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Azido-meta-hemipinic Acid: An "Introverted" Acid?

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Abstract

Reaction of Azido-m-hemipinic acid (IV) with N-hydroxy Succinimide (NHS) and Dicyclohexyl Carbodiimide (DCC) has been investigated. The product (IV) has been identified as a tri-NHS adduct of the elusive O-acylisourea intermediate in DCC catalyzed reactions of carboxylic acids and alcohols/amines. Based on these observations, a question is posed whether 'azido-m-hemipinic acid' is to be considered as an "introverted" acid?.

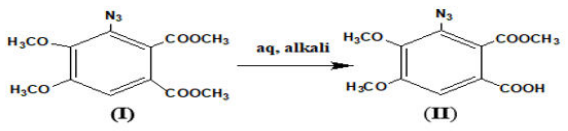
Keywords: Carboxylic acid; O-acylisourea; Dicyclohexyl carbodiimide; N-hydroxysuccinimide; Mass spectrometry

Abbreviations:

NHS: N-hydroxy Succinimide; DCC: Dicyclohexyl Carbodiimide; NMR: Nuclear Magnetic Resonance; DCM: Dicholoromethane.

Introduction

'Meta-hemipinic acid' and 'nor-meta-hemipinic acid' are classical compounds obtained by degradation of the alkaloid Papaverine are constituents of soil humus/humic acids. This paper is a part of our continued studies on 'dimethyl-azido-m-hemipinate', 'azido-dimethyl succinylosuccinate and azido meta-meconine [1-6]. We have earlier carried out the alkaline hydrolysis of 'dimethyl-azido-m-hemipinate' (I) and surprisingly found that only one of the two methoxycarbonyl ester groups got hydrolyzed to give the acid-ester (II) (Scheme 1). The question that arose was whether the ester group adjacent to the azide group was sterically hindered and whether it was yet another example of an "introverted" carboxylic acid?

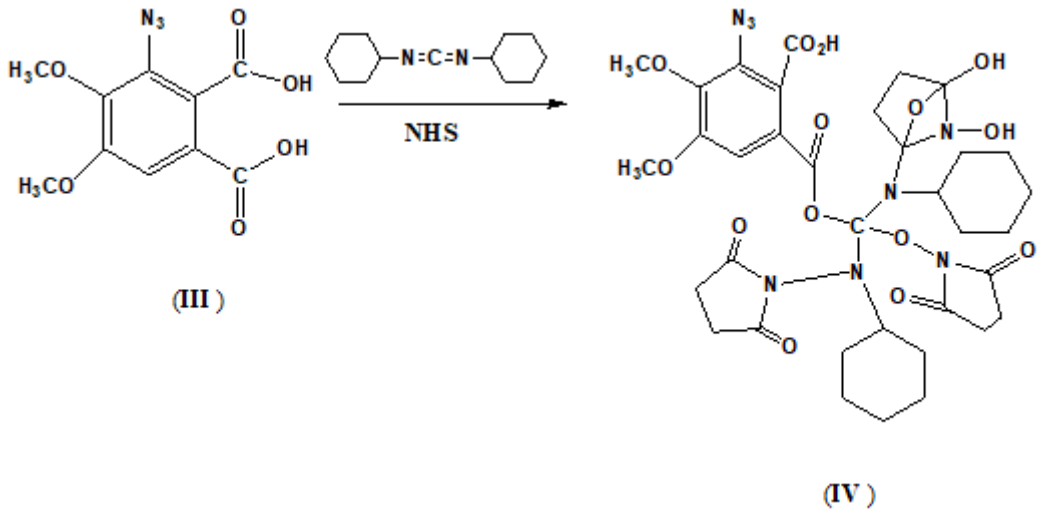


(I) (II)

Scheme 1: Conversion of azido-m-hemipinate (I) to the corresponding acid-ester (II).

An example of an "introverted" acid has been reported in a cleft like structure pointing inwards into a cavity and it has been stated that "carboxylic acids are especially difficult to place in a sterically demanding environment [7] their oxygen is exposed, and convoluted molecular architectures must be created to bring intramolecular elements near them in space". This paper refers to the isolation of covalent adduct using Diisopropyl carbodiimide and it was suggested that "it is either the elusive O-acylisourea or the commonly encountered N-acyl urea". In view of the above statements, this work thus remains inconclusive, as the authors further state that "only crystallography can resolve the structure of the adduct as neither NMR nor IR is up to the task and further progress in this admittedly narrow investigation must now await the growth of suitable crystal for X - ray analysis". These authors further state: "Support for this intermediate comes from kinetic methods and stereochemical probes, rather than direct observation. A report involving direct NMR observation was subsequently shown to be a mis-assignment, but an old claim of TLC separation of an O-acylisourea persists, and a more recent publication describes the crystallization of a series of O-acylisoureas from coumarin-3-carboxylic acid derivatives and DCC".

With this background, we carried out a reaction of 'azido-m-hemipinic acid'(III) with DCC and NHS. To our surprise we obtained a rather intriguing product with the proposed structure (IV). It was evident that (III) had reacted with DCC and more than one NHS unit. Based on the above, structure (IV) is proposed for this compound (Scheme 2).



Scheme 2: Tri-NHS adduct of O-acylisourea intermediate (IV) obtained from the reaction of 'azido-m-hemipinic acid' with N-hydroxysuccinimide (NHS) with dicyclohexyl carbodiimide (DCC).

Experimental

General

All the chemicals were purchased from Aldrich Chemical Company and were used without any further purification unless specified. 'Azido-m-hemipinic acid' was prepared from 'azido-m-hemipinate' or 'azido-m-meconine' by hydrolysis or oxidation (loc. cit.), the O-acylisourea intermediate was purified by Preparative Thin Layer Chromatography (Prep.-TLC). MALDI-MS, MS/MS spectra were recorded on AB SCIEX TOF/TOF 5800; FT-IR spectrum was recorded on TENSOR 27, UV spectrum was recorded on Perkin Elmer Lambda 35. ^{1}H-NMR spectrum was recorded in house on a Bruker ASCENT (400 MHZ) instrument. Deuetrated NMR solvents were obtained from Merck KGaA, 64271 Darmstadt, Germany and used without any further purification. MALDI-MS time-of- flight reflection experiments (MALDI-MS) were performed on an Agilent ionization time of flight mass spectrometer.

Synthesis of the tri-NHS adduct of the elusive O-acylisourea intermediate

To a mixture of 'azido-m-hemipinic acid' (50 mg, 0.518 mmol) and N-hydroxy succinimide (31.5 mg, 0.52 mmol) in 10 ml of Dicholoromethane (DCM) was taken in a round bottom flask with a magnetic needle, DCC (67.5 mg, 0.52 mmol) in diethyl ether (5 ml) was added in small amounts. The reaction was then set up on a magnetic stirrer. After overnight stirring the reaction mixture was filtered and solid DCC- urea obtained was filtered off. The filtrate was then evaporated vaccum. An off-white solid (36 mg) was obtained, which was dried in a vaccum desiccator over P_2O_5. It was then recrystallized from hot petroleum ether. Another fraction of the off-white solid (28 mg) was obtained from the petroleum ether insoluble fraction.

M. P. 146°C; HRMS: Calculated for $C_{35}H_{44}N_8O_{14}$: m/z 800.2977; Obs. 800.2507; FT-IR: (Solid; methanol, 3335.86, 3305.09, 2119.32, 1724.43, 1702.03, 1626.36, 1590.35, 1495.96, 1445.29, 1415.85, 1350.96, 1298.95, 1266.51, 1211.78, 1162.93, 1032.58, 995.40, 719.10, 661.70, 627.35 cm^{-1}); UV: (Methanol, 203.61, 206.25, 209.10, 211.32, 213.54, 216.64, 218.22, 222.22, 224.32, 20.72 nm); ^{1}H-NMR (400 MHz, $CdCl_3$) δ=6.2 (s, ^{1}H), δ=4.10 (s, ^{3}H), δ=4.12 (s, ^{3}H), δ=2.95 (m, ^{12}H), δ=2.83 (s, ^{1}H), δ=2.71 (s, ^{1}H), δ=1.33-1.63 (m, ^{22}H).

Results and Discussion

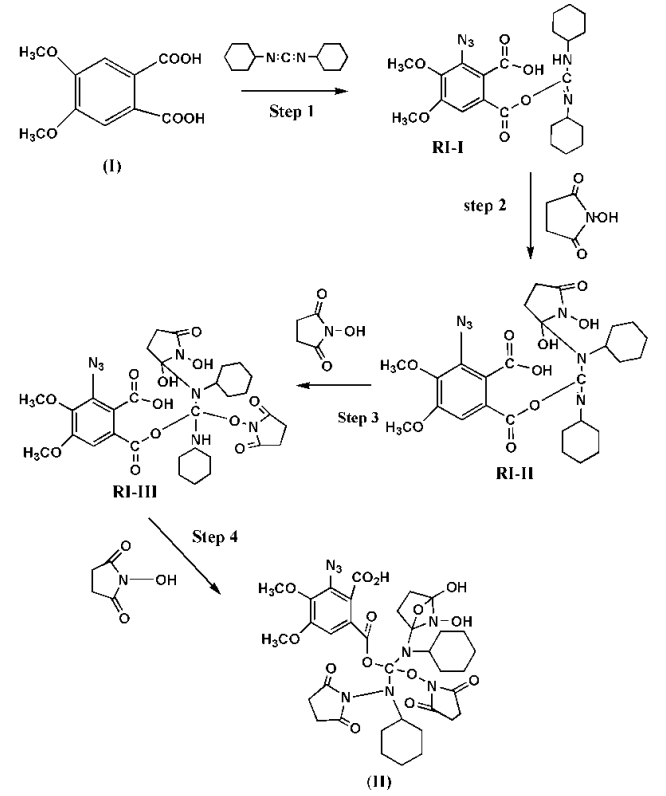
DCC brings about coupling of carboxylic acid with alcohols/amines, yielding esters/amides along with insoluble DCC-urea. DCC has been extensively used in peptide/nucleotide synthesis and their automated versions [8-10]. The mechanism of such coupling reactions is well understood. The accepted mechanism involves the attack by the carboxylic group on central carbon atom of DCC. This is followed by the attack of the nucleophile on the carbonyl group yielding the esters/amides and DCC-urea. It may be noted that the attack on the nucleophile is a prerequisite for formation of esters/amides/peptides.

Compound (IV), m.pt. 146°C has a molecular weight with M^+ value of m/z 800.2507. Other spectral data for the compound are FT-IR spectrum showing a broad absorption from 3700 to 2200 cm^{-1} and 1702 cm^{-1} due to the carboxyl group; a peak at 2119 cm^{-1} due to the azide group and a strong absorption at 1724 cm^{-1} attributed to the NHS ester (SI-I). Its UV spectrum showed multiple absorption peaks between 208 to 230 nm. Its ^{1}H-NMR spectrum showed signals for one aromatic proton, two methoxy groups and three NHS moieties along with the signals due to two cyclohexane ring protons of the DCC skeleton. Comparison of the intensity of the aromatic/methoxy signals with that of the protons of the NHS near δ 2.9 indicated towards the presence of up to three NHS units in (IV) (SI-II). This was further corroborated by MALDI-MS investigations, which showed the presence of the molecular ion (M^+) at m/z 800.2507 (SI-III) and the base peak at m/z 533.1589, which possibly arises out of fragmentation of the molecule (IV) it being a tertiary ester could undergo ready cleavage. MS/MS spectrum (SI-IV) of the m/z 800 peak showed peaks at m/z 726.416 (loss of 74 amu) and 508.231 (loss of 292 amu), which

further lost a mass of 292 amu yielding a fragment with m/z 298.128, which confirms the molecular ion to be 800.2507. The central carbon in (IV) is a new asymmetric (chiral) carbon atom and so optical rotation measurements were undertaken using a digital polarimeter. Indeed (IV) was found to be optically active with specific rotation of $[\alpha]_D$=-17.5° methanol, 2 × 103 g/ mL).

Proposed steps involved in formation of (IV)

It is proposed (scheme 3) that the carboxylic acid, away from the azide groups attacks the central carbon of DCC (step 1) which is not sufficiently exposed for a nucleophilic attack to occur (the nucleophile here is the NHS molecule), blocking the formation of any ester. The newly formed amino group in (RI-I) then adds (step 2) to one the carbonyl groups of a NHS unit (RI-II). This is followed by the attack of a second NHS unit (step 3) on the central carbon atom of DCC generating once again yet another amine (RI-III). This finally undergoes a DCC assisted dehydrated- coupling to give (IV). It is thus clear that no DCC-urea formation occurred. This is to be explained by the inability of the NHS unit to approach the carbonyl group as happens in the well accepted mechanisms of DCC catalyzed coupling reactions. Further in the formation of (IV), the carboxylic acid group adjacent to the azide group did not or could not participate in the reaction.



Scheme 3: Proposed steps involved in the formation of the O-acylisourea intermediate (IV).

Compound (IV) represents a tri-NHS adduct of the elusive O-acylisourea intermediate. Is the carboxylic acid ortho- to the azide functionality to be termed as an "introverted acid"? In our opinion, the carboxylic acid group adjacent to the azide group in 'azido m-hemipinic acid' does not itself react and further hinders the attack of the nucleophile (NHS in this case) on the carbonyl group of the other carboxylic acid attached to the DCC central carbon atom, so necessary for the nucleophile (usually an alcohol or an amine) and to attack for the release of DCC-urea. These steps do not occur in our case and therefore 'azido-m-hemipinic acid' constitutes yet another example of an "introverted" carboxylic acid.

It is pertinent to point out that many other pathways and structures were duly considered before arriving at structure (IV). However, none of these other structures could satisfactorily explain the molecular ion observed at m/z 800.2507. Thus Step-2 leading to the intermediate (RI-I) alone could explain the presence of two additional hydrogen atoms in the final product.

Recently we have used (IV) for crosslinking studies using it as a new heterobifunctional crosslinker based on an "introverted" carboxylic acid and analyzed the data generated using ESI-MS and bioinformatics software StavroX 3.6.0.1[10,11]. CXL-MS is an emerging technique for preparation of antibody- drug conjugates [12] which has been described as a "pinnacle of achievement" in this field. CXL-MS is also gaining importance in cryo-electron microscopy (cryo-EM) studies [13], which could provide 3D structure of protein complexes in living cells at molecular resolutions.

'Azido-m-hemipinic acid' thus constitutes yet another example of a rare "introverted" carboxylic acid, which prevents the conventional pathway in DCC catalyzed reactions, from being followed in our case.

Conclusion

'Azido-m-hemipinic acid' (III) could possibly considered as an "introverted" acid as the carboxylic acid adjacent to the azide group is unreactive in nature. Reaction of [III] with DCC and NHS leads to the formation of (IV) which is possibly a tri-NHS adduct of the elusive O-acylisourea intermediate proposed n DCC catalyzed reactions. The four possible steps involved in the formation of (IV) have are also proposed. Compound (IV) has been used successfully by us for chemical crosslinking-mass spectrometric (CX-MS) studies. Results reported in the current paper are supported by FT-IR, UV, 1H-NMR, MALDI-MS and MS/MS investigations. Apart from providing a better understanding of the use of DCC in coupling reactions, the work presented here could impact diverse fields, e.g., studies on protein-protein interactions, systems and systems and structural biology. These could also be used in photo-micro/nanolithography; for making soluble nanocarbon materials and for making solar cells. Azides are considered as "green reagents" [14] as their reactions often involve only a benign loss of nitrogen, which is not an environment pollutant. On the other hand, reactions involving retention of all the three nitrogen atoms of the azide group provide a simple route for bio-orthogonal chemistry in cells. Thus, nucleophilic displacement of the NHS group by proteins, amino group carrying drugs (e.g., Doxorubicin and cis-Platin) followed by reaction with fluorescently labeled and alkyne group modified proteins/ monoclonal antibodies could pave the way for the use of 'click' chemistry [15-18].

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