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# Synthesis and antiviral properties of tricyclic amides derived from α-humulene and β-caryophyllene

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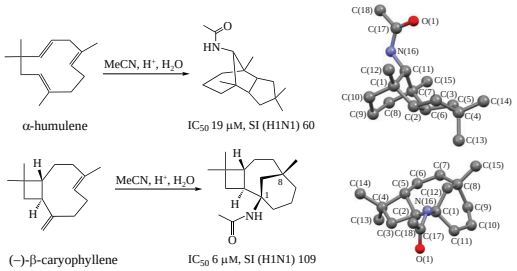
**The Ritter reaction of humulene with acetonitrile occurs as the biomimetic process to afford the amide having the skeleton of a natural alcohol. The structures of the amides obtained from humulene and caryophyllene were confirmed by XRD data. The activity of some cage compounds against influenza virus allowed one to suggest the mechanism of antiviral action based on interfering with membrane fusion activity of viral hemagglutinin.**




*Keywords*: humulene, caryophyllene, acetonitrile, Ritter reaction, amides, antiviral agents, influenza, molecular modeling.

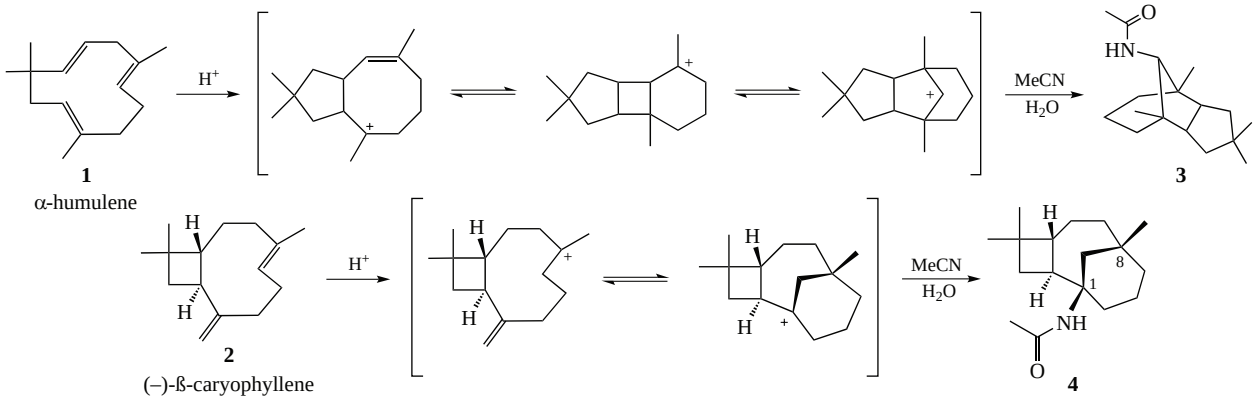
Terpenes are the most important source of lead compounds for the flavour, fragrance, and pharmaceutical industry. Essential oils and their components are used in treatment of upper respiratory tract diseases as expectorants and antiviral agents. Many studies have been devoted to investigation of the antiviral activity of total plant extracts, where monoterpenoids are the major components.[1] Chemical modification of the natural mono- and sesquiterpenoids with additional pharmacophore groups can significantly increase the antiviral activity.[2] The aim of this work was to synthesize acetamides based on sesquiterpene compounds, humulene **1** and caryophyllene **2**, and to study the antiviral activity of the resulting polycyclic amides. α-Humulene is acyclic sesquiterpene with three double bonds and six allylic positions, which is found in the essential oils of *Humulus lupulus* (Common Hop). The anti-inflammatory[3] and anti-cancer[4] activity of humulene has been shown. Caryophyllene, the most common representative of bicyclic sesquiterpenes, is present in many essential oils, especially in *Eugenia caryophyllata*,[5] *Myrica gale*[6] and *Comptonia peregrina*[7] ones. As a rule, in essential oils caryophyllene is accompanied by isomeric (*Z*)-β-caryophyllene (isocaryophyllene), humulene and β-caryophyllene epoxide.[8] We previously studied the transformations of caryophyllene under the Ritter reaction conditions and showed that the main product was a tricyclic acetamide of caryolanic type.[9] In the present work we have subjected α-humulene to the Ritter reaction with acetonitrile and examined the antiviral properties of compounds derived from α-humulene and β-caryophyllene.

Recently the interest in the Ritter reaction has emerged since varying alcohols and nitriles provided great diversity of various amides.[10] In addition, the study of the transformations of natural compounds under the Ritter reaction conditions can reveal some biomimetic transformations.[11] We have shown herein that dissolution of α-humulene **1** in acetonitrile containing 5% sulfuric acid afforded tricyclic acetamide **3** as the major product having the skeleton of natural alcohol Apollan-10-ol, α-caryophyllene alcohol[12] (Scheme 1). Product **3** was isolated from the reaction mixture by crystallization in acetonitrile in 57% yield; its structure was confirmed by X-ray diffraction analysis,† as well as 1D ($^1$H, $^{13}$C) and 2D NMR

† *Crystallographic data for* N-*(2,2,4,8-tetramethyldecahydro-4,8-methanoazulen-9-yl)acetamide* **3**. $C_{17}H_{29}NO$, $M$ = 263.41, monoclinic, *C*2/*c*, $a$ = 15.659(1), $b$ = 21.839(1) and $c$ = 9.6459(4) Å, $\beta$ = 99.708(3)°, $V$ = 3251.4(3) Å$^3$, $Z$ = 8, $d_{calc}$ = 1.076 g cm$^{-3}$, $\mu$(MoKα) = 0.065 mm$^{-1}$, $F$(000) = 1168.0, ($\theta$ = 1.62–28.78°, completeness 99.9%), $T$ = 296(2) K, colourless, (0.95 × 0.12 × 0.06) mm$^3$, transmission 0.8790–0.9281, 48340 measured reflections in index range –21 ⩽ $h$ ⩽ 19, –29 ⩽ $k$ ⩽ 28, –12 ⩽ $l$ ⩽ 12, 3870 independent ($R_{int}$ = 0.0387), 177 parameters, $R_1$ = 0.0610 [for 3030 observed $I > 2\sigma(I)$], $wR_2$ = 0.2137 (all data), GOOF = 1.192, largest diff. peak and hole 0.628 and –0.798 e A$^{-3}$.

*Crystallographic data for* N-*[(1*R*,2*S*,5*R*,8*R*)-4,4,8-trimethyltricyclo[6.3.1.0$^{2,5}$]dodec-1-yl]acetamide* **4**. $C_{17}H_{29}NO$, $M$ = 263.41, tetragonal, $P4_3$, $a = b$ = 10.3159(5) and $c$ = 15.5864(7) Å, $V$ = 1658.7(1) Å$^3$, $Z$ = 4, $d_{calc}$ = 1.055 g cm$^{-3}$, $\mu$(MoKα) = 0.064 mm$^{-1}$, $F$(000) = 584.0, ($\theta$ = 3.08–24.65°, completeness 99.9%), $T$ = 296(2) K, colourless, (0.61 × 0.11 × 0.07) mm$^3$, transmission 0.9387–0.9881, 17065 measured reflections in index range –12 ⩽ $h$ ⩽ 12, –12 ⩽ $k$ ⩽ 12, –18 ⩽ $l$ ⩽ 16, 2705 independent ($R_{int}$ = 0.0504), 176 parameters, $R_1$ = 0.0720 [for 2705 observed $I > 2\sigma(I)$], $wR_2$ = 0.2238 (all data), GOOF 1.212, largest diff. peak and hole 0.465 and –0.786 e A$^{-3}$.

The X-ray diffraction experiments were carried out on a Bruker KAPPA APEX II diffractometer (graphite-monochromated MoKα radiation). Reflection intensities were corrected for absorption by




**Scheme 1** *Reagents and conditions*: MeCN, $H_2SO_4$ (cat.), room temperature, 20 min, then $Na_2CO_3$ (aq.).

spectroscopy (homonuclear $^1$H–$^1$H COSY, $^1$H–$^1$H NOESY and heteronuclear $^1$H–$^{13}$C HSQC, $^1$H–$^{13}$C HMBC, see Online Supplementary Materials, Figure S1).

Compound **4** was obtained similarly in 42% yield, as described earlier.[9] Its crystals were grown from hexane and confirmed by X-ray diffraction analysis. Our X-ray studies allowed us to clarify the location of the substituents in the 1 and 8 positions of amide **4**.†

The *cis*-fused five-membered rings in compound **3** have an *envelope* conformations, the six-membered ring adopts a *chair* conformation that is typical for such tricycle skeleton.[13] The orientation of the acetamido group is determined by the torsion angle C(17)–N(16)–C(11)–C(1) of 107.9(2)°. The presence of this group leads to formation of the hydrogen bonded chains [see Online Supplementary Materials, Figure S2(*a*)], directed along the axis *c* [Figure 1(*a*)]. The classical hydrogen bond N(16)–H···O(1) with parameters H···O and N···O of 2.03 and 2.887(2) Å, and N–H···O of 171° additionally strengthened by weak bonds C(8)–H···O(1) and C(18)–H···O(1) with parameters: 2.58, 3.437(2) Å, 148° and 2.56, 3.414(2) Å, 148°, respectively, are observed. The six-membered ring of compound **4** adopts a distorted *sofa* conformation, the seven-membered one has the conformation of distorted *chair* in contrast to the same tricycle skeleton of compounds from Cambridge Crystallographic Data Centre demonstrating *chair* conformation for six-membered and *boat* for seven-membered one.[14,15] The envelope of *trans*-fused four-membered ring has a puckering angle of 29.9°. The acetamido group is almost in the plane of C(1)–C(12)–C(8) bridge, torsion angle C(17)–N(16)–C(1)–C(12) is –165.9(3)°. The presence of this group also leads to formation of the intermolecular hydrogen bonds N–H···O that organize the molecules into helical chains about the $4_3$ screw axis along the axis *c* [see Figure S2(*b*)]. The parameters of hydrogen bond N(16)–H···O(1) are as follows: distances H···O and N···O are 2.1 and 2.942(4) Å, angle N–H···O is 166°.

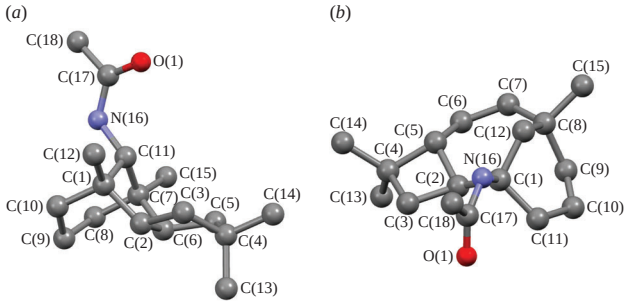



**Figure 1** The molecular structure of (*a*) compound **3** and (*b*) compound **4** (the hydrogen atoms are omitted for clarity).

Recently, we have shown that tricyclic acetamide obtained by the Ritter reaction of isocaryophyllene exhibited extremely high activity against influenza virus, strain H1N1.[16] We named that compound Ginsamide because it had the backbone of the natural alcohol Ginsenol. It is of note that compounds containing the amide bond are promising antiviral agents in terms of medicinal chemistry.[17,18] Amides **3** and **4**, as well as the initial sesquiterpenes humulene **1** and caryophyllene **2** were tested herein as influenza virus inhibitors (Table 1).

The obtained tricyclic acetamides **3** and **4** truly possess a significant virus-inhibitory activity while remaining low-toxic in *in vitro* experiments. At the same time, the activity of amides **3** and **4** is essentially lower than that of ginsamide. Notably, the initial sesquiterpenoids humulene and caryophyllene showed no activity in this test, being both non-toxic and inactive. Meanwhile, the described compounds possess the suppressing activity against virus A/Puerto Rico/8/34 (H1N1), that is, like vast proportion of currently circulating influenza strains, resistant to another cage compounds, amantadine and rimantadine.[19] These drugs block virus-specific ion channel M2 that is responsible for acidification of virion interior and further providing dissociation of viral genome from the envelope. Previously, we showed that ginsamide activity was due to binding to influenza virus hemagglutinin; we also obtained a ginsamide-resistant strain and identified mutations. Considering the structural similarity of amides **3** and **4** with the framework Ginsamide, we suggested that these compounds could also exhibit activity against influenza

**Table 1** Antiviral activity of compounds **1**–**4** against influenza virus A/Puerto Rico/ in MDCK cells.

| Compound | $CC_{50}$[a]/μM | $IC_{50}$[b]/μM | SI[c] |
|---|---|---|---|
| α-Humulene **1** | >1470 | >1470 | <1 |
| (–)-β-Caryophyllene **2** | >1470 | >1470 | <1 |
| Amide **3** | >1140 | 19.01 ± 2.6 | 60 |
| Amide **4** | 707 ± 35 | 6.46 ± 0.4 | 109 |
| Ginsamide[d] | >1140 | 0.152 ± 0.03 | 7500 |
| Rimantadine | 344 ± 20 | 72 ± 6 | 5 |
| Amantadine | 266 ± 19 | 60 ± 8 | 4 |

[a] $CC_{50}$ – cytotoxic concentration; the concentration affording 50% death of cells. [b] $IC_{50}$ – effective concentration; the concentration affording 50% inhibition of virus replication. [c] SI – selectivity index, ratio $CC_{50}/IC_{50}$. [d] Biological data from ref. 16.

SADABS program.[23] The structures were solved by direct methods using the SHELXS-97 program and refined by anisotropic (isotropic for all H atoms) full-matrix least-squares method against $F^2$ of all reflections by SHELX-97.[24] The positions of the hydrogen were calculated geometrically and refined in riding model.

CCDC 2152454 and 2152455 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

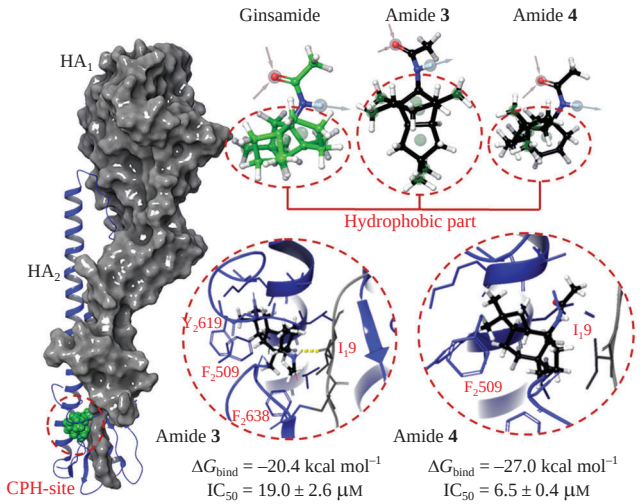



**Figure 2** Molecular docking results: one protomer of HA is presented. Protein visualization is based on PDB code 1RU7.[22] CPH-site is presented as a green region. Hydrophobic parts of Ginsamide and amides **3**, **4** are highlighted by dark-red dotted lines. The hydrogen bond between H-atom of amide **3** and O-atom $Ile_1$9 is shown by the yellow dotted line.

virus hemagglutinin. According to reported data,[16] Ginsamide binds to the $HA_2$ at the Camphecene[20,21] binding site (CPH-site). The pharmacophore profiles of Ginsamide and amides **3** and **4** are similar (Figure 2). Amides can bind in the CPH-site, predominantly forming hydrophobic contacts with surrounding amino acids. Additionally, hydrogen bond between amide **3** and $Ile_1$9 is formed. The binding energy of amide **4** is almost 7 kcal $mol^{-1}$ lower than the binding energy of amide **3**. In other words, the affinity of amide **4** to CPH-site is higher than that of amide **3**. This result correlates with data of biological tests.

To conclude, the Ritter reaction of humulene proceeds as biomimetic transformation with the formation of a cage amide having a natural alcohol Apollan-10-ol skeleton. The activity of amides **3** and **4** against influenza virus was assessed; the mechanism of action was suggested. Based on the data presented, it can be concluded that these agents have a good potential as highly effective antiviral compounds.

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*Online Supplementary Materials*

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.09.013.

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