Chemistry of Succinimido Esters. IV*1.

A Facile Preparation of N-Succinimidyl Carboxylates
Using N,N'—Disuccinimidyl Carbonate

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A facile preparation of N-succinimidyl carboxylates (N-succinimidyl esters of fatty, substituted benzoic and aliphatic dicarboxylic acids) using N,N'-disuccinimidyl carbonate (DSC) was developed. The reactions of the acids with DSC in the presence of pyridine in acetonitrile gave the corresponding esters in good yield. N-Hydroxysuccinimide formed during a reaction and pyridine could be easily removed by treatment with water. No by-products, of course, could be detected by HPLC and TLC analyses. The present method has advantages of high yields and purities of the desired esters, and of convenience for preparation of the N-succinimidyl esters, compared with the method using N,N'-dicyclohexylcarbodiimide (DCC). The structures of the resulting esters were determined by mass spectrometry.

1 Introduction

N-Acloyxsuccinimidides (1) involving long chain acyl residue [CN=8~18, CN : carbon number of the acyl group in (1)] react specifically with amino group in sphingolipid bases such as sphingosine and dihydrosphingosines to give N-acyl compounds (ceramides)11,12. It is reported that (1) can be prepared by the coupling reaction of fatty acid and HOSu12 in the presence of DCC12 in aprotic solvents. However, (1) involving short chain acyl residues, especially CN=3~7, were not reported because the purification was not easy. In the previous papers33,34, it has been reported that (1) (CN=3~7) could be purified by the fractional crystallization, silica gel column chromatography, and recrystallization from an appropriate solvent and/or the combined treatment, and that (1) (CN=2~8) could be applied to N-acylation of amino compounds such as amino acids14 and aliphatic primary amines35, and the chemical modification of residual Lys groups in proteins35 as N-acyl reagents. However, yields of (1) by the method using DCC were not satisfactory (< 40%).

On the other hand, it is reported that amino acid esters react with DSC12 to give the corresponding N-succinimidyl esters without formation of any by-products16. In the present paper, we have studied the reaction of fatty acid with DSC in order to establish a convenient method for the preparation of (1), and...
applied it to the preparation of N-acyloxy succinimides (1) and N,N'-disuccinimidyl dicarboxylates [(2) and (3)].

2 Experimental

2.1 Materials

DSC (SP grade), acetonitrile (SP grade), hexane (SP grade) and aliphatic dicarboxylic acids (GR or EP grade) were purchased from Wako Pure Chemicals. Fatty acids (GR grade) and substituted benzoic acids (GR grade) were purchased from Tokyo Kasei or Nakarai Chemicals. Other solvents were purified by the ordinary method. Eluents for HPLC analyses were filtered with a 0.45 μm membrane filter (Toyo Roshi). Pre-coated TLC plates (Kieselgel 60) were purchased from Merck.

2.2 Analytical methods

TLC analyses were carried out by the previously reported method. HPLC analyses were preformed by using the same apparatus in the previous paper. Columns : Finepak SIL C18 (5 μm) 4.6×150 mm (JASCO) and FineSIL (10 μm) 4.6×250 mm (JASCO), flow rates : 0.5 and 1.0 mL/min for Finepak SIL C18 and 2.0 mL/min for FineSIL, detection wavelength : 210 or 254 nm, mobile phases : acetonitrile and acetonitrile containing 0.1% phosphoric acid for Finepak SIL C18, and 2% ethanol-hexane for FineSIL. MS analyses were performed by using the JEOL D-300 Mass Spectrometer. GC-MS (EI-MS) : column

2.3 Products

2.3.1 N-Acylloxy succinimides (1)

DCC Method — (1) were prepared by the same method in the previous paper.

DSC Method — A mixture of fatty acid (50 mmol), pyridine (50 mmol), DSC (50 mmol) and acetonitrile (150 ml) was stirred at room temperature. Since the acids were not dissolved completely in the cases of (1 g)−(1 k), the reactions were performed at 50°C. DSC was consumed for about 3~5 h of stirring. After 12~18 h of stirring, the reaction mixture was concentrated to about 50 g and the residue was treated as follows : In the cases of (1 a) and (1 e)−(1 k), the concentrated solution was poured into 300 ml of cold water, precipitating colorless crystals which were filtered, dried in vacuo and recrystallized from an appropriate solvent. In the cases of (1 b)−(1 d), the concentrated solution was poured into a 100 ml of cold water. The compounds were extracted with three 100 ml portions of chloroform. The extracts were washed with five 100 ml portions of water, dried over anhydrous sodium sulfate, evaporated and dried in vacuo.

2.3.2 N-Succinimidyl benzoates (2)

DCC Method — To a mixture of HOSu (0.1 mol), substituted benzoic acid (0.1 mol) and dioxane (100~400 ml) was added a DCC (0.1 mol/100 ml dioxane) solution, precipitating colorless urea compound. The mixture was stirred for further 10~16 h. The reaction mixture was filtered to remove the urea, and washed with three 30 ml portions of dioxane. The combined filtrates were evaporated to dryness. The residue was recrystallized from ethanol.

DSC Method — A mixture of substituted benzoic acids (50 mmol), pyridine (50 mmol), DSC (50 mmol) and acetonitrile (150 ml) was stirred at room temperature. In the cases of (2 j), (2 m) and (2 k), the reactions were carried out at 60°C because the acids were not dissolved in acetonitrile at room temperature. In the other cases, the reactions were performed at room
temperature. In some cases [(2 e), (2 h) and (2 i)], the esters were precipitated during the reaction. The crystalline products were filtered and combined with the residue of the reaction solution described below and treated with water. The reaction solution was concentrated under reduced pressure. To the concentrated solution was added water (300 ml). The mixture was stirred for 1～3 h at room temperature and then filtered, washed with five 50 ml portions of water and dried in vacuo. The purities of the esters were more than 99% (by HPLC analysis).

2.3.3 N,N' -Disuccinimidyl dicarbonates(3)

DCC Method—To a mixture of dicarboxylic acid (50 mmol) and HOSu (100 mmol) dissolved in dioxane (200～400 ml) was added a DCC solution (100 mmol/100 ml dioxane). After stirring for 12～15 h, the reaction mixture was worked up as for (2). The residue was suspended in 200 ml of ethanol. The crystalline products were filtered and recrystallized from a mixture of ethanol and acetonitrile.

DSC Method—A mixture of dicarboxylic acid (25 mmol), pyridine (50 mmol), DSC (50 mmol) and acetonitrile (150 ml) was stirred at room temperature. In the cases of (3 f ) and (3 g), the reactions were carried out at 50°C because the acids were not dissolved in acetonitrile at room temperature. The mixture was worked up in the same manner as for (2).

3 Results and Discussion

3.1 N-Acyloxysuccinimides (1)

The reaction of fatty acid with DSC in acetonitrile gave N-acyloxysuccinimides (1) and HOSu. In the cases of (1 g)～(1 k), the reactions were performed at 50°C because the acids were not dissolved completely in acetonitrile at room temperature. The results are summarized in Table-1. The imide (HOSu) which was formed in the reaction and the added pyridine were very easily removed by the water treatment (2-3). The yields and purities of (1) were much better than those prepared by the method using DCC. Table-1 demonstrates that the present method also has an advantage of easiness for preparation of (1). The mass spectra (GC-MS) of (1) prepared by the DCC method were accordance with those by the DCC method3'.

\[
\text{RCOOH} + \text{DSC} \quad \text{Pyridine} \quad \text{Acetonitrile} \rightarrow (1) + \text{HOSu} + \text{CO}_2
\]

3.2 N-Succinimidyl benzoates (2)

The reactions of substituted benzoic acids with HOSu in the presence of DCC in dioxane gave the corresponding N-succinimidyl esters (2). The crude (2) was contaminated with some by-products which were removed by recrystallization (Table-2). The yields of ortho-substituted esters were fairly low compared with the meta- and para-substituted esters.

On the other hand, the DSC method gave high yields and purities even for ortho-substituted (2) as shown in Table-2. In the case of DSC, HOSu and pyridine were easily removed by the water treatment as for (1). Purities were more than 99% after the water treatment. This suggests that the DSC method is much more convenient than the DCC method. In the cases of (2 j), (2 m) and (2 k), the acids were not dissolved in acetonitrile at room temperature. This problem was also avoided by carrying out the reactions at an elevated temperature (60°C).

The structures of the resulting esters (2) were determined by GC-MS (EI-MS) at 70
The molecular weight ions ($M^+$) were detected for all esters, although the relative intensities were very weak (<5%). The characteristic ions, $M-114$, which were formed by the elimination of $SO_2$ from (2), were observed as base peak ions. The ion, $M-114$ and $m/e=98$ (Su) were the base peak ions for (1) (2 < CN < 11) and (1) (12 < CN < 22), respectively. Thus, Table-2 suggests that the fragment ion pattern of (2) corresponds to the former because arylcarbonyl groups are not so big as long chain acyl groups. Besides the fragment ions in Table-2, M−97 and M−142 were also observed for (2).

3.3 $N,N'$-Disuccinimidyl dicarboxylates (3).

The reactions of aliphatic dicarboxylic acids with HOSu in the presence of DCC in dioxane gave the desired $N,N'$-disuccinimidyl esters together with the formation of considerable amounts of by-products. The by-products could be removed by the ethanol treatment and recrystallization. However, the purities of (3) were not satisfactory (Table-3).

On the other hand, the reactions of the acids with DSC gave much more pure esters only by the water treatment (Table-3). As for the preparation of (3 f) and (3 g), the reactions were carried out at 50°C in the cases of (3 f) and (3 g).

The structures of the esters (3) were determined by mass spectrometry (Table-3). Since El-MS (GC-MS) analyses did not give the molecular weight ion ($M^+$), FD-MS analyses were applied to (3). The ions, $M+1$ and $M−114$...
Table 3: Yields and physical properties of N,N'-disuccinimidyl dicarboxylates (3).

<table>
<thead>
<tr>
<th>Esters</th>
<th>DSC method</th>
<th>DCC method</th>
<th>TLCna</th>
<th>mp°C</th>
<th>Solvent for recrystallizationb</th>
<th>FD-MS</th>
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<tbody>
<tr>
<td>(3a)</td>
<td>3</td>
<td>78</td>
<td>100</td>
<td>59</td>
<td>98</td>
<td>0.29</td>
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<tr>
<td>(3b)</td>
<td>4</td>
<td>85</td>
<td>100</td>
<td>21</td>
<td>98</td>
<td>0.31</td>
</tr>
<tr>
<td>(3c)</td>
<td>5</td>
<td>77</td>
<td>100</td>
<td>21</td>
<td>98</td>
<td>0.36</td>
</tr>
<tr>
<td>(3d)</td>
<td>6</td>
<td>82</td>
<td>99</td>
<td>21</td>
<td>98</td>
<td>0.38</td>
</tr>
<tr>
<td>(3e)</td>
<td>7</td>
<td>89</td>
<td>99</td>
<td>11</td>
<td>99</td>
<td>0.41</td>
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<tr>
<td>(3f)</td>
<td>8</td>
<td>87</td>
<td>99</td>
<td>11</td>
<td>99</td>
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<tr>
<td>(3g)</td>
<td>10</td>
<td>90</td>
<td>100</td>
<td>48</td>
<td>92</td>
<td>0.47</td>
</tr>
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a) before recrystallization.
b) after recrystallization.
c) CHCl3 : EtOH : AcOH = 100 : 5 : 1 (vol/vol/vol).

e) base peak ion (m/e=114 : SO).

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References


スクシンイミドエステルの化学（第4報）

N,N'-ジスクシンイミジルカルボネート（DSC）を用いたN-スクシンイミジルカルボキシレートの簡易合成法

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