Araştırma Makalesi / Research Article

Investigation Into The Molecular Stability, Synthesis Mechanism, and Formation of Some Norcantharimide Derivatives Using AcCl or Ac₂O: A Mechanism-Based Study

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Abstract
The aim in this study was first to explain, in detail the conversion of diacetates over time into chlorinated monoacetates following ether cleavage with AcCl of norcantharimide derivatives with the help of the NMR technique and second, to verify this conversion theoretically and computationally. Ether cleavage reactions of N-methyl, N-benzyl, and N-acetoxyethyl-substituted norcantharimide derivatives were performed with Ac₂O or AcCl in the presence of H₂SO₄, and the mechanisms of these reactions were elucidated in detail. According to the ¹H NMR analyses of aliquots from the reactions with AcCl, trans-1,4-diacetates formed firstly. Upon the continuation of the reaction, trans-1,4-diacetates transformed into trans-1,2-chloroacetates via an S₅' mechanism. Additionally, this explanation was further supported by the soft theoretical and physical calculations.

Keywords
Chlorinated products; Norcantharimide; Amines; S₅' mechanism

1. Introduction
Cantharidin is a natural compound, and its use as a potential anticancer agent dates back to 1264 (Nickolls and Teare 1954, Wang 1989, Lin et al. 1998). This molecule belongs to the terpenoid class, which is used for curative purposes in numerous treatments in traditional Chinese medicine (Wang 1989). Cantharidin and its structural derivatives norcantharidin (1) and, norcantharimide (2) and their analogs 3 and 4 (Figure 1) attract biomedical interest due to their small size, easy modifiability, and ability to pass through cell membranes without requiring energy. Although cantharidin is suitable for use for chemotherapeutic purposes, it has limited usage due to its known toxic effects (Tagwireyi et al. 2000). Therefore, there is a need for new cantharidin derivatives with increased...
therapeutic effect and reduced toxicity. For this purpose, scientists have long focused on the synthesis of these new derivatives and on studies to improve the products’ therapeutic index.

Figure 1. Molecular structures of norcantharidin (1), norcantharimide (2), isoindoline-1,3-dione (3), and norcantharimide analogue 4.

Norcantharidin (1), norcantharimide (2), isoindoline-1,3-dione (3) and its analogs 4 are preferred due to their small molecular structures, ability to be synthesized by short-step syntheses, using cheap and simple chemical methods, and the above mentioned biochemical properties. It is possible to synthesize norcantharimides from cantharidin and norcantharidin, which are structurally anhydrides, using primary aromatic/aliphatic amines and thus the synthesis of norcantharimide derivatives having potentially different bioactivity becomes possible. Recently, Kara and co-workers synthesized norcantharimide analogs containing different functional groups on the cyclohexane ring starting from 3-sulfolene. They also investigated the synthetic routes for norcantharimide derivatives and their photophysical properties (Tan et al. 2011 and 2014). In this study, the ether cleavage reaction mechanism of norcantharimide derivatives using Ac₂O and AcCl was examined in detail. The compounds used were synthesized and characterized by the NMR technique previously (Köse et al. 2017 and 2020). Here, detailed NMR spectroscopy studies depending on the specified reaction time intervals, conversions between of trans-1,4-diacetates and trans-1,2-chloroacetates, and detailed and descriptive synthesis mechanisms of the products were discussed and explained, unlike in the previous work (Köse et al. 2020). Moreover, the products that formed at specified time intervals were identified and the data from these conversions were graphed. All reactions are solvolysis reactions. In contrast to our previous work, in this study, soft density functional theory (DFT) studies and computational calculations were performed. Computed energies of the products in the reactions were calculated using theoretical data (Köse et al. 2020). Physical and theoretical formation energy calculations were performed to better explain the mechanisms of the products obtained.

2. Materials and Methods

The experimental procedures for all products have been reported in detail in our previous papers (Köse et al. 2017 and 2020). The reader is invited to access this information for additional data concerning this article.

2.1 General

All chemical solvents and reagents were used as received (Sigma-Aldrich). NMR spectra were recorded in CHCl₃-d₄ using a 400 MHz Bruker spectrometer for ¹H NMR and a 100 MHz Bruker spectrometer for ¹³C NMR. Thin-layer chromatography (TLC) was visualized using UV light.

2.2 General procedure for the ether cleavage step with Ac₂O

Corresponding tricyclic imides (1.0 g) were dissolved in Ac₂O (5 mL) and 3-4 drops of H₂SO₄ was added at room temperature. The resulting solution was stirred at the times indicated in Figures 5a and 5b at rt. Aliquots were occasionally taken from the reaction medium to determine the consumption of tricyclic imide. Upon completion of diacetate formation, the mixture was concentrated under reduced pressure. The crude product was crystallized from dichloromethane/hexane. The crystals were obtained by filtration.

2.3 General procedure for the ether cleavage step with AcCl

To a stirring solution of corresponding tricyclic imides (1.0 g) in CH₂Cl₂ (10 mL) were added AcCl (4 mL) and 3-4 drops of H₂SO₄ at room temperature. The resulting solution was stirred at the times indicated in Figures 5a and 5b at rt. Aliquots were taken from the reaction medium to determine the
conversion from diacetate to chloroacetate. After the conversion of diacetate (indicated by $^1$H NMR), the mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was diluted with CH$_2$Cl$_2$ and crystallized with hexane. The crystals were filtered and washed with fresh hexane.

3. Results and Discussions

3.1. Overview

The aim in this study was to prove that the chlorinated monoacetate products (5b-9b) forming during the ether cleavage reaction with AcCl proceed through the diacetate products (5a-9a), which form firstly, and to demonstrate this transformation spectroscopically. In accordance with this purpose, Ac$_2$O only was firstly used for the ether cleavage of the starting compounds (5-9) and it was determined that trans-1,4-diacetates were the products. When these starting compounds were treated with AcCl, diacetates, which formed in the previous reaction, were determined first. For this, aliquots were taken from the reaction medium with AcCl at specific times and their NMR spectra were examined. A careful examination of the spectra showed that the diacetate product formed in the reaction medium firstly. As the reaction proceeded, the diacetate transformed into the chloroacetate gradually and then finally was completely consumed. This conversion, which was determined using NMR integration data, was observed with N-methyl-, N-benzyl- and N-acetoxyethyl-substituted norcantharimide derivatives. The other two derivatives (N-ethyl and N-phenyl) were excluded from this work since they have not been studied.

Baran et al. conducted cleavage reactions with internal ethers in an acidic medium. In their research, instead of 1,2-chloroacetates, 1,4-chloroacetates were formed as the sole product. They performed an $S_{N}$ reaction and cis-1,4-
chloroacetates were synthesized (Baran et al. 2003 and 2004). By application of their methodology, the etheric bonds in norcantharimide derivatives were subjected to cleavage reactions using Ac₂O or AcCl. The products and their formation mechanisms resulting from the ring-opening reactions with Ac₂O and AcCl appear to be as described in mechanisms 1 and 2 in Figure 2. Since the formation of the product in the cleavage reaction with AcCl took a long time, a detailed study was designed to establish how this product formed, unlike in the previous article (Köse et al. 2020). Surprisingly, it was found with the help of the ¹H NMR spectra that the trans-1,2-chloroacetate formed in this reaction according to the SN₂’ mechanism progressed from the trans-1,4-diacetate that formed first as in the cleavage reaction with Ac₂O, as well.

Figure 3. The structure of the expected stereoisomer products 16a-20a, 16b-20b, 16c-20c, and 16d-20d at the end of the reaction time in the reaction with AcCl.

First, when the NMR results were analysed, it was thought that the reaction mechanism proceeded as in Figure 3 because the NMR results and the expected products were in agreement. However, due to doubt, the structures were crystallized and X-ray analysis was performed (Köse et al. 2020). Here, it was seen that the diastereomeric products in Figure 4 were formed instead of the expected diastereomeric products in Figure 3.

Looking at the structures of the products of 5-9 it can be clearly seen that this tricyclic compound has an axis of symmetry and therefore bridgehead carbons and protons are identical and have the same steric area (Figure 4). Due to this feature, AcCl attacked both bridgehead carbons equally and the symmetry was broken. Following the formation of enantiomeric 5a'-9a' and 5a''-9a'', a chlorination reaction occurred (Figure 4). If the chloride had attacked the carbons where the acetates were attached according to the expected synthesis routes in Figure 3, the formation of the regioisomers in Figure 3 would have been required. However, the reaction did not progress that way. The chloride ion attacked the olefinic carbons according to the mechanism in Figure 4 and the products were formed as an enantiomeric mixture according to the SN₂’ mechanism. The attack direction of the chloride was determined by X-ray analysis in our previous study. Based on the results of that analysis, we proved that the five membered ring and chlorine atom were trans in that article. Moreover, the steric hindrance is lower at the endo-directed attack of chloride. If chloride had attacked from the exo face of 5a'-9a' and 5a''-9a'', product 17 would have been formed (for 17 see table 1). There was some information lacking about the detailed mechanism of the formation and conversion from 5a'-9a' and 5a''-9a'' of these enantiomers (5b'-9b' and 5b''-9b'') in the earlier paper (Köse et al. 2020). In this paper, this mechanism was elucidated. The conversion times and quantitative ratios of the trans-1,2-chloroacetate products were determined by NMR spectroscopy. Additionally, the reason why the
expected products/isomers in Figure 3 did not occur was proved by DFT analysis.

3.2 Detailed Reaction Tracing

To further clarify the mechanism and conversion, aliquots were taken from the reaction medium at regular periods during the formation of the chloroacetate with AcCl and NMR analyses of these samples were performed. It was seen that the diacetate product formed in the reaction medium firstly. As the reaction continued, the heights of peaks belonging to the diacetate decreased and the heights of peaks belonging to the chloroacetate increased. Because of this conversion, the chloroacetate formed as a single product after consumption of the diacetate. It was deduced that the chloride performed a nucleophilic attack at C5 and C6 on $5a'-9a'$ and $5a''-9a''$ in the actual synthetic route instead of at C7 and C4 as in the expected synthetic route and therefore the chloroacetate formed following an $S_N2'$ mechanism as an enantiomeric mixture (Figure 4). Here it is clearly explained when the chloroacetate began to form and how long completion of the conversion took.

In the reaction that used AcCl, the reaction mixture was initially colourless, then turned light green, and after 8-10 h turned dark green under the daylight. This coloration indicated that the reaction was progressing from the diacetate product to the chloroacetate product. The work was carried out with internal ethers 5, 8, and 9.

Figure 5a. This column shows $^1$H NMR spectra taken from the reaction of 8 with AcCl at 1, 2, 7, 19, 24, 32 and 40 hours.

Figures 5a and 5b show the $^1$H NMR spectra of 5a, 5b and 8a, 8b, respectively, at the specified reaction times. The $^1$H NMR spectra seen in the Figure 5a and 5b were obtained from the reactions with AcCl of the two compounds (5 and 8) substituted with methyl and benzyl groups on the nitrogen under the same conditions. The spectra seen in the Figure 5a belong to compound 5 at reaction times 1, 7, 24, 32, 44, 67, and 90 hours in the presence of AcCl.
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Figure 5b. This column shows $^1$H NMR spectra taken from the reaction of 5 with AcCl at 1, 7, 24, 32, 44, 67, and 90 hours.

Those seen in the figure 5a belong to compound 8 at reaction times 1, 2, 7, 19, 24, 32, and 40 hours with AcCl. Before analysing these spectra, it was considered useful to determine which peaks belonged to which protons.

3.3 Analyses of NMR spectra

The signal at 6.3 ppm seen in both columns as a doublet of doublet (dd) belonged to the olefinic proton for both trans-1,2-chloroacetates (5b and 8b) and trans-1,4-diacetates (5a and 8a). The other olefinic protons H5 in 5a and 8a and H7 in 5b and 8b resonated at 6.05 ppm as a multiplet (m) and the signals overlapped. In the spectra in the left column, the signal at 5.5 ppm belonged to H4 for 5b. In the spectra in the right column, the signal of this proton was seen at 5.45 ppm for 8b (Figures 5a and 5b). This H4 proton belonged to the trans-1,2-chloroacetates (5b and 8b) and was also geminal to the acetate group. The signals seen at 5.4 ppm and 5.2 ppm in the left spectra belonged to H7 and H4 protons for 5a and 8a, respectively. These signals resonated at 5.37 ppm and 5.12 ppm in the right spectra. It was determined that trans-1,4-diacetate and trans-1,2-chloroacetate in 4:1 stoichiometry for an hour in the reaction of 9a and 9b. As the reaction continued, it was seen that the heights of the signals belonging to trans-1,4-diacetate decreased. Simultaneously, the heights of the signals belonging to trans-1,2-chloroacetate increased. According to this result, an intermolecular transformation from trans-1,4-diacetate to trans-1,2-chloroacetate was occurring and it was detected that trans-1,2-chloroacetate was synthesized from trans-1,4-diacetate. Moreover, based on the integrations in the NMR spectra, the ratio of trans-1,4-diacetate to trans-1,2-chloroacetate in the reaction mixture was observed to decrease proportionally with the reaction time. Integration bars are not specified in the spectra shown in Figures 5a and 5b. The reader is invited to refer to the spectra with integration provided in the supporting information section of the online version of this article.

As mentioned in detail in the mechanism part, the location of the double bond in 5a-9a shifted with the intermolecular transformation via the S_{N2}' mechanism. In spite of this migration, no substantial chemical shift was observed in the olefinic signals in the NMR spectra during the reaction. The signal at 4.5 ppm belonging to the H5 proton in 5b and 8b, which was in the geminal position with the chlorine atom and is shown in brown, is not specified in Figures 5a and 5b. Nevertheless, it was determined that the height of this signal increased...
proportionally with the signal of H4 at the same time.

3.4 Graphic depiction of the products

It was demonstrated by 1H NMR spectroscopy that the trans-1,2-chloroacetate product was synthesized in the ether cleavage reaction with AcCl formed from a trans-1,4-diacetate intermediate, which formed firstly for all reactions. The graphs display the concentration (%) of the conversion of trans-1,4-diacetate to trans-1,2-chloroacetate over time (Figure 6). These graphs were designed using the integration data in the 1H NMR spectra of the synthesized products because of the cleavage reactions of compounds 5, 8, and 9 with AcCl. A careful examination of the graphs shows that the concentrations of trans-1,4-diacetate and trans-1,2-chloroacetate were 1:3 for 5b and 5a, respectively, at the end of one hour of reaction time. This ratio was 1:1 between 8a and 8b and between 9a and 9b. As indicated in graph A, the concentration of trans-1,4-diacetate product 5a was 73% in the first hour and after 26 hours the concentration decreased to 22%. In the other graphs, the concentration of trans-1,4-diacetate product 8a was 13% for 8a and 6% for 9a at this time. This result indicates that it could be said that the synthesis of the trans-1,2-chloroacetate 5b was slower than that of other trans-1,2-chloroacetates 8b and 9b. The reason was thought to be the intramolecular Van der Waals interactions for 8. The extended synthesis time of trans-1,2-chloroacetate 8a from 8 showed that this interaction was quite strong compared to that of 8 and 9. The intermolecular conversion from trans-1,4-diacetate to trans-1,2-chloroacetate required 4 days for the reaction of 5, 40 h for the reaction of 8, and 32 h for the reaction of 9. As outlined in graphic C in Figure 6, the synthesis of 9b took less time compared to the syntheses of 5b and 8b, even though 9b was the most sterically bulky and substituted heteroatoms at nitrogen. Nevertheless, the formation time of 9b showed that this steric effect did not hinder the reaction. Additionally, when the amount of acid was increased, the synthesis time of trans-1,2-chloroacetate decreased but the aromatized product began to form.

3.5 Computational Part
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To further explain the formation of 5b and better understand the behaviour of 5a, DFT with the B3LYP functional (Lee et al. 1988) was used via the program Gaussian 09 (Frisch et al. 2009). All energies reported in the results and discussion were calculated at the B3LYP/6-311G(d,p) level and include unscaled zero-point vibrational energies.

Computational studies showed that the energy of trans-1,2-chloroacetate resulting from the transformation was lower than that of trans-1,4-diacetate, which first occurred in the reaction. The trans-1,2-chloroacetate product formed because of the reaction with AcCl, and the formation energies of the isomers of this structure are given in Table 1 in kcal/mol. This table shows that in the ongoing reaction with AcCl the attack aspects of the chloride ion that formed in the same reaction medium was supported exactly. Moreover, the stereochemistry and absolute configuration of these chlorinated products, formed as an enantiomeric mixture following the Sn2' mechanism, were verified by computational calculations. These computational results clearly revealed that compound 5b was preferable and the difference in energies between them proved this theoretically.

Table 1. Formation energies of all possible products (in hartree/particle), including Zero-Point corrections and relative energy differences (kcal/mol) for 5b, 17, 16a, and 18 respectively.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Computed structures</th>
<th>Energy values</th>
<th>Formation energy differences (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>-1242.26581230</td>
<td>9.1</td>
</tr>
</tbody>
</table>

4. Conclusion

In this study, the ether bonds of 5, 8, and 9 were subjected to cleavage reactions to give 5a, 8a, 9a, 5b, 8b, and 9b in the presence of Ac2O or AcCl and H2SO4 as catalyst. First, the ether cleavage reaction was performed using Ac2O and trans-1,4-diacetates (5a, 8a, and 9a) were synthesized. The mechanism of these reactions was explained in detail. Second, the ether cleavage reactions of 5, 8, and 9 were performed using AcCl and it was determined that trans-1,2-chloroacetates 5b, 8b, and 9b were the products instead of the expected cis-1,4-chloroacetates 16a-d and 20a-d. Studies such as 1H NMR and theoretical calculations were conducted to determine the mechanism. The results of these studies were discussed in detail. First, the mechanism of the reaction of 5, 8, and 9 with AcCl was explained by identifying and monitoring the
reaction intermediates and products with $^1$H NMR spectroscopy. It was determined that 5b, 8b, and 9b formed through the trans-1,4-diacetate intermediates 5a, 8a, and 9a in the reaction medium over time. The stoichiometric proportions of the products (trans-1,4-diacetate and trans-1,2-chloroacetate) formed because of the ether cleavage reaction with AcCl of 5, 8, and 9 were measured via integrals of $^1$H NMR spectra of aliquots taken from the reaction at certain time points. Based on these findings, trans-1,2-chloroacetate began to occur from the corresponding trans-1,4-diacetate over time via an $S_{N}2'$ mechanism. These results were reinforced by theoretical calculations. The formation energies of 5b and its isomers were calculated and it was determined that the formation of 5b was preferential computationally.

Caution: During the method described here, when an excess amount of AcCl is removed under reduced pressure, suffocating AcCl and HCl gases are released from the vacuum pump if not adequately trapped or ventilated in a fume hood. Appropriate precautions should be taken during this procedure.

Supporting Information: Aliquot NMR spectra, characterized spectra of the compounds, and computations with the B3LYP/6-311G(d,p) statistics for the optimized structures are provided in the Supporting Information

5. References


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