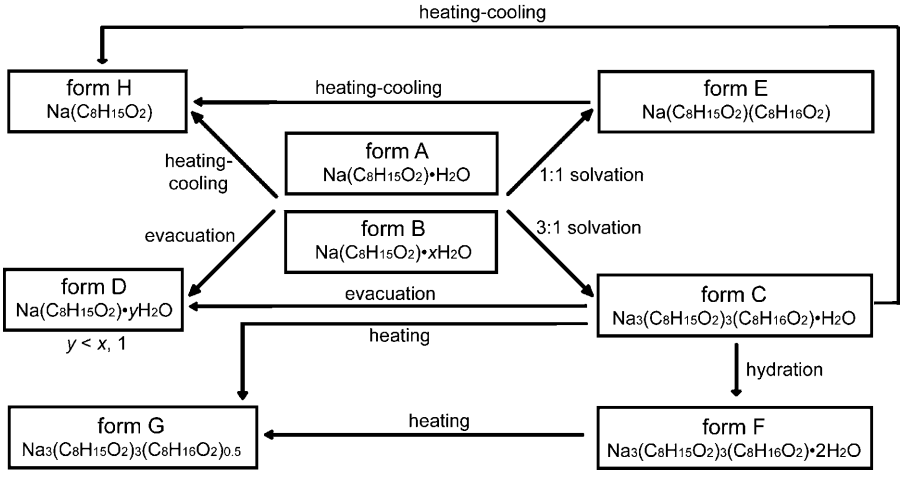


Figure 13. Profile of the relative energy as a function of the dihedral angle (C3–C1–C2–O10) of the carboxylate group.

3. Conclusion

The detailed screening for solid forms of sodium valproate showed that the compound can be present as eight solid forms, including one metastable intermediate produced during thermal decomposition. The structural relations among these compounds are presented in Scheme 3. Two hydrates (A and B) are obtained by slow evaporation from organic solvents and an additional lower hydrate (D) can be prepared by evacuation. Three solvates of sodium valproate with valproic acid are prepared by thermally assisted solvolysis (C) or by direct reaction with the acid (E and F). The anhydrous salt (H) is obtained by heating and subsequent cooling of forms A, B, C and E. A semisolvate with valproic acid (G) is obtained as an unstable intermediate by heating forms C and F. The instability and pronounced affinity towards moisture in air of some of these compounds burdens their complete structural characterization. The attempts to calculate the cell parameters using powder X-ray diffraction in those cases were also unsuccessful, due to the poor crystallinity of the compounds and the inherent absence of sufficient number of strong and well resolved peaks in their diffraction patterns (for example, see the diffraction patterns deposited as figure S3), and the result did not improve even by using high-intensity synchrotron X-ray radiation.

Of particular interest to pharmaceutical applications is the relative stability of the solid-state forms of sodium valproate. Under ambient conditions, forms A and B as well as the com-



Scheme 3. Routes of interconversion among the various solid-state forms of sodium valproate.

mercially available compound appear as very hygroscopic white powders. Form C, which crystallizes as prismatic crystals, is less hygroscopic, while forms E and F are not hygroscopic and are stable at ambient conditions. Form F cannot be dehydrated by moderate evacuation. Partial stabilization of forms A and B can be practically achieved by evacuation and pressing, which results in the lower hydrate D, or after a heating–cooling cycle, resulting in crystallization from the melt of the anhydrous form H. Addition of one molecule of valproic acid and saturation with one molecule of water of the remarkably hygroscopic forms A and B results in the less hygroscopic form C. Addition to form C of a second water molecule affords form F which is not hygroscopic and is indefinitely stable.

Examination of the orientation preferences of the carboxylate group relative to the alkyl backbone studied by extensive DFT calculations[25] shows very low energy differences among the various conformations of the alkyl chains. The flexibility of the aliphatic chains, together with the structural misfit imposed by the shape of the anion, can cause temporary opening of channels in the structure for penetration of water molecules, which appears as strong affinity for absorption of humidity. Various degrees of saturation of the hydrogen bonding potential of the valproate carboxylate group(s) with protons from the carboxylic group(s) of the acid, which can be achieved by co-crystallization of the salt with valproic acid or other acids, can result in partial or complete stabilization of the compound.

Experimental Section

Materials: Sodium valproate of more than 98% purity was purchased from Wako Chemical Co. (Japan). The purity of the commercial sample was confirmed by drying and potentiometric titration, yielding 0.2% water and 99.8% of valproate. Valproic acid was prepared by treating 0.5 g sodium valproate with 4 mL hydrochloric acid (1.9 $mol \cdot L^{-3}$) and heating at 318–323 K for 20 min. The acid was recovered by separation of the upper organic layer or by extraction with pentane. The purity of the product was confirmed by comparison of the IR spectrum with that of a commercially available sample and with the spectra stored in the SDBS spectral database.[26]

Preparation of the solid forms of sodium valproate

Form A: Form A was prepared by dissolving 0.15–0.20 g sodium valproate in 2 mL stirred ethanol or THF at 333 K without saturation, followed by warm decantation and slow evaporation at 280 K. The dry salt is very soluble in ethanol and slightly less in THF. Prior exposure to air or moisture results in significantly decreased solubility. The first crystals of the product appear immediately after cooling of the solution, and the process is completed after the solvent has evaporated completely after about two weeks. White needle-like crystals of poor quality were obtained (figure S4), which are very hygroscopic and appear unstable in air above 288 K. Saturation of the ethanol or THF solution with the salt results in a syrupy mass without crystals. Fast evaporation in an ice bath did not improve the result.

Form B: Hot saturated acetone solution of sodium valproate was prepared by dissolving the salt until saturation in 4 mL warm acetone which was vigorously stirred at 318 K. The suspension was quickly filtered and the filtrate was stored at 208 K. The first crystals appeared in several hours, and the solvent evaporated completely after about one week. As the white powdery residue after the evaporation did not contain any crystalline material, it was redissolved in 4 mL hot acetone and slowly evaporated under the same conditions. The product thus obtained was a mixture of form B appearing as white powder and colorless crystals of form C (figure S4). Above 288 K both forms are hygroscopic in air and rapidly turn into a semi-solid mass. Mechanical separation of the two phases proved very difficult, and neither preparation of unsaturated solutions nor repeated recrystallization improved the result. The conditions of crystallization were carefully optimized to obtain high-quality crystals of form C. While it was possible to isolate crystals of form C with sufficient quality for X-ray diffraction, separation of the pure form B was precluded by the remarkable hygroscopicity of the mixture.

Form C: Pure form C was prepared by slow evaporation from hot saturated solution of sodium valproate in ethyl acetate. Sodium valproate was gradually added to 3 mL stirred cold ethyl acetate, the mixture was gently heated up to 303 K and then more of the salt was added to saturation. The suspension was filtered quickly and the solution was stored at 280 K. Small white needle-like crystals of poor quality appeared after several days (figure S4).

Form D: Whenever forms A, B and C were pressed and evacuated with KBr using oil pump (for IR measurements), they all transformed to the same form, denoted form D.

Form E: Form E was prepared by scaling down the previously described procedure.[14] To 0.83 g sodium valproate, 5 mL hot acetone (323 K) was added and the mixture was gently shaken while heated. Valproic acid (0.39 mL) was added to the solution, whereupon the precipitate dissolved. The clear solution was ice-cooled, resulting in a voluminous precipitate after 10–15 min. The solid was filtered out and washed several times with cold acetone (273 K). After being dried over silica gel for an hour, the product appeared as white crystalline aggregates of insufficient quality for X-ray diffraction (figure S4). The substance is stable in air and is not hygroscopic under ambient conditions.

Form F: In absence of details on the synthesis of this compound in the literature,[14] a similar synthetic procedure as in the case of form E was applied. 3 mL hot acetone (323 K) were added to 0.315 g sodium valproate. The heated mixture was shaken, treated with 0.1 mL valproic acid and ice-cooled, resulting in white gelatinous product after ~15 min. The solid was filtered out and treated in the same way as form E. The product appears as amorphous white powder (figure S4). The reactant ratio is critical for the success of the procedure; IR examination of the reproducibility showed that occasionally poorly crystallized form E has been obtained. Form F does not appear hygroscopic under ambient conditions.

Form G: Form G is a valproic acid semisolvate obtained as an unstable intermediate by heating form C or form F and detected during the TG analysis.

Form H: Form H was obtained as anhydrous salt obtained by heating and subsequent cooling of forms A, B, C and E (figure S4). The samples were cycled between room temperature and 453 K (vide infra).

IR spectroscopy: The FTIR spectra were recorded from KBr pellets and Nujol mulls at ambient temperature with Perkin–Elmer System 2000 spectrometer. In most cases, the spectra depended on the

sample preparation. The spectrum of valproic acid was recorded from a small amount of liquid cast on KBr pellet.

Thermal analysis: The DSC measurements were carried out under dynamic nitrogen atmosphere with Perkin–Elmer DSC7 instrument using open aluminum pans, with heating–cooling cycles from 253 K up to 453 K and back to 253 K at heating/cooling rate of 10 K min^{-1}. The TG, DTG and DTA curves were recorded in the 303–523 K range under dry nitrogen atmosphere using a Perkin–Elmer Diamond TG/DTA analyzer with ceramic/aluminum sample pans.

Elemental analysis: The C and H contents were determined by standard methods at Osaka University on samples that have been stored in refrigerator. Atomic Absorption Spectrometry analysis of the sodium content of forms E and F was conducted with Varian SpectrAA 55B atomic absorption spectrometer using sodium lamp (589.0 nm, $I = 5$ mA, slit = 0.5 nm) and acetylene/air flame. The metal content was determined by using a standard calibration curve (figure S5).

Theoretical calculations: Around 30 molecular structures of the valproate ion were constructed by multi-step geometry changes which included systematic rotations of the alkyl groups and initial optimization using the MM$^+$ procedure. Each structure was then re-optimized at the AM1 level, inspected for negative vibrational frequencies by single point runs and a set of unique real geometrical minima was built. This set of structures was used as input for further optimization with Gaussian03 series of programs[25] employing the Becke–Lee–Yang–Parr method with the 6-31G(d,p) basis set [B3LYP/6-31G(d,p)]. The geometry optimizations afforded eight molecular structures (including two mirror isomers) of the valproate ion, for which the harmonic vibrational spectra were calculated. The raw vibrational frequencies were linearly scaled by 0.98, a factor obtained by comparison of the theoretical and experimental value of the carbonyl stretching frequency. The barriers for conformational changes of the valproate ion and the respective geometries in gas phase were examined by relaxed scans of the potential energy by a 360° rotation of the carboxylate group (i.e. around the dihedral angle C3–C1–C2–C10 in Figure 10) in 10° increments and complete optimization of the rest of the molecule.

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Keywords: anticonvulsants · clusters · hydrates · polymorphism · sodium valproate · valproic acid

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