ECO-FRIENDLY MICROWAVE-ASSISTED SYNTHESIS OF BIOLOGICALLY ACTIVE NAPHTHENIC ACID *N*-CYCLOHEXYL AMIDES

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Abstract
Inside the framework of green chemistry, a noticeable results were obtained in microwave-assisted solvent-free synthesis of biologically active *N*-cyclohexyl amides of naphthenic acids (NAs). Naphthenic acid amides were synthesized directly from free carboxylic acids in the absence of solvent and catalyst. Synthesized *N*-cyclohexyl amides of naphthenic acid were evaluated for their auxin activity.

Introduction
The stable and polar amide functionality is an important unit among the organic molecules present in natural-occurring materials (e.g., peptides and proteins). It is also found in many synthetic substances as intermediates or as active pharmaceutical products or prodrugs [1]. Due to its interest in organic synthesis, the preparation of amides from the corresponding amines is an important and well-known transformation, but the main drawbacks of these reactions are long reaction time, low yield, use of organic solvents and expensive or toxic reagents [2,3,4].
Over the last years, a large number of publications have clearly shown that many types of chemical transformations can be carried out successfully under microwave irradiation [5]. Most importantly, microwave processing frequently leads to dramatically reduced reaction times, higher yields, easier work-up matching with the goal of green chemistry, atom economy, and selectivity of reactions. In addition to their wide application, utilization of microwave technology in the amide solvent-free synthesis is not frequently described in the literature [6,7,8].
Napthenic acids (NAs) represent a complex mixture of alkyl-substituted aliphatic and cyclic monocarboxylic acids of the general formula $C_nH_{2n-z}O_2$, where n is the number of carbon atoms and z the hydrogen deficiency due to ring formation, obtained from oil and oil derivatives by alkaline extraction. In addition to a wide application in the chemical industry, these compounds exhibit biological activity. Low concentrations (up to 0.5 mg/L) of NAs and their salts have been studied for a long time as substances exhibiting biological activity, such as plant growth hormones [9,10] but at high concentrations (above 50 mg/L), NAs are corrosive and toxic substances [11] and for these reasons they represent serious contaminants of refinery wastewaters and act as environmental pollutants.
Having in mind the nature of naphthenic acids and amide group as an important functionality due to its presence in great number of biomolecules, the aim of the present work was to designed simple, eco-friendly method of forming biologically active amide derivatives of naphthenic acids by using microwave irradiation.

Experimental

The Velebit naphthenic acids (VNA) were extracted from the atmospheric gas oil fraction (distillation interval 168-290 °C) of Vojvodina crude oil [10].

All reagents and solvents were obtained from commercial suppliers and used without further purification, as well as Aldrich naphthenic acids (ANA). Microwave-assisted reactions were carried out in CEM Discover BenchMate single-mode microwave reactor. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (Silica gel 60 F_{254}). Purification of products was carried out by flash column chromatography using Kieselgel 60 (0.040–0.063, Merck). NMR spectra were recorded on a Bruker AC 250 E instrument.

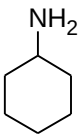
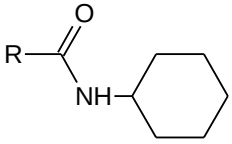
Microwave-assisted synthesis of naphthenic acid *N*-cyclohexyl amides were carried in a 10 mL Pyrex reaction vial. A mixture of NAs (5 mmol) and amine (5 mmol) was heated at 270 °C for 2 min of microwave irradiation with stirring. Upon cooling to 45 °C the reaction mixture was dissolved in CH_2Cl_2 and washed with 1 M HCl, 5% $NaHCO_3$ and H_2O. After removing the solvent with rotavapor, the residue was purified by flash column chromatography on silica gel (dichloromethane:ethyl acetate=9.5:0.5) to afford the pure product **1** (1.40 g, 78%) and **2** (1.27 g, 78%) as a yellow oil.

The auxin activity of the amides was determined by the test of inhibition of white mustard (*Sinapis alba*) germination based on counting the germinated seeds after treatment with VNK, ANK and naphthenic acid *N*-cyclohexyl amides solution (**1** and **2**, 10^{-5}-10^{-7} M) and the corresponding concentrations of 3-indoleacetic acid (IAA, 10^{-5}-10^{-7} M). The experiments were repeated two times. Germination was performed in dark, under the temperature of 25 °C for 24h. The results are presented as mean value ± standard deviation.

Results and discussion

Napthenic acids (NAs), complex mixture of carboxylic acids isolated from middle and higher fractions of the Vojvodina crude oil Velebit and comercial Aldrich napthenic acids, reacted efficiently with cyclohexylamine under high-temperature heating in closed-vessel system of microwave reactor. The maximal operative temperature for these reaction systems was 270 °C. Microwave-assisted synthesis of NA amides was completed in 2 minutes of microwave irradiation leading to a 78% of isolated yield (Table 1).

Table 1. Microwave-assisted synthesis of Velebit (VNA) and Aldrich (ANA) naphthenic acid *N*-cyclohexyl amides

Entry	NAs	Amine	Product (**1**/**2**)	Yield (%)
1	VNA			78
2	ANA			78

The study of biological activity of the type of the plant hormones auxin are presented in Figure 1 and 2. Since the rooting occurs in the presence of plant hormones as it is indol-3-acetic acid, a natural auxin, it has been used as control.

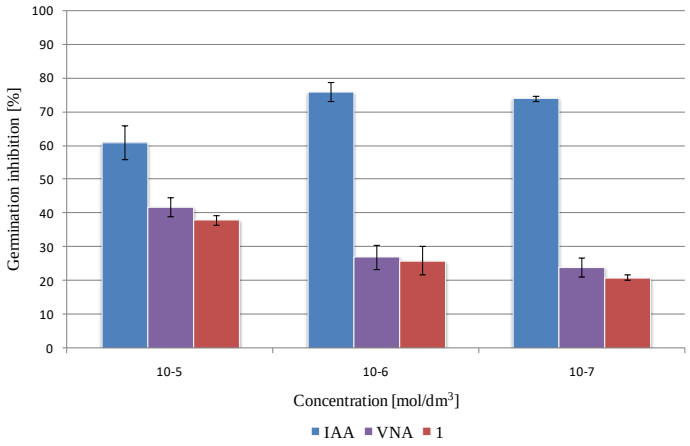


Figure 1. Auxin activity of VNA and their *N*-cyclohexyl amides

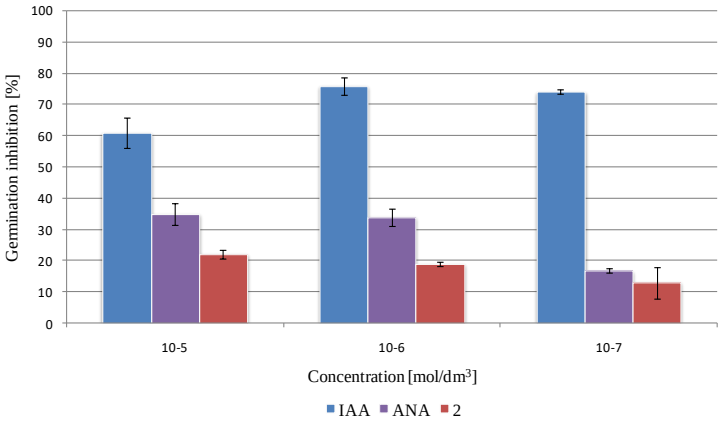


Figure 2. Auxin activity of ANA and their *N*-cyclohexyl amides

Based on the test of inhibition of germination (higer inhibition - higher auxin activity) of white mustard it is obvious that NAs Velebit and Aldrich showed somewhat lower auxin activity which corresponds to an IAA concentration of 10^{-5} M. The lowest auxin activity was exhibited by the concentration of 10^{-7} M.

As is evident from Figure 1 and 2, the treatment of white mustard seeds with NA *N*-cyclohexyl amides (**1** and **2**) caused lower inhibition of germination, which may be a result of an activity similar to that of plant hormones of the auxin-type.

Conclusion

In conclusion, directly from non-activated carboxylic acids and amines and in the absence of coupling reagents and solvents, NA *N*-cyclohexyl amides were successfully synthesized uner microwave irradiation. Starting from the equimolar mixture (atom-economical synthesis) of amine and complex mixture of NAs, high yields of NA *N*-cyclohexyl amides were obtained. Biological activity studies of NAs and synthesized amide derivatives as stimulators of plant growth revealed their auxin activity but the chemical transformation of carboxylic functional group didn't lead to increasing of germinaton inhibition.

Acknowledgements

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