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Isolation, characterization and semi-synthesis of natural products dimeric amide alkaloids

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ABSTRACT

Isolation, characterization of natural products dimeric amide alkaloids from roots of the Piper chaba Hunter. The synthesis of these products using intermolecular [4+2] cycloaddition reaction has been described. Obtained products were characterized using IR, ^{1}HNMR, ^{13}CNMR and Mass Spectroscopy.

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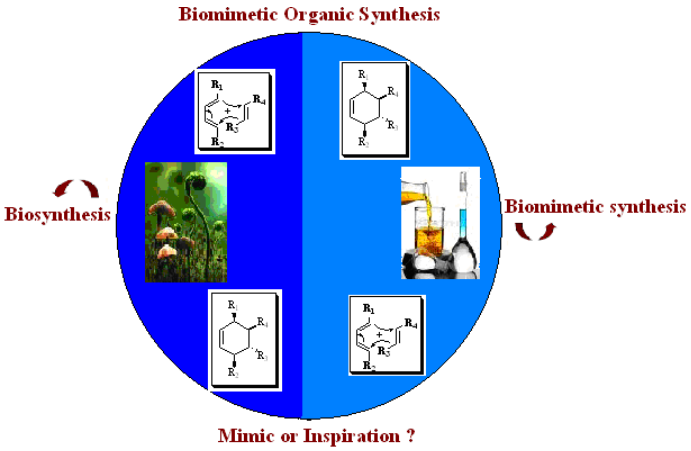
Introduction

The awesome structural diversity and complexity of natural products inspire many chemists to consider how nature creates these molecules. Nature's biosynthetic enzymes offer a powerful and practical route to many organic compounds, and synthetic chemists sometimes seek to imitate the efficiency and elegance of the biosynthetic machinery by designing biomimetic reactions that approximate natural reaction pathways. Probably the most astonishing biomimetic reactions[1] are tandem processes that combine several transformations in sequence and produce complicated structures from comparably simple starting materials in a single laboratory operation. Biosynthesis is described as "the reaction or reaction sequence occurred in organism or its immediate environment will be viewed as biosynthesis" where as biomimetic synthesis describes as "A specific reaction or a sequence of reactions that mimic a proposed biological pathway is defined as bimimetic synthesis. An early example is Sir Robert Robinson's landmark synthesis of tropinone in 1917.[2] Forty-two years later, Gilbert Stork and Albert Eschenmoser independently proposed that the steroid ring system could be formed by tandem cation-π cyclizations of a polyene in an ordered transition state.[3] A non-enzymatic version of this reaction type was demonstrated in W. S. Johnson's classic synthesis of progesterone in 1971.[4] Chapman's synthesis of carpanone is a striking example of the power of biomimetic strategies.[5] In 1980, Black proposed that the endiandric acids could arise biosynthetically from linear polyenes.[6] In 1982, K. C. Nicolaou gave chemical support to Black's hypothesis by chemically synthesizing endiandric acids A-G.[7] Biomimetic Synthesis of Natural Products which involves, The biomimetic polyene carbocyclizations reaction, The biomimetic cycloaddition reaction, The biomimetic electrocyclization reaction, The polyether biomimetic synthesis, The biomimetic oxidative coupling of phenol, Some other interesting biomimetic synthesis, The present biomimetic synthesis of chabamides or dimeric amide alkaloids involves cycloaddition reactions.



The Diels Alder reaction

In the Diels-Alder reaction a six membered ring is formed through fusion of a 4 π component, usually a diene and a 2 π component which is commonly referred to as the

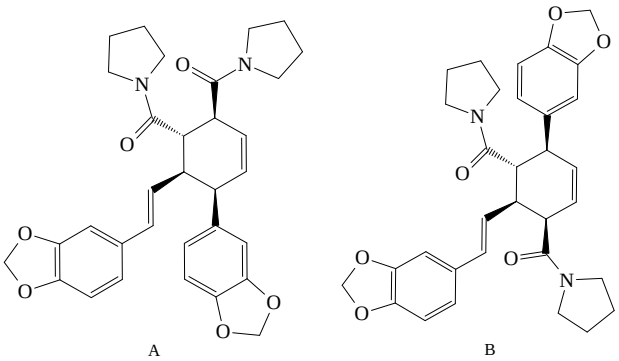


Figure 1.

dienophile. The Diels Alder reaction has proven to be great synthetic value, forming a key-step in the construction of compounds containing six-membered rings. Cyclohexene ring generated all the way through the formation of two new σ-bonds and one π bond with four adjacent stereocenters. The reaction is named after Otto Diels and Kurt Alder, two German chemists who studied the synthetic and theoretical aspects of this reaction in great detail.[8] Their efforts have been rewarded with the 1950 Noble prize.

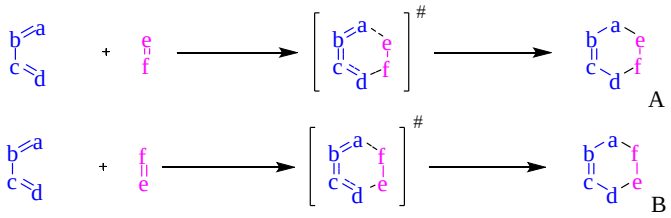


Figure 2 Schematic representation of the Diels-Alder reaction.

Cis principle

In Diels-Alder reactions, the stereoselectivity is generally high due to the "*cis* principle", which states that Diels-Alder reactions require a cisoid conformation for the diene and suprafacial-suprafacial mode of reaction, meaning that both ends of the diene attack from the same face of the dienophile in a *syn* fashion.

Frontier Molecular Orbital (FMO) Approach

Diels-Alder rections can be devided into, normal electron demand and inverse electron demand additions. This difference is based on the way the rate of the reaction responds to the introduction of electron withdrawing and electron donating substituents. Normal electron demand Diels-Alder reactions are promoted by electron donating substituents on the diene and electron withdrawing substituents on the dienophile. In contrast, inverse electron demand reactions are accelerated by electron withdrawing substituents on the diene and electron donating ones on the dienophile. There also exists an intermediate class, the neutral Diels-alder reaction, which is accelerated by both electron withdrawing and donating substitutents. The way the substituents affect the rate of the reaction can be rationalized with aid of Frontier Molecular Orbital (FMO) theory. This theory was developed during a study of the role of orbital asymmetry in pericyclic reactions by Woodward and Hoffmann[9] and, independently, by fukui[10] Later, Houk contributed significantly to the understanding of the reactivity and selectivity of these processes.[11]

The FMO theory states that a reaction between two compounds is controlled by the efficiency with which the molecular orbitals of the individual reaction partners interact. The interaction is most efficient for the reactivity is completely determined by interactions of the electrons that are highest in energy of the of the reaction partners (those in the Highest Occupied Molecular Orbital, the HOMO) with the Lowest Unoccupied Molecular Orbital (LUMO) of the other partner, applied to the Diels-alder reactions, two modes of interaction are possible. The reaction can be controlled by the interaction of the HOMO of the diene and the LUMO of the Dienophile (normal electron demand), or by the interaction between the LUMO of the diene and the HOMO of the dienophile (inverse electron demand), as illustrated in Fig-B. In the former case, a reduction of the diene-HOMO and dienophile-LUMO energy gap can be realized by either raising the energy of the HOMO of the diene by introducing electron donating substituents or lowering the energy of the dienophile LUMO by the introduction of electron donating substituents or lowering the energy of the dienophile LUMO by the introduction of electron withdrawing substituents. A glance at Fig-A confirms that in the formation of two new bonds, orbital symmetry is conserved so that, according to Woodward and Hoffmann, the reaction is concerted. In other words, no intermediate is involved in the pericyclic process such as the Diels-Alder reaction.[12] This conclusion is consistent with a number of experimental observations. The *cis* or *trans* conformation of the dienophile is fully conserved in the configuration of the cycloadduct, which proves that there is no intermediate involved with a lifetime long enough to allow rotation around C-C bond.

Selectivity can arise when substituted dienes and dienophiles are employed in the Diels-Alder reaction. Two different cycloadducts denoted as *endo* and *exo* are possible. Under the usual conditions their ratio is kinetically controlled. Alder and Stein already discerned that there usually exists a preference for formation of the *endo* isomer i.e formulated as tendency of maximum accumulation of unsaturation, (the Alder-Stein rule)[13] Indeed, there are only very few examples of Diels-Alder reactions where the *exo* isomer is major product.[14] The interactions underlying this behavior have been subject of intensive research. Since the reactions leading to *endo* and *exo* product share the same initial state, the difference between the respective transition-state energies fully account for the observed selectivity. These differences are typically in the range of 10-15

kJ per mole.[15] Woodward and Katz[16] suggested that secondary orbital interactions are of primary importance. These interactions are illustrated in fig-B for the normal electron demand (HOMO-diene, LUMO-dienophile controlled). The symmetry allowed overlap between π-orbital of the carbonyl group of the dienophile and the diene-HOMO is only possible in the *endo* activiated complex. Hence, only the *endo* transition state is stabilized so that the reaction forming the *endo* adduct is faster than that yielding *exo* product.

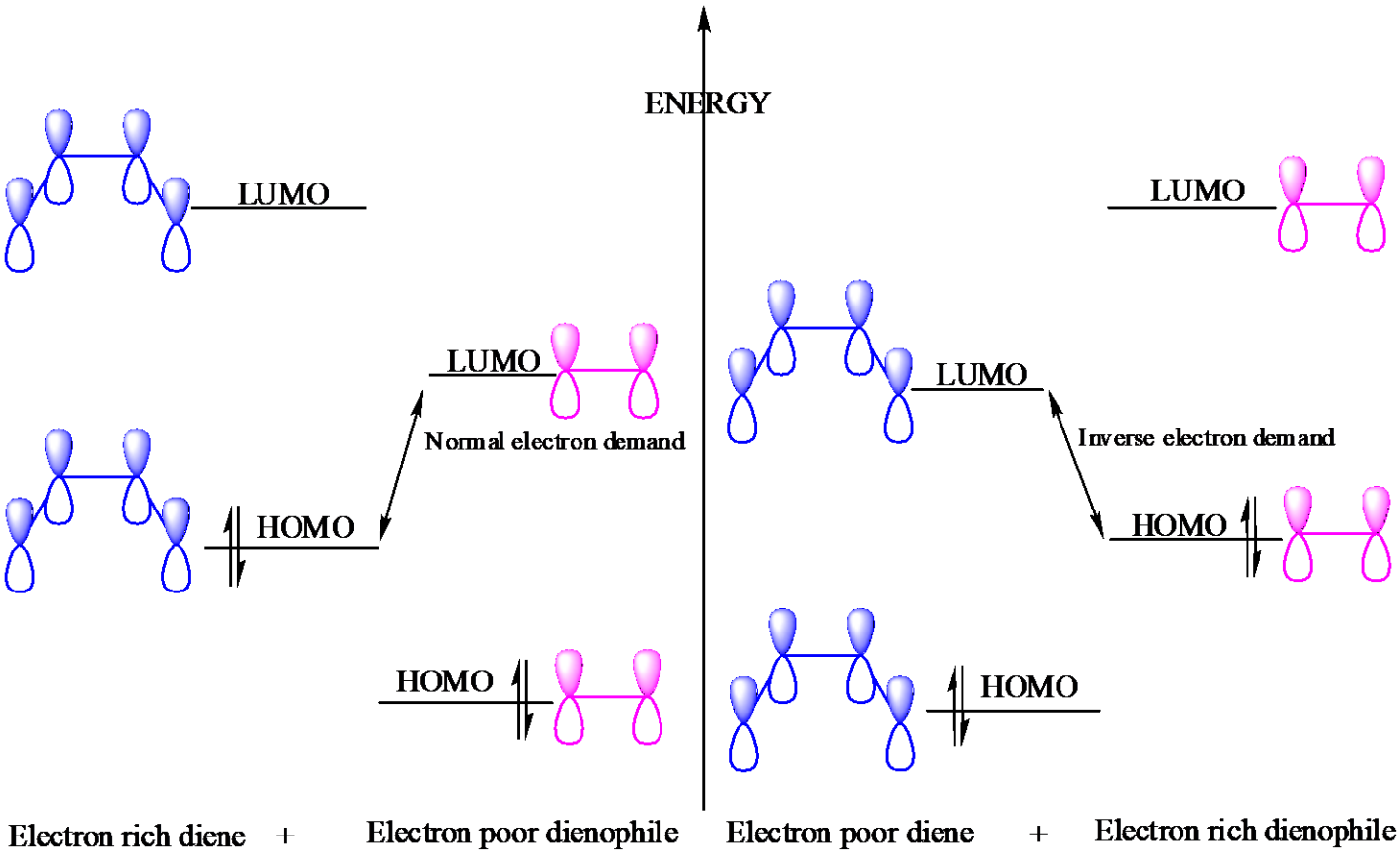
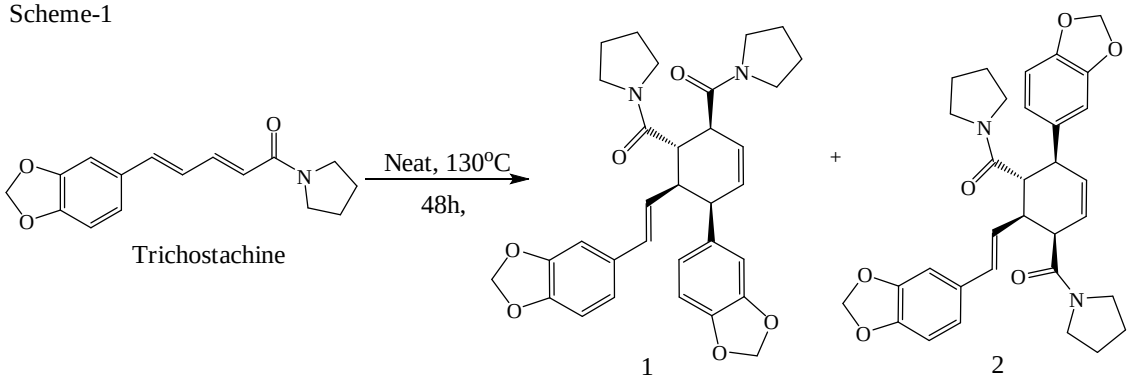
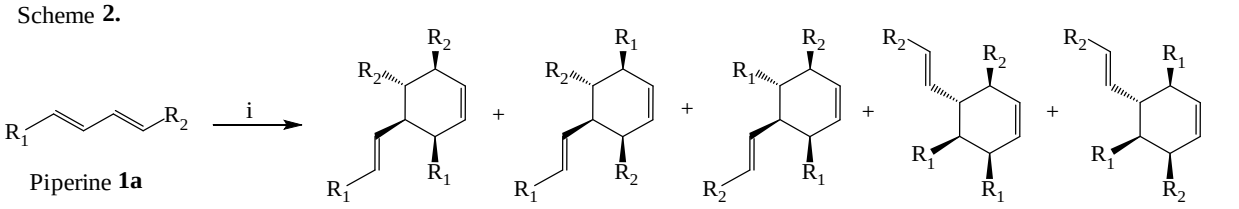


Fig-B. Orbital correlation diagram illustrating the distinctions between normal electron demand (left side) and inverse electron demand (right side) Diels alder reactions.

Scheme-1



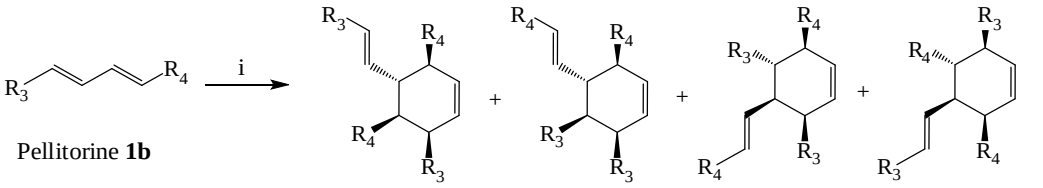
Scheme **2.**



Piperine **1a**

2a (*ortho-exo*) **3a** (*meta-exo*) **4a** (*meta-exo*) **5a** (*ortho-endo*) **6a** (*meta-endo*)

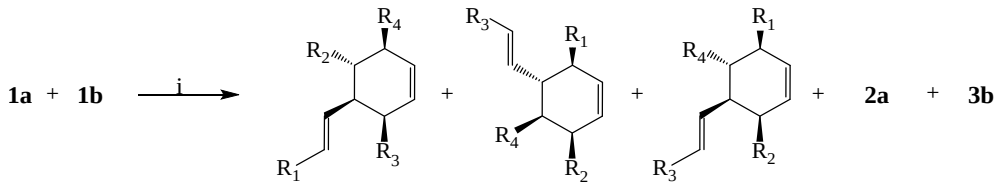
Scheme **3.**



Pellitorine **1b**

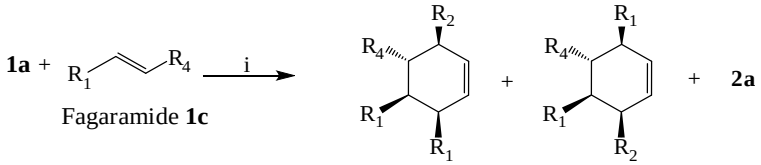
2b (*meta-endo*) **3b** (*ortho-endo*) 4**b** (*meta-exo*) nigramide O 5**b** (*meta-exo*)

Scheme **4.**



2c (*ortho-exo*) **3c** (*ortho-endo*) **4c** (*meta-exo*) nigramide N

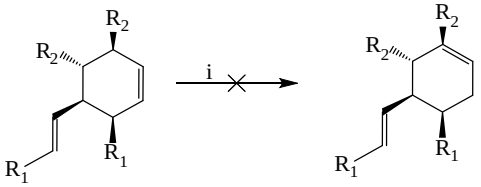
Scheme **5.**



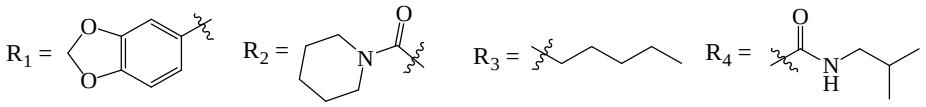
Fagaramide **1c**

2d (*ortho*) **3d** (*meta*)

Scheme **6.**



2a Isomerised product



Scheme 2, 3, 4, 5 & 6
Reagents and conditions:(i) Cu $(NO_3)_2$, Reflux temp, 70 h, H_2O

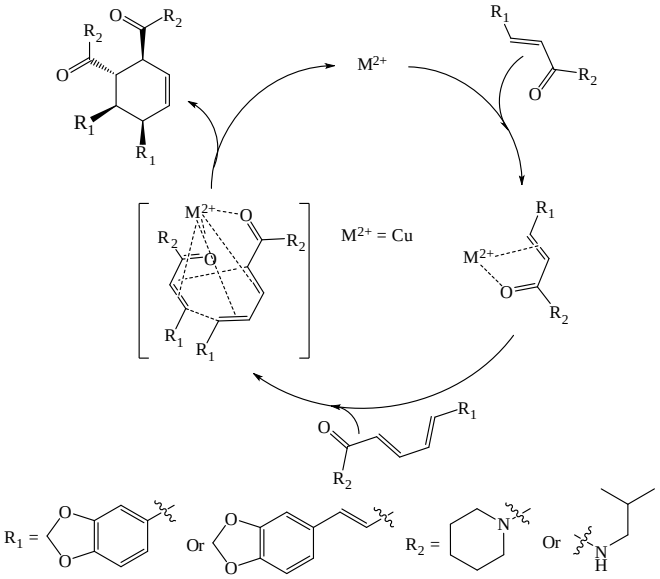
This interpretation has been criticized by Mellor, who attributed the *endo* selectivity to steric interactions. Steric effects are frequently suggested as important in determining the selectivity of Diels-Alder reactions, particularly of α-subsituted dienophiles, and may ultimately lead to *exo*-selectivity. [17] For other systems, steric effects in the *exo* activated complex can enhance *endo* selectivity. [18] In summary, it seems for most Diels-Alder reactions secondary orbital interactions afford a satisfactory rationalization of the *endo*-*exo* selectivity. However, since the *endo*-*exo* ratio is determined by small differences in transition state energies, the influence of other interactions, most often steric in origin and different for each particular reaction is likely to be felt. The compact character of the Diels-Alder activated complex (the activation volume of the retro Diels-Alder reaction is negative) will attenuate these effects.[19]

Results and Discussions

Chabamides F & G as dimeric amide alkaloids were isolated from this plant Piper *chaba* Hunter. These two dimers were formed by Diels-Alder reaction employing monomer trichostachine. This hypothesis was further confirmed by the mass spectrum, which showed a significant peak at *m/z* 294.113 [M^++Na], assigned to the trichostachine ion arising by the Retro-Diels–Alder cleavage of molecular ion into two halves. Finally, to confirm the existence of the compounds **F** and **G**, we extracted the roots of *P.chaba* with MeOH at room temperature followed HPLC/electron spray ionization (ESI) MS experiments. In HPLC/ESIMS of the MeOH extract showed the presence of peaks at *m/z* 563 [M^++Na] and 543 [M^++1] at about 8.8 min and 10.6 min of LC retention time, respectively. To prove this biosynthetic hypothesis we have carried out the intermolecular [4+2] cycloaddition reaction with the trichostachine under solvent free conditions (Scheme **1**). Reaction mixture was analysed by the LC-MS, which clearly indicted the presence of the compounds **1** and **2** (retention time and mass). In HPLC analysis, retention times of the synthetic **1** and **2** were identical to those of chabamide **F** and **G**, confirming the structure and stereochemistry are same as that of isolated alkaloids. Based on above result during Diels-Alder reaction of trichostachine, we developed two kinds of methodologies for this biomimetic synthesis of dimeric amide alkaloids based on catalytic. On the basis of a biosynthetic hypothesis (described in Chapter I) by the intermolecular Diels-Alder reaction, we chosen piperine (**1a**), pellitorine (**1c**) and *trans*-fagaramide (**1c**) as substrates to perform the biomimetic synthesis of the dimeric chabamides (Compound **H**-**K**) and this study also identified plausible products between piperine (**1a**) and pellitorine (**1c**). This study not only explains formation of cyclo adducts but also explains the different mechanistic aspects in Diels-Alder reaction (*endo* and *exo* products) of copper salts in aqueous medium. Under normal conditions only combinations of dienes and dienophiles that have FMO's of similar energy can be transformed into a Diels-Alder adduct. When the gap between the FMO's large, forcing conditions are required, and undesired side reactions and retro Diels-Alder reactions can easily take over. These cases challenge the creativity of the organic chemist and have led to the invention of a number of methods for promoting reluctant Diels-Alder reactions under mild conditions.[20]

Plausible mechanism for Diels-Alder reaction:

Sijbren Otto. et. al studied extensively on copper (II) catalyzed Diels-Alder reactions on various moieties. [25, 26] Based on these reports we proposed plausible mechanism for this copper catalyzed Diels-Alder reaction. The first step in the cycle comprises rapid coordination of the lewis acid to the dienophile leading to a complex in which the dienophile is activated for reaction with the diene. The cycloadduct has dissociated from the lewis acid in order to make the catalyst available for another cycle. However we didn't carry any kinetic studies to prove this mechanism.



Plausible mechanism of Diels-Alder reaction catalyzed by copper (II) salts

Use of lewis acids in Diels-Alder reaction is to lower LUMO dienophile energy to result in the decrease of the LUMO dienophile-HOMO diene gap (normal electron demand) or reduce LUMO diene energy to result in the decrease of the LUMO diene-HOMO dienophile gap (inverse electron demand). The presence of Lewis acids, the Diels-Alder dimerization of piperine, pellitorine, piperine with fagaramide, peperine with pellitorine, gave much lower combined yields in neat conditions. *Wie et al.* previously reported [21, 22] Diels-Alder reaction of piperine and in both thermal and by lewis acid of Co(II) $Cl_2.6H_2O$/P(Ph)$_3$/Zn (1:10:10 mol %) in 3-octanol at 170^{o}C with isomerised product (24 %) and 77 % over all yield.

To find the optimum conditions towards the catalyst, piperine (**1a**) was taken to perform the Diels-Alder reaction in presence of variety of lewis acids and metal salts (Table **1**). The highest catalytic activity was attained for the reaction using 10 mol % of Cu (II) salts. The role of copper salts in this reaction can be attributed to its Lewis acid ability, which enhances both the electron donating capacity of diene and electron withdrawing

capacity of the dienophile (required for normal electron demand for Diels-Alder reaction). The The catalytic activitiy of Lewis acids like Cu^{+2} mainly relies on their coordinating character to assemble both dienophile and diene to such a way that promote the reaction to wards the reaction barrier.

To find the optimum conditions towards the solvent several reactions were carried out under the solvents like benzene, toluene, xylene, water and results were tabulated (Table **2**). Among organic solvents xylene is better to get considerable yield with copper salts. Later water was found to be the best for both yield and selectivity of this cycloaddition.

Cycloaddition reactions of piperine (**1a**):

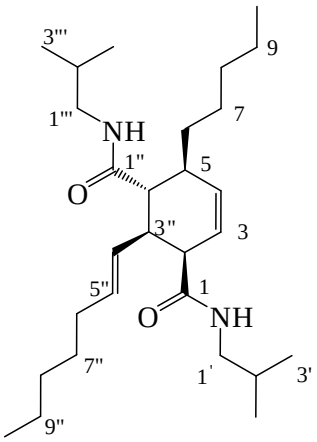
Lewis acids catalyzed cycloaddition reactions (Scheme **2**) of piperine (**1a**) under organic and aqueous solvent conditions to give resultant cycloadducts **2a, 3a, 4a, 5a and 6a,** among them **2a** is major *ortho-exo* cyclohexene type dimeric amide alkaloid and also known as chabamide, which is previously isolated[23] from this plant, isomer **3a** is previously isolated from *Piper nigrum*[21] Remaining isomers (**4a**-**6a**) were synthesized from piperine by Diels-Alder reaction by *Kun Wei. et al*. its physical and spectroscopic data were identical with reported data[22] (^{1}H-NMR, ^{13}C-NMR & Mass spectra). In the cycloaddition of piperine (**1a**), solvents toluene, xylene and water were used in presence of cuper (II) salts. Reaction showed good overall yield and more *exo* selectivity in organic solvent like xylene. Water catalyzed reactions were ended with good overall yield and minute decrease in *exo* selectivity, infinitesimal increase in *endo* selectivity (Table 2). This reaction showed completely regioselectivity (yield of **2a**+**3a**>**4a**+**5a**+**6a**) due to maximum involvement of α-double bond rather than γ-double bond of **1a** during Diels-Alder reaction.

Cycloaddition reactions of pellitorine (**1b**):

Same catalytic and solvent conditions were employed for pellitorine (**1b**) as used in piperine (**1a**) for the biomimetic synthesis (Scheme **3**) of **chabamide J** & **K** (Chapter-II)**.** These dimers were plausibly generated by monomer pellitorine by cycloaddtion reactions in biosynthesis. During cycloaddition of pellitorine (**1b**), solvents like toluene xylene and water were used in presence of cupper (II) salts. In former catalyzed reaction showed good overall yield and more *endo* selectivity in both organic (xylene) and water. Increase in *endo* selectivity is more in aqueous medium rather than organic solvent like xylene (Table **2**). Cycloaddition of pellitorine under above said catalytic conditions gave corresponding cycloadducts **2b**, **3b**, **4b** and **5b**. Physical and spectral data of adducts **2b** & **3b** are identical with compound **J** & **K** (**chabamide J** & **K** mentioned in Chapter-II) and all physical and spectral data of adduct **4b** is identical with **nigramide O** which is isolated previously from *piper nigrum*.[21] The structure of **5b** a new cycloadduct formed during this biomoimetic synthesis employ pellitorine as monomer, its structure was elucidated by 1D and 2D spectral data. This reaction showed completely regioselectivity (yield of **2b**+**5b**≈**3b**+**4b**) due to maximum involvement of α-double bond rather than γ-double bond of **1b** during Diels-Alder reaction.

Structure elucidation of compound 5b:

Compound **5b** was obtained as a pale yellow oil, had the molecular formula of $C_{28}H_{50}N_2O_2$, as deduced from the HRESIMS (Fig-9) *m/z*, 447.3958 [M^++H]. IR spectrum (Fig-**1**) implied the presence of carbonyl (1648 cm^{-1}) and NH (3304 cm^{-1}).



Compound **5b**

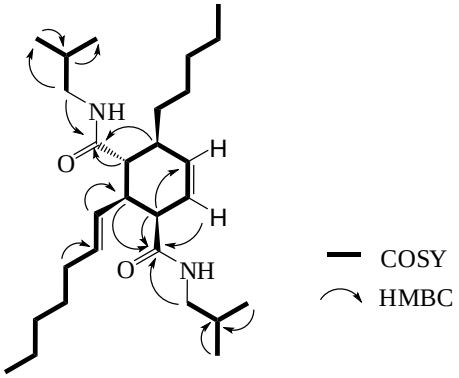
The ^{1}H NMR spectrum of **5b** revealed the presence of a *trans* double bond at δ 5.28 (dd, J = 15.0, 10.0 Hz, H-4"), 5.63 (m, H-5"), two isobutylamide groups at δ 3.15 (m), 3.17 (m), 3.17 (m, H2-1'), 1.74 (m, H-2'), 0.91 (d, J = 6.7 Hz, H-3'), 0.90 (d, J = 6.7, H- 3'), 5.53 (br t, J = 5.7 Hz, NH) and δ 2.96 (m, H1-1'''), 2.97 (m, H2-1'''), 1.73 (m, H-2'''), 0.87 (d, J = 6.7 Hz, H-3'''), 0.86 (d, J = 6.7 Hz, H-3'''), 3.15 (br t, J = 6.0 Hz, NH), *n*-amyl group and 1-heptene unit at δ 1.96 (m, H-6), 1.40 (m, H-7), 1.20 (m, H-8), 1.27 (m, H-9), 0.86 (t, J = 6.5 Hz, H-10) and δ 5.28 (dd, J = 15.0, 10.0 Hz, H-4"), 5.63 (m, H-5"), 1.89 (m, H-6"), 1.30 (m, H-7"), 1.28 (m, H-8"), 1.27 (m, H-9"), 0.88 (t, J = 6.5 Hz, H-10"), respectively (Table **3**). The ^{13}C NMR spectrum (Fig-**3**) displayed the presence of 28 carbon atoms and were further classified by DEPT experiments (Fig-**4**) into categories of 6 methyls, 10 methylenes, 10 methines and 2 quaternary carbons including two carbonyls (δ 173.80 and 173.04).

The analyses of the ^{1}H and ^{13}C NMR spectral data of **5b** showed a high degree of similarity to dimeric alkaloid, **compound J** naturally isolated from this plant (Chapter-II) compound is *meta-endo* while **5b** is *meta-exo* product. Furthermore, the detailed elucidation of the 2D NMR data (COSY, HSQC and HMBC) had determined the planar structure of **5b**.

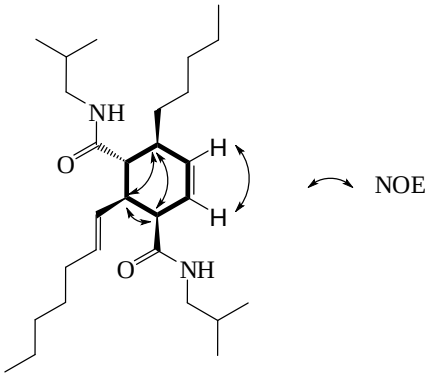
Table 3: ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data for compound 5b ($CDCl_3$)

No.	**^{13}C, δ**	**Compound 5b**
		^{1}H, δ (mult, J, Hz)
1	173.80	-C-
2	51.79	2.45 (m)
3	124.01	5.56 (ddd, 10.0, 4.3, 2.6)
4	133.91	5.98 (dt, 10.0, 1.8)
5	37.04	2.41 (m)
6	32.59	1.96 (m)
7	28.56	1.40 (m)
8	31.97	1.20 (m)
9	29.74	1.27 (m)
10	14.12	0.86 (t, 6.5)
1'	46.96	3.15 (m) & 3.17 (m)
2'	28.56	1.74 (m)
3'	20.10	0.91 (d, 6.7) 0.90 (d, 6.7)
NH	-	5.53 (br t, 5.7)
1"	173.04	-C-
2"	49.98	2.68 (dd, 10.3, 10.0)
3"	36.92	2.82 (ddd, 10.3, 10.0, 5.0)
4"	131.31	5.28 (dd, 15.0, 10.0)
5"	133.05	5.63 (m)
6"	32.32	1.89 (m)
7"	28.35	1.30 (m)
8"	31.38	1.28 (m)
9"	29.02	1.27 (m)
10"	14.04	0.88 (t, 6.5)

1'''	46.74	2.96 (m) & 2.97 (m)
2'''	28.35	1.73 (m)
3'''	20.20 20.20	0.87 (d, 6.7) 0.86 (d, 6.7)
NH	-	3.15 (br t, 6.0)



Key COSY and importent HMBC correlations of compound **5b**



Key NOE correlations of compound **5b**

The ^{1}H homodecoupling NMR (Fig-**7**) experiments of **5b** revealed the connectivities H-2 (δ 2.45, m) to H-3 (δ 5.56, ddd, J = 10.0, 4.3, 2.6 Hz) to H-4 (δ 5.98, dt, J = 10.0, 1.8 Hz) to H-5 (δ 2.41, m) to H-2"(δ 2.68, dd, J = 11.3, 10.0 Hz) to H-3" (δ 2.82, ddd, J = 10.1, 10.0, 5.0 Hz) *via* cyclohexene ring protons. The *meta*-orientation of the carbonyl and isobutylamide groups were established by HMBC (Fig-**6**) correlations for δ 2.45 (m, H-2), 5.56 (ddd, J = 10.0, 4.3, 2.6 Hz, H-3), 2.82 (ddd, J = 10.3, 10.0, 5.0 Hz, H-3")/δ 173.80 (C-1) and δ 2.68 (dd, J = 10.3, 10.0 Hz, H-2"), 2.41 (m, H-5), 2.82 (ddd, J = 10.3, 10.0, 5.0 Hz, H-3")/δ 173.04 (C-1").

Furthermore, the ^{1}H-^{1}H COSY (Fig-**7**) cross-peaks between δ 2.82 (ddd, J = 10.3, 10.0, 5.0 Hz, H-3") and δ 5.28 (dd, J = 15.0, 10.0 Hz, H-4"), and δ 5.63 (m, H-5") and δ 2.41 (m, H-5), 1.96 (m, H-6), 1.40 (m, H-7), coupled with the HMBC correlation for δ 5.63 (m, H-5") to δ 28.35 (C-7"), δ 1.40 (m, H-7) to δ 37.04 (C-5) established the attachment of the 1-heptene and *n*-amyl groups at C-3" and C-5, respectively.

The analysis of the ^{1}H-^{1}H coupling constants and NOESY (Fig- **8**) data allowed us to determine the relative stereochemistry of compound **5b**. The coupling constants of H-2"/H-5 and H-2"/H-3" (10.3 Hz) indicated anti relations of H-2"/H-5 and H-2"/H-3". In the NOESY spectrum correlations were observed at δ 2.45 (H-2) δ 2.82 (H-3") and δ 2.41 (H-5) and correlations were not observed at δ 2.68 (H-2") with δ 2.82 (H-3") and δ 2.68 (H-2") with δ 2.41 (H-5). These data were in agreement with the β-orientation for H-2" and α-orientation for H-3" and H-5. Thus, based on these spectral data the stereostructure of **5b** was confirmed and trivially named as **chabamide L**.

Cycloaddition reaction between piperine (1a) and pellitorine (1b):

Our aim of this cycloaddition reaction is to explain to study different cycloadducts and selectivity of diene among piperine and pellitorine (Scheme **4**). This biomimetic synthesis will **explain** the probability of diene, which participated in Diels-Alder reaction between piperine (**1a**) and pellitorine (**1b**) both were isolated from same plant (*P. chaba*). **Nigramide N**, which is formed biosynthetically via cycloaddition reaction between piperine and pellitorine, this adduct previously isolated from roots of *P. nigrum* [21] by *Wei. et. al*.

Lewis acid catalyzed cycloaddition reactions of piperine (**1a**) and pellitorine (**1b**) under organic and aqueous solvent conditions to **give** resultant cycloadducts **2c**, **3c**, **4c, 2a** and **3b.** Cycloadduct **2c** and **3c** is new cycloadducts and their structures were illustrated by 1D and 2D spectral data.

Structure elucidation of compound 2c:

Compound **2c** was obtained as pale yellow liquid. The molecular formula of **2c** was established as $C_{31}H_{44}N_2O_4$ by HRESIMS (Fig-**18**), which provided a molecular ion peak at *m/z* 509.3381 [M^++H], in conjunction with its ^{13}C NMR spectrum (Fig-**12**). The IR spectrum displayed absorption bands diagnostic of carbonyl (1640 cm^{-1}) (Fig-**10**). The 300 MHz ^1H NMR spectrum (in $CDCl_3$) indicated the presence of two signals at δ 5.86 (dd, J = 15.6, 10.1 Hz) and 6.27 (d, J = 15.6 Hz), which were assigned to *trans*-olefinic protons by the coupling constant of 15.6 Hz. It also displayed aromatic protons due to two 1, 3, 4-trisubstituted aromatic rings at δ 6.82 (1H, br s), 6.76 (1H, dd, J = 7.8, 1.4 Hz), 6.75 (1H, d, J = 7.8 Hz) (Fig-**11**), (Table 4).

In addition to the above-mentioned moieties, combined inspection of ^1H NMR and ^1H–^1H COSY revealed the presence of cyclohexene ring, one isobutylamide and one pyrrolidine ring.

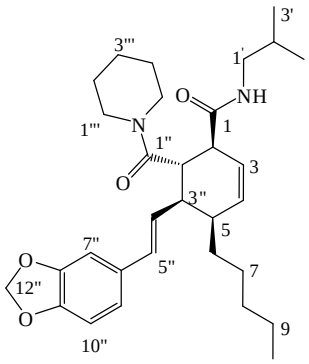
The ^{13}C NMR spectrum displayed the presence of 31 carbon atoms and were further confirmed by DEPT experiments into categories of 11 methylenes, 12 methines and 5 quaternary carbons including two carbonyls (δ 173.01 and 172.50). On the basis of these characteristic features, database and literature search led the skeleton of compound **2c** as a dimeric alkaloidal framework.

A comprehensive analysis of the 2D NMR data of compound **2c** facilitated the proton and carbon assignments. ^1H–^1H COSY spectrum suggested the sequential correlations of δ 3.51 (dq, J = 5.0, 2.6 Hz)/5.62 (dt, J = 9.8, 2.6 Hz)/6.10 (ddd, J = 9.8, 1.5 Hz)/2.20 (m)/2.72 (ddd, J = 11.1, 10.1, 5.2 Hz)/3.35 (dd, J = 11.1, 9.8 Hz) assignable to H-2-H-3-H-4-H-5-H-3"-H-2" of the cyclohexene ring.

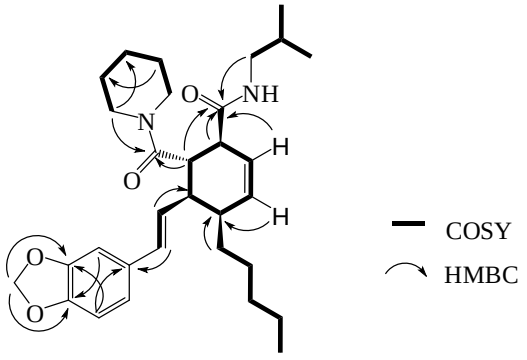
Table 4: ^1H & ^{13}C NMR data of cycloadduct **2c** in $CDCl_3$ (300 MHz, δ in ppm, mult, J in Hz)

No.	**^{13}C, δ**	**Compound 2c**
		^1H, δ (mult, J, Hz)
1	173.01	-C-
2	47.73	3.51 (dq, 5.0, 2.6)
3	124.31	5.62 (dt, 9.8, 2.6)
4	132.63	6.10 (ddd, 9.8, 1.5)
5	39.12	2.20 (m)
6	32.20	1.35 (m)
7	27.23	1.22 (m)
8	31.92	1.23 (m)
9	22.69	1.28 (m)
10	14.08	0.08 (t, 5.5)
1'	46.90	3.09 (t, 5.8)
2'	28.54	1.53 (m)
3'	20.07 20.07	0.85 (d, 6.5) 0.88 (d, 6.5)
1"	172.50	-C-

2"	39.49	3.35 (dd, 11.1, 9.8)
3"	45.17	2.72 (ddd, 11.1, 10.1, 5.2)
4"	127.32	5.86 (dd, 15.6, 10.1)
5"	131.53	6.27 (d, 15.6)
6"	132.03	-
7"	105.36	6.82 (br s)
8"	147.91	-
9"	146.87	-
10"	108.26	6.75 (d, 7.8)
11"	120.74	6.76 (dd, 7.8, 1.4)
12"	100.99	5.95 (br s)
1'"	47.21	3.52 (m)
2'"	26.62	1.62 (m)
3'"	24.51	1.66 (m)
4'"	25.61	1.55 (m)
5'"	43.09	3.50 (m)
NH	-	2.30 (t, 7.1)



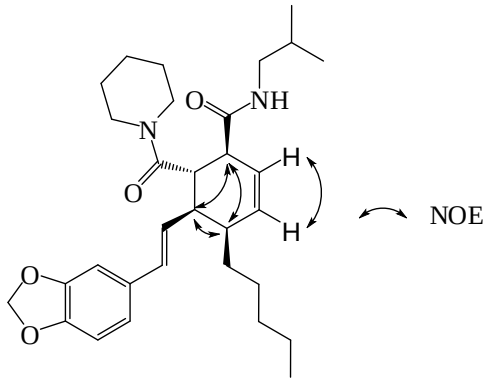
Compound **2c**



COSY and importent HMBC correlations of compound **2c**

Concerning the connections of the *n*-amyl and 3, 4-methylenedioxy styryl groups, HMBC spectrum (Fig-**15**) showed correlations of H-4, H-6, H-7/C-5; H-5", H-4"/C-3", which implies that these units were bonded to the cyclohexene ring at C-5 and C-3". Further, HMBC correlations of two methylene protons at δ 5.95 with 147.91 (C-8"), 146.87 (C-9"),

confirmed the location of methylenedioxy group at C-8", and C-9". Remaining units, isobutylamine and pyrrolidine (rings) were connected through carbonyl groups at C-2 and C-2", which was confirmed by HMBC correlations of H-2 and H-1' to C-1 (δ 173.01) and H-2" and H-1'" to C-1" (δ 172.50).

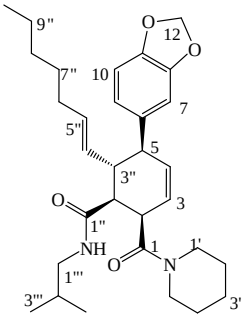


Key NOE correlations of compound **2c**

The assignment of the relative configuration of compound **2c**, and confirmation of overall structure were achieved by the interpretation of the NOESY spectral data and by analysis of ^{1}H NMR coupling constants. The large vicinal coupling constants of H-2"/H-2 (11.1 Hz) and H-2"/H-3" (11.1 Hz) indicated anti-relations of H-2"/H-2 and H-2"/H-3" and the axial orientations for these protons. In the NOESY spectrum (Fig-**17**), the occurrence of the correlations between H-2/H-3" and the absence of NOE effects between H-2/H-2" and H-2"/H-3" supported the above result. This data indicated β-orientation for H-2" and α-orientation for H-2 and H-3". The α-orientation of H-5 was suggested by the coupling constant of H-5/H-3" (5.2 Hz) and the absence of the NOESY correlations between H-3" and H-2". On the basis of these spectral data, the structure of compound **2c** was unambiguously established and trivially named as **chabamide M**.

Structure elucidation of compound 3c:

Compound **3c** was obtained as pale yellow liquid. The molecular formula of **3c** was established as $C_{31}H_{44}N_2O_4$ by HRESIMS (Fig-**27**), which provided a molecular ion peak at *m/z* 509.3391 [M^++H], in conjunction with its ^{13}C NMR spectrum (Fig-**21**). The IR spectrum displayed absorption bands diagnostic of carbonyl (1624 cm^{-1}) moiety (Fig-**19**). The 300 MHz ^{1}H NMR spectrum (in $CDCl_3$) indicated the presence of two signals at δ 4.63 (dd, J = 15.6, 10.0 Hz) and 5.46 (dt, J = 15.6, 6.8 Hz), which were assigned to *trans*-olefinic protons by the coupling constant of 15.6 Hz. It also displayed aromatic protons due to two 1, 3, 4-trisubstituted aromatic ring at δ 6.75 (1H, br s), 6.73 (1H, d, J = 7.8, 1.4 Hz), 6.71 (1H, d, J = 7.8 Hz) (Fig-**20**). In addition to the above-mentioned moieties, combined inspection of ^{1}H NMR and ^{1}H–^{1}H COSY revealed the presence of cyclohexene ring, one isobutylamide and one pyrrolidine ring. The ^{13}C NMR spectrum displayed the presence of 31 carbon atoms (Table **5**), and were further classified by DEPT experiments (Fig-**22**) into categories of 11 methylenes, 12 methines and 5 quaternary carbons including two carbonyls (δ 173.34 and 173.88). On the basis of these characteristic features, database and literature searches led the skeleton of compound **3c** as a dimeric alkaloidal framework.

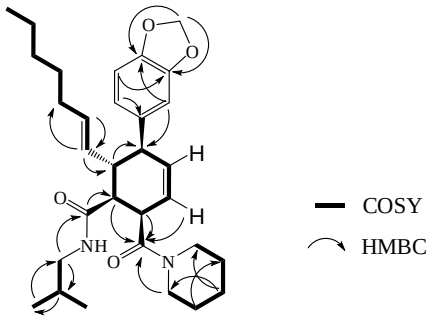


Compound **3c**

Table 5: ^{1}H & ^{13}C NMR data of cycloadduct **3c** in $CDCl_3$ (300 MHz, δ in ppm, mult, J in Hz)

No.	**^{13}C, δ**	**Compound 3c**
		^{1}H, δ (mult, J, Hz)
1	171.34	-C-
2	44.20	2.82 (m)
3	124.68	5.63 (dt, 9.7, 1.9)
4	131.30	5.82 (ddd, 9.7, 4.8, 1.9)
5	42.49	3.94 (dq, 10.0, 1.9)
6	133.66	-

7	107.88	6.75 (br s)
8	147.42	-
9	146.49	-
10	111.40	6.71 (d, 7.8)
11	123.72	6.73 (dd, 7.8, 1.4)
12	101.07	5.92 (d, 1.4)
1'	47.04	3.54 (m)
2'	26.76	1.63 (m)
3'	24.61	1.67 (m)
4'	25.73	1.55 (m)
5'	43.37	3.53 (m)
1"	173.88	-C-
2"	45.90	3.36 (dt, 11.7, 4.8)
3"	43.33	2.76 (dt, 11.7, 10.0)
4"	129.91	4.63 (dd, 15.6, 10.0)
5"	133.18	5.46 (dt, 15.6, 6.8)
6"	32.41	1.87 (q, 6.8)
7"	29.03	1.70 (m)
8"	31.38	1.23 (m)
9"	22.53	1.25 (m)
10"	14.06	0.85 (t, 5.8)
1"'	46.65	2.90 (m)
2"'	28.41	1.62 (m)
3"'	20.05	0.80 (d, 6.5) 0.82 (d, 6.5)
NH	-	5.80 (t, 5.8)

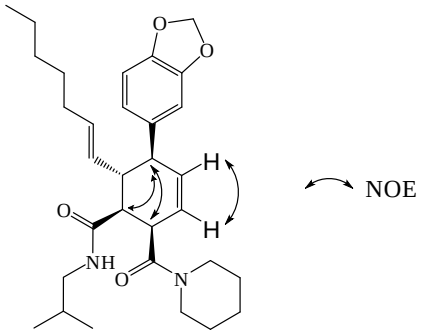


Key COSY and HMBC correlations of compound **3c**

A comprehensive analysis of the 2D NMR data of compound **3c** facilitated the proton and carbon assignments. ^{1}H–^{1}H COSY spectrum (Fig-**25**) suggested the sequential correlations of δ 2.82 (m)/5.63 (dt, J = 9.7, 1.9 Hz)/5.82 (ddd, J = 9.7, 4.8, 1.9 Hz)/3.94 (dq, J =.10.0, 1.9 Hz)/2.76 (ddd, J = 11.7, 10.0 Hz)/3.36 (dt, J = 11.7, 4.8 Hz) assignable to H-2-H-3-H-4-H-5-H-3"-H-2" of the cyclohexene ring. Concerning the connections of the 3, 4-methylenedioxyphenyl and 1-heptene groups, HMBC spectrum (Fig-**24**) showed correlations of H-7, H-11, H-3"/C-5; H-5", H-4", H-5/C-3", which implies that these units were bonded to the cyclohexene ring at C-5 and C-3". Further, HMBC correlations of two methylene protons at δ 5.92 with 147.42 (C-8"), 146.49 (C-9"), confirmed the location of methylenedioxy group at C-8", and C-9".

Remaining units, pyrrolidine and isobutylamine were connected through carbonyl groups at C-2 and C-2", which was confirmed by HMBC correlations of H-2 and H-1' to C-1 (δ 171.34) and H-2" and H-1'" to C-1" (δ 173.88). The assignment of the relative configuration of compound **3c**, and confirmation of overall structure were achieved by the interpretation of the NOESY spectral data and by analysis of ^{1}H NMR coupling constants. The large vicinal coupling constants of H-3"/H-2" (11.7 Hz) and H-5/H-3" (10.0 Hz), indicated anti-relations of H-3"/H-5 and H-3"/H-2" and the axial orientations for these protons.

In the NOESY spectrum (Fig-**26**), the occurrence of the correlations between H-2"/H-5 and the absence of NOE effects between H-3"/H-2" and H-3"/H-5 supported the above result. These data indicated β-orientation for H-2" and α-orientation for H-2 and H-3". The α-orientation of H-2 was suggested by the coupling constant of H-2/H-2" (4.8 Hz) and the occurrence of the NOESY correlations between H-2" and H-2. On the basis of this spectral data, the structure of compound **3c** was unambiguously established and trivially named as **Chabamide N**.



Key NOE correlations of compound **3c**

Cycloaddition reaction between piperine (1a) and E-fagaramide (1c)

Lewis acid catalyzed cycloaddition reactions (Scheme **5**) of piperine (**1a**) and *trans*-fagaramide (**1c**) under aqueous solvent conditions to give resultant cycloadducts **2d**, **3d** and **2a.** To carry this biomimetic synthesis to explain **compound H** and **I** (mentioned in chapter-II), we taken piperine (**1a**) which is isolated from same plant and *trans* fagaramide was synthesized by reported method.[24] Cycloaddition reaction between **1a** and **1c** end up with overall yield 70% in xylene and 75% in water. In both solvents *ortho* products were formed dominantly compared with *meta* products. Spectral data 1D and 2D of cycloadducts **2d** & **3d** were identical with compound **H** & **I** (see chapter I, compound H & K). Cycloadduct **2a** is identical with chabamide. This cycloaddition reaction practically proved as biomimetic synthesis for compound H and I.

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