

Preparation of some derivatives of (+)-usnic acid with aromatic amines under microwave irradiation condition

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ABSTRACT

Up to now, derivatives of usnic acid were only prepared by classical methods of refluxing in organic solvents for hours. In this study, we examined the condensation reaction of usnic acid with *o*-toluidine under

microwave irradiation condition which gave good yield in a short time. The obtained optimal condition was applied to prepare some other derivatives of (+)-usnic acid.

Key words: (+)-Usnic acid, *o*-toluidine, microwave irradiation condition, condensation reaction, cyclopentyl methyl ether.

INTRODUCTION

Usnic acid (**1**), 2,6-diacetyl-7,9-dihydroxy-8,12-dimethyl-1,3-(2*H*,12*H*)-dibenzofurandione, is a naturally occurring dibenzofurane derivative found in several lichen species. Usnic acid was identified in many genera of lichens including *Alectoria*, *Cladonia*, *Evernia*, *Parmotrema*, *Ramalia*, and *Usnea*, and it exists in one of two enantiomers which differ in the orientation of the methyl group located in position 12. It was first isolated by the German scientist W. Knop in 1844 [1].

Usnic acid possesses a wide range of interesting biological properties. It is a potent antibiotic effective against Gram-positive bacteria, including *Mycobacterium tuberculosis*, *Staphylococcus*, *Streptococcus* and *Pneumococcus*. It also exhibits antiviral, antiprotozoal, antimetabolic, anti-inflammatory and anticancer [2].

In 2006, Sophie Tomasi and co-workers [3] performed condensation reaction of usnic acid with some amines, diamines and triamines. In 2008 and 2009, similar processes were performed by some authors [4].

All reactions on usnic acid were done by refluxing for some hours in organic solvents such as tetrahydrofuran (THF), benzene,... which were commonly harmful. For that reasons, we

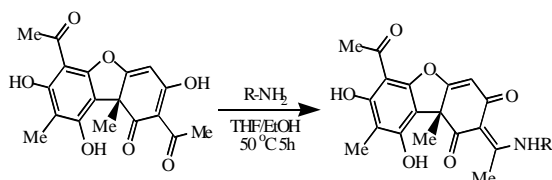


Fig 1. Condensation reaction of (+)-usnic acid with some amines.[3, 4]

examined these reactions under microwave irradiation condition with the aim obtaining new compounds by using the reaction conditions which were more friendly with the environment.

EXPERIMENTAL

Materials

THF (99 %), ethanol (99.7 %), *o*-toluidine were purchased from Kanto-Japan, cyclopentyl methyl ether (CPME) was from Aldrich, and methanol for HPLC was purchased from Labscan. (+)-Usnic acid (**1**) ($[\alpha]_D + 451.4$, c' 0.327 g/100 mL in EtOH: CHCl₃ 4:1) was isolated from lichen *Parmotrema praesorediosum* with the purity of 98.5 % determined by HPLC. Amines were purchased from Aldrich.

Equipments

Discover oven (CEM). HPLC Agilent 1100 series, C₁₈ column Agilent Zorbax 15 mm x 4.6 mm x 3 μm, temperature 40 °C, detector UV, wavelength 282 nm. The column was maintained at 40 °C, mobile phase A was water, mobile phase B was methanol. The gradient parameters were 60 % B for 10 minutes, 80 % B for 15–20 minutes, and 100 % B for 25–30 minutes. NMR spectra were measured on a Bruker Avance III spectrometer, at 500 MHz for ¹H NMR and 125 MHz for ¹³C NMR, using residual solvent signal as internal reference: chloroform-*d* δ_H 7.24, δ_C 77.23. The HR-ESI-MS were recorded on a HR-ESI-MS MicroOTOF-Q mass spectrometer.

Experimental procedures

Microwave irradiation method.

In a 10 mL test tube of a dedicated microwave oven, **1** (x mM), *o*-toluidine (y mM), and solvent (ethanol, THF or CPME) were added. The solution attained naturally pH 5, the optimal pH condition for condensation reactions. Then the

solution was irradiated at a power of 60 W. The reaction conditions were changed such as: temperature, time, ratio of substrate and reactant and kind of solvents. After the reaction, the percentage (H %) of the performed product (**3**) was determined by HPLC.

$$H = \frac{H_a \times a}{0.05 \times M_p} \%$$

Where:

a: The mass of the crude obtained product.

M_p: The molecular weight of the product (**3**).

H_a : The percentage of the product (**3**) in HPLC spectrum.

0.05: The molar concentration (mM) of the starting material (**1**) of the infected solution

After the reaction, the mixture was cooled and the light yellow precipitate was filtered off, washed with hot water, and dried for 24 hrs in a dessicator. The results were presented in Table 1.

The obtained optimal condition was applied to prepare some derivatives (Table 2) of (+)-usnic acid.

Conventional heating refluxing method.

A solution of **1** (0.1 mM) and *o*-toluidine (0.1 mM) in CPME (5 mL) was refluxed and stirred at 500 rounds/min. Twelve different experiments were prepared in order to determine the yield of the product, determined by HPLC, owing to time (1–12 hours).

RESULTS

Condensation reaction of (+)-usnic acid with *o*-toluidine

Effect of the reaction temperature

The yield increased when the temperature of the solution was raised from 80 to 90 °C (entries 1, 2 of Table 1), but from 90 °C to 110 °C, the yield

began to decrease (entries 3, 4). This result was due to the formed side-product (**4**). Therefore, the good yield of reaction was achieved at 90 °C. The

result showed that the higher the temperature was, the lower reaction selectivity took place.

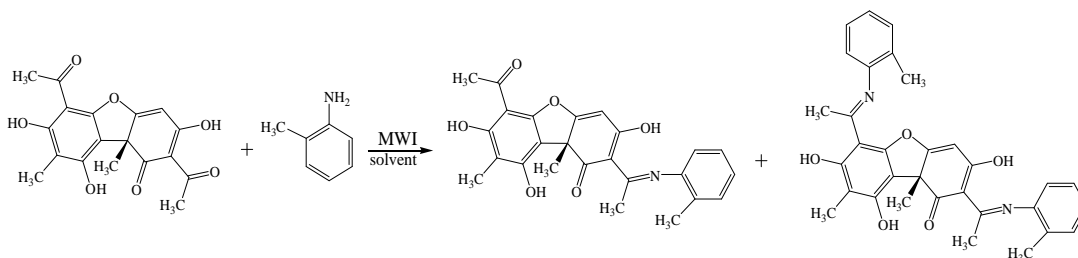


Fig 2. Condensation reaction of (+)-usnic acid with *o*-toluidine

Effect of the reaction time

In order to investigate the role of the reaction time, the reaction was carried out within 15–20 minutes at pH 5 and other conditions were kept unchanged (entries 2, 5, 6 of Table 1). The good yield of reaction was achieved in 20 minutes (entry 2).

*Effect of the quantity of *o*-toluidine*

The reaction of **1** and *o*-toluidine with the ratio of (1:1) gave highest yield of 93.9 %. When the ratio of *o*-toluidine was increased, the excess amine would react with both carbonyl groups, C-14 and C-17 of usnic acid, to create the side product **4**.

Effect of solvent

Under microwave irradiation condition,

CPME, ethanol or TMF gave appropriate results and among them, CPME gave the best. CPME is a green solvent and is no harmful to the human health and the environment, therefore, it was chosen for the following experiments.

Conventional heating refluxing method

As time increased from 1 to 9 hours, the yield also increased from 11.2 % (1 hour) to 58.2 % (9 hours) and reached the highest 62.2 % at 10 hours. More than 10 hours, the yield was not be ameliorated (60.2 %).

The results showed that the reaction yield performed under microwave irradiation condition was higher and quicker than under the conventional method, therefore this condition was applied to prepare some derivatives of (+)-usnic acid.

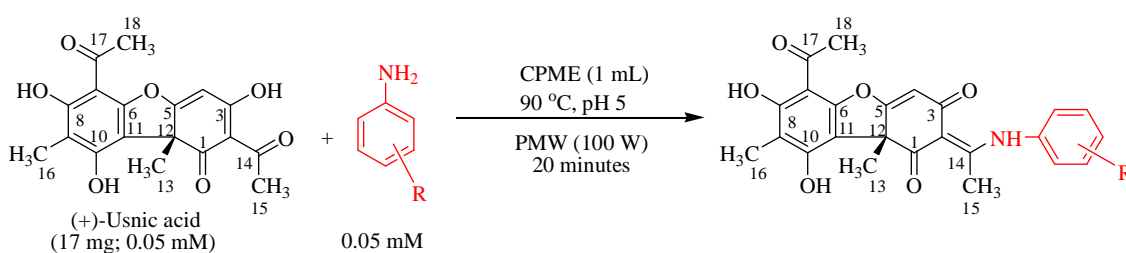
Table 1. Condensation reaction (+)-usnic acid with o-toluidine under microwave irradiation condition.

| Entry | Temp. (° C) | Time (min.) | 1 : 2 ^a (x : y) | Solvent (1 mL) | pH | HPLC (%) | | | | Yield ^b (%) |
|-----------|----------------|----------------|---------------------------------------------|-------------------|----------|----------|----------|-------------|------------|---------------------------|
| | | | | | | 1 | 2 | 3 | 4 | |
| 1 | 80 | 20 | 1 : 2 | Ethanol | 5 | – | 2.6 | 62.9 | 34.5 | 58.1 |
| 2 | 90 | 20 | 1 : 2 | Ethanol | 5 | – | 3.6 | 86.0 | 10.4 | 83.4 |
| 3 | 100 | 20 | 1 : 2 | Ethanol | 5 | – | 1.7 | 84.0 | 14.3 | 76.4 |
| 4 | 110 | 20 | 1 : 2 | Ethanol | 5 | – | 1.5 | 76.6 | 21.9 | 74.7 |
| 5 | 90 | 15 | 1 : 2 | Ethanol | 5 | – | 2.0 | 45.7 | 52.3 | 41.2 |
| 6 | 90 | 25 | 1 : 2 | Ethanol | 5 | – | 2.5 | 66.1 | 31.4 | 64.6 |
| 7 | 90 | 20 | 1 : 1.5 | Ethanol | 5 | – | 3.6 | 89.0 | 7.4 | 85.3 |
| 8 | 90 | 20 | 1 : 1 | Ethanol | 5 | – | – | 96.0 | 4.0 | 92.7 |
| 9 | 90 | 20 | 1 : 1 | THF | 5 | – | – | 95.0 | 5.0 | 91.0 |
| 10 | 90 | 20 | 1 : 1 | CPME | 5 | – | – | 98.2 | 1.8 | 96.9 |

^a Molar ratio^b The yield of **3** was determined based on the percentage of HPLC

Preparation of some derivatives of (+)-usnic acid

The obtained optimal condition was applied to prepare some derivatives of (+)-usnic acid. The results were presented in Table 2.

**Fig 3.** Process for preparing some derivatives of (+)-usnic acid

DISCUSSIONS

The condensation reaction of usnic acid and amine at the ratio of (1:1) gave the enamine-adduct performed at the carbonyl group C-14 of usnic acid. If the molar ratio was (1:2), the side-product was performed in which the two carbonyl groups at C-14 and C-17 were attacked. These results were compatible with published data in the literature [3, 4]. The structures of all adducts were confirmed by 1D and 2D-NMR and HR-ESI-MS. Some HMBC experiments, of UA and UT, were presented in Fig. 4. All adducts from aromatic

amines showed no correlations of the signal at δ_{H} 14.93 (NH) to the neighboring carbons of usnic acid (C-2,14,15), but happily the HMBC experiment of UT, the adduct from an aliphatic amine, showed clear correlations of the signal at δ_{H} 3.44 (H-19) to the neighboring carbon of usnic acid (C-14).

The chemical structure of adducts in the enamine form was well confirmed by the chemical shifts values of some carbons, as the followings:

C-3, as a phenolic carbon with δ_C 191.88 in usnic acid, was changed to a conjugated quinone with δ_C 190–195 in enamine-adducts.

C-14, as a ketone group with δ_C 200.4 in usnic acid, was changed to the enamine group with δ_C 174–175.

C-15, as a methyl group adjacent to a ketone group with δ_C 27.95 in usnic acid, was changed to

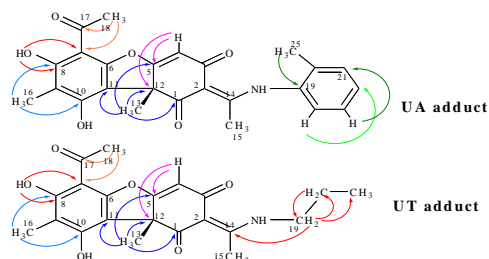


Fig 4. Keys of HMBC correlations of UA and UT

a methyl adjacent to an alkene group with δ_C 18.05–18.46.

Furthermore, the calculation of the active energy of two structures by DFT:B3LYP with the basis 6-31G(d,p) method in methanol, tetrahydrofuran and gas, the energy of the enamine form is lower than that of the imine one. These results showed that obtained adducts were in the enamine form.

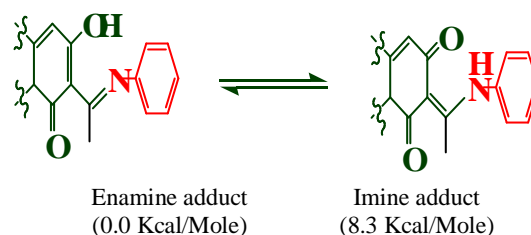
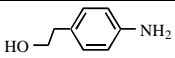
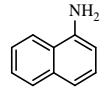


Fig 5. Active energy calculated by DFT:B3LYP method of two tautomers

Table 2. Results of condensation reaction (+)-usnic acid with 11 aromatic and 1 aliphatic amines.

| Entry | Ar-NH ₂ | Code of compound | Yield (%) |
|-------|--------------------|------------------|-----------|
| 1 | | UR | 64.8 |
| 2 | | UA | 96.9 |
| 3 | | UB | 96.7 |
| 4 | | UC | 75.5 |
| 5 | | UQ | 87.4 |
| 6 | | UE | 94.7 |
| 7 | | UF | 91.8 |
| 8 | | UD | 89.7 |
| 9 | | UH | 68.8 |

| | | | |
|----|-----------------------------------------------------------------------------------|----|------|
| 10 |  | UK | 67.6 |
| 11 |  | UI | 65.9 |
| 12 | H ₃ C-CH ₂ -CH ₂ -NH ₂ | UT | 95.5 |

Spectral data of obtained products

• **UA:** HR-ESI-MS: m/z 456.1418 [M+Na]⁺. ¹H NMR (CDCl₃), δ (ppm): 1.80 (s, 3H), 2.13 (s, 3H), 2.31 (s, 3H), 2.53 (s, 3H), 2.72 (s, 3H), 5.91 (s, 1H), 7.14 (d, 1H, $J = 7.0$ Hz), 7.33 (m, 3H), 11.91 (s, OH), 13.38 (s, NH), 14.93 (s, OH). ¹³C NMR (CDCl₃), δ (ppm): 7.61 (C-16), 18.05 (C-15), 20.03 (C-25), 31.41 (C-13), 32.15 (C-18), 57.53 (C-12), 92.81 (C-4), 101.53 (C-7), 102.49 (C-11), 105.09 (C-2), 108.30 (C-9), 126.53 (C-21), 127.16 (C-23), 128.80 (C-20), 131.49 (C-22), 134.07 (C-24), 135.20 (C-19), 155.95 (C-6), 158.35 (C-10), 163.69 (C-8), 166.36 (C-14), 174.81 (C-5), 195.86 (C-3), 198.83 (C-1), 200.78 (C-17).

• **UB:** HR-ESI-MS: m/z 434.1614 [M+H]⁺. ¹H NMR (CDCl₃), δ (ppm): 1.43 (s, 3H), 1.75 (s, 3H), 2.10 (s, 3H), 2.57 (s, 3H), 2.69 (s, 3H), 5.88 (s, 1H), 7.14 (d, 2H, $J = 8.5$ Hz), 7.45 (d, 2H, $J = 8.5$ Hz), 11.70 (s, OH), 13.34 (s, NH), 15.09 (s, OH).

• **UC:** HR-ESI-MS: m/z 436.1419 [M+H]⁺. ¹H NMR (CDCl₃), δ (ppm): 1.76 (s, 3H), 2.11 (s, 3H), 2.57 (s, 3H), 2.69 (s, 3H), 2.70 (s, 3H), 5.88 (s, 1H), 6.92 (d, 2H, $J = 8.5$ Hz), 7.06 (d, 2H, $J = 8.5$ Hz), 11.89 (s, OH), 13.36 (s, OH), 14.83 (s, NH).

• **UD:** HR-ESI-MS: m/z 498.07 [M+H]⁺. ¹H NMR (CDCl₃), δ (ppm): 1.76 (s, 3H), 2.11 (s, 3H), 2.57 (s, 3H), 2.69 (s, 3H), 5.88 (s, 1H), 7.08 (d, 2H, $J = 8.5$ Hz), 7.61 (d, 2H, $J = 8.5$ Hz), 11.70 (s, OH), 13.35 (s, OH), 15.09 (s, NH).

• **UE:** HR-ESI-MS: m/z 450.1566 [M+H]⁺. ¹H NMR (CDCl₃), δ (ppm): 1.76 (s, 3H), 2.11 (s, 3H), 2.56 (s, 3H), 2.69 (s, 3H), 3.85 (s, 3H), 5.87 (s, 1H), 6.97 (d, 2H, $J = 8.5$ Hz), 7.11 (d, 2H, $J = 8.5$ Hz), 11.88 (s, OH), 13.35 (s, NH), 14.87 (s, OH).

• **UF:** HR-ESI-MS: m/z 450.1569 [M+H]⁺. ¹H NMR (CDCl₃), δ (ppm): 1.77 (s, 3H), 2.11 (s, 3H), 2.54 (s, 3H), 2.69 (s, 3H), 3.88 (s, 3H), 5.87 (s, 1H), 7.03 (t, 2H, $J = 7.5$ Hz), 7.15 (d, 1H, $J = 7.5$ Hz), 7.36 (t, 1H, $J = 7.5$ Hz), 11.96 (s, OH), 13.36 (s, OH), 14.78 (s, NH).

• **UH:** HR-ESI-MS: m/z 454.1059 [M+H]⁺. ¹H NMR (CDCl₃), δ (ppm): 1.76 (s, 3H), 2.11 (s, 3H), 2.57 (s, 3H), 2.69 (s, 3H), 5.88 (s, 1H), 7.14 (d, 2H, $J = 8.5$ Hz), 7.45 (d, 2H, $J = 8.5$ Hz), 11.71 (s, OH), 13.35 (s, OH), 15.09 (s, NH).

• **UI:** HR-ESI-MS: m/z 470.1610 [M+H]⁺. ¹H NMR (CDCl₃), δ (ppm): 1.85 (s, 3H), 2.14 (s, 3H), 2.55 (s, 3H), 2.74 (s, 3H), 5.98 (s, 1H), 7.38 (d, 1H, $J = 7.0$ Hz), 7.58 (t, 1H, $J = 7.5$ Hz), 7.63 (m, 2H), 7.86 (m, 1H), 7.98 (m, 2H), 11.88 (s, OH), 13.40 (s, NH), 15.34 (s, OH).

• **UK:** HR-ESI-MS: m/z 464.1703 $[M+H]^+$. 1H NMR ($CDCl_3$), δ (ppm): 1.77 (s, 3H), 2.11 (s, 3H), 2.59 (s, 3H), 2.69 (s, 3H), 2.93 (t, 2H, $J = 5.0$ Hz), 3.92 (t, 2H, $J = 5.0$ Hz), 5.88 (s, 1H), 7.14 (d, 2H, $J = 8.5$ Hz), 7.35 (d, 2H, $J = 8.0$ Hz), 11.83 (s, OH), 13.35 (s, NH), 15.02 (s, OH).

• **UQ:** HR-ESI-MS: m/z 436.14 $[M+H]^+$. 1H NMR ($CDCl_3$), δ (ppm): 1.68 (s, 3H), 2.03 (s, 3H), 2.56 (s, 3H), 2.66 (s, 3H), 5.76 (s, 1H), 7.01 (t, 1H, $J = 7.5$ Hz), 7.06 (d, 1H, $J = 8.0$ Hz), 7.11 (d, 1H, $J = 8.0$ Hz), 7.31 (t, 1H, $J = 7.5$ Hz), 11.43 (s, OH), 13.31 (s, OH), 14.36 (s, NH).

• **UR:** HR-ESI-MS: m/z 420.1417 $[M+H]^+$. 1H NMR ($CDCl_3$), δ (ppm): 1.76 (s, 3H), 2.11 (s, 3H), 2.58 (s, 3H), 2.69 (s, 3H), 5.88 (s, 1H), 7.20 (d, 2H, $J = 7.5$ Hz), 7.40 (t, 1H, $J = 7.5$ Hz), 7.48 (t, 2H, $J = 7.5$ Hz), 11.83 (s, OH), 13.36 (s, OH), 15.06 (s, NH).

• **UT:** HR-ESI-MS: m/z 386.10 $[M+H]^+$. 1H NMR ($CDCl_3$), δ (ppm): 1.07 (t, 3H, $J = 7.5$ Hz), 1.69 (s, 3H), 1.77 (m, 2H), 2.07 (s, 3H), 2.62 (s, 3H), 2.65 (s, 3H), 3.44 (m, 2H), 5.76 (s, 1H), 11.96 (s, 10-OH), 13.33 (s, 8-OH). ^{13}C NMR ($CDCl_3$), δ (ppm): 7.58 (C-16), 11.52 (C-21), 18.46 (C-15), 22.44 (C-20), 31.37 (C-13), 32.14 (C-18), 45.81 (C-19), 57.13 (C-12), 101.46 (C-7), 102.29 (C-2), 102.60 (C-4), 105.23 (C-11), 108.04 (C-9), 156.02 (C-6), 158.42 (C-10), 163.58 (C-8), 174.16 (C-5), 175.00 (C-14), 190.25 (C-3), 198.31 (C-1), 200.79 (C-17).

CONCLUSION

Condensation reaction of usnic acid with *o*-toluidine under microwave irradiation condition gave better yield in a short time comparing to the conventional heating method. The best condition of condensation reaction between (+)-usnic acid and *o*-toluidine (1:1) was irradiated at 90 °C within 20 minutes. Cyclopentyl methyl ether is a good

solvent for the reaction with the yield of 96.9 %. Some new derivatives of (+)-usnic acid were prepared and this is the first time their NMR spectral data are reported.

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Điều chế một số dẫn xuất của acid (+)-usnic với amine thơm trong điều kiện chiếu xạ vi sóng

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TÓM TẮT

Cho đến nay, dù acid usnic và một số dẫn xuất của nó được biết có những hoạt tính

sinh học hấp dẫn, tuy nhiên, số lượng công trình nghiên cứu về các điều chế dẫn xuất của

acid usnic còn nhiều hạn chế và hầu như các phản ứng đều được thực hiện bằng cách đun nóng trong dung môi hữu cơ theo phương pháp cổ điển. Trong bài báo này, chúng tôi khảo sát phản ứng ngưng tụ giữa acid (+)-usnic với o-toluidine trong điều kiện chiếu xạ vi sóng, sau đó, áp dụng điều kiện tối ưu để

điều chế một số dẫn xuất mới của acid usnic. Đây là lần đầu tiên các dẫn xuất này được điều chế và được trình bày về số liệu phổ NMR. Phần thực nghiệm có thực hiện lại phản ứng ngưng tụ trong điều kiện theo phương pháp cổ điển để có số liệu so sánh giữa hai phương pháp.

Từ khoá: Acid (+)-usnic, o-toluidine, điều kiện chiếu xạ vi sóng, phản ứng ngưng tụ, cyclopentyl methyl ether.

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