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Andrographolide undergoes modification after illumination by blue laser in the presence of sodium bicarbonate

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Andrographolide is the marker compound of *Andrographis paniculata* (Kalmegh), a herb that constitutes many traditional Asian remedies. In the present photochemical approach to modify its 12-en-14-hydroxyl moiety into 13-enone, a methanol solution of andrographolide has been illuminated with blue laser for 6 hours in the presence of sodium bicarbonate. After the illumination, it has been discovered that the 12-en-14-hydroxyl moiety undergoes modification into the 11-enone.

Keywords: Andrographolide, modification, illumination, blue laser, sodium bicarbonate

Andrographolide (**1**; Figure 1), a labdane-type diterpenoid, is the marker compound of *Andrographis paniculata* (Kalmegh), a herb that constitutes many traditional Asian remedies[1]. The compound itself was found to exhibit many effects: anti-inflammatory, anti-hyperglycemic, anti-hyperlipidemic, anti-cancer, anti-viral, and immunomodulatory ones [1]. Due to these multi-effects, it is interesting to modify the compound for structure-activity relationship studies.

Modifications of **1** had been carried out by others on various functional groups[2,3]. However, all these modifications relied on the thermal approach. To the best of our knowledge, there has been no report on the utilization of photochemical approaches for the modification of **1**.

Photochemical approaches have the advantage over thermal one in that they could access products, which are energetically inaccessible by thermal one, throughout the excitation of the starting molecule[4]. In this regard, illumination by laser is the most efficient photochemical approach, since the laser is monochromatic, unidirectional, and coherent[5].

Compound **1** appears colorless. However, it was found to absorb visible electromagnetic waves at 400 – 600 nm[6]. This absorption might be due to the carbonyl group at C-16 of **1** which is in conjugation with the C=C bond at C-12 (Figure 1). Because of this absorption, we assumed **1** to be photochemically reactive under illumination by blue laser (450 nm).

Previously, Pandeti and colleagues modified the 12-en-14-hydroxyl moiety of **1** into 13-enone of **2** (Figure 1) in a two-step thermal approach, using methanol as the solvent, in the presence of sodium carbonate (Na_2CO_3) at the second step[7]. The calculated partition coefficient (log P) of **2** (2.39 +/- 0.45) is higher than that of **1** (1.62 +/- 0.45), but the calculated molecular weight of **2** (334.4498 g/mol) is lower than that of **1** (350.4492 g/mol). Therefore, this approach serves as a method to obtain a more hydrophobic analog of **1** without increasing the molecular weight; thus keeping the analog under drug-like properties[8]. In our photochemical approach to obtain the same modification of **1**, we illuminated a methanol solution of **1** by blue laser for 6 hours, in the presence of sodium bicarbonate ($NaHCO_3$). Herewith is a short communication of the research.

Results and Discussion

During the illumination, no change in the temperature of the solution was detected.

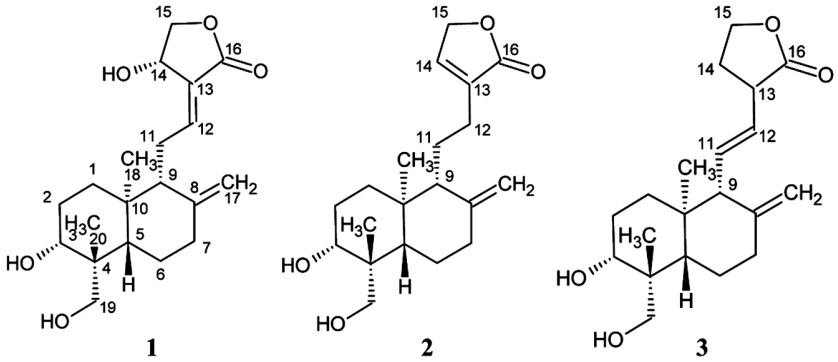

Figure 1 — Structure of andrographolide (**1**)[1], **2**[7], and **3**

Therefore, the solution of **1** was free from thermal involvement.

After the illumination and subsequent purifications, colorless powder (0.3356 g) was obtained. This product, upon elution on a silica gel 60 F_{254} plate with ethyl acetate – *n*-hexane (2:1), ended up as a single spot. Its retention factor (0.56) was significantly higher than that of **1** (0.26). These results indicated that **1** had undergone a modification into a more hydrophobic compound, which is in agreement with the calculated log P of **2**.

The ^{1}H NMR spectra of the product revealed the two most deshielded peaks at 6.59 ppm (1 ^{1}H, *dd*, 3.6 Hz) and 5.52 ppm (1 ^{1}H, *dd*, 3.6 Hz) (Supplementary 1). These chemical shifts suggest that each of those peaks belongs to one ^{1}H nucleus which is attached to a C=C bond. The only ^{1}H nucleus like that in **2** is ^{1}H-14 (Figure 1). However, the ^{1}H nucleus of 6.59 ppm correlates to another one of 2.32 ppm only, and the ^{1}H nucleus of 5.52 ppm correlates to another one of 3.06 ppm only (Figure 2); while ^{1}H-14 in **2** should have correlated to two – instead of one – neighboring geminal hydrogen nuclei (Figure 1). This indicates that the product is not **2**.

Since the ^{1}H NMR peaks at 6.59 and 5.52 ppm have a common *J* value (3.6 Hz), it is possible that their represented ^{1}H nuclei are next to each other and connected by a C=C bond. One way to have this is to put the C=C bond at C-11, as depicted in structure **3** (Figure 1 and Figure 2). In this way, the peaks at 2.32 ppm, 6.59 ppm, 5.52 ppm, and 3.06 ppm represent ^{1}H-9, ^{1}H-11, ^{1}H-12, and ^{1}H-13, respectively. For this structure, the ^{13}C-11 should be represented at around 140 ppm, and ^{13}C-12 should be represented at around 100 ppm, which are in agreement with the ^{13}C- and HMQC-NMR spectra of the product (Supplementary 3 and 4). Structure **3** was further supported by a long-

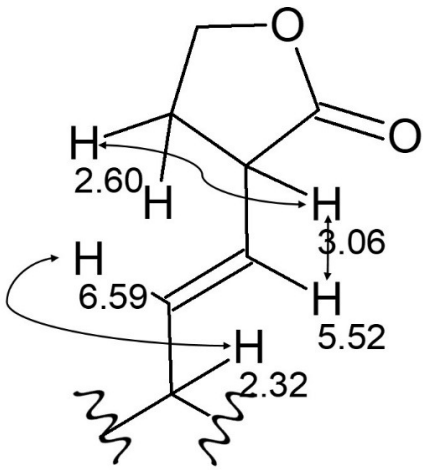

Figure 2 — ^{1}H-^{1}H correlations on **3**

range correlation between the ^{1}H-12 (represented at 5.52 ppm) and the ^{13}C nucleus (represented at around 170 ppm) which presumably belongs to the carbonyl group of **3** (Supplementary 5).

To explain how **1** could have undergone modification into **3**, we assumed that at the beginning, the 12-en-14-hydroxyl moiety of **1** might have rearranged into 12-hydroxyl-13-en upon protonation of the 14-hydroxyl group of **1** by methanol (a weak acid) (Figure 3). This rearrangement was favorable because the resulted C=C bond at C-13 was still in conjugation with the carbonyl group at C-16. Then, the 12-hydroxyl group might undergo elimination with the help of sodium bicarbonate (a weak base), resulting in a C=C bond at C-11 which was in conjugation with the C=C bond at C-13. Finally, with the help of the blue laser, the C=C bond at C-13 was hydrogenated by methanol (a hydrogen donor) to afford **3**. The C=C bond at C-13 was the only one that was hydrogenated because it was the only one that

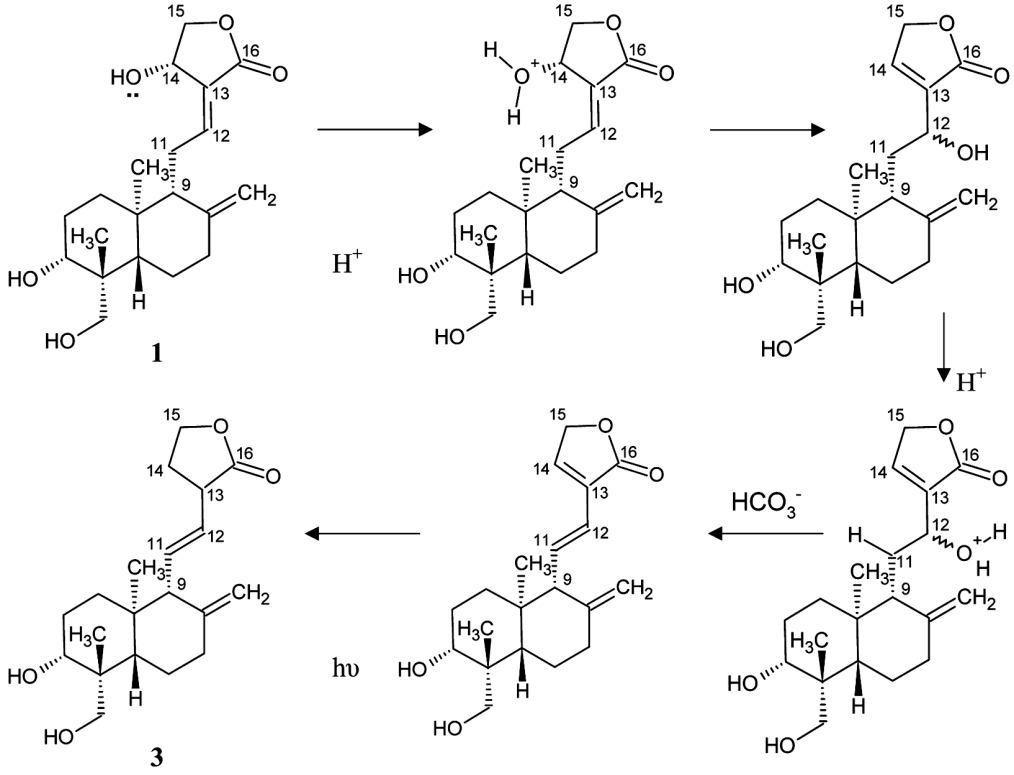

Figure 3 — Proposed mechanism of modification of **1** into **3**

was in conjugation with the carbonyl group at C-16, where the excitation by blue laser (n → π*) might have taken place.

To examine the importance of sodium bicarbonate in the modification of **1** into **3**, we illuminated a methanol solution of **1** with the blue laser for 6 hours, without the presence of sodium bicarbonate. After the illumination, no change in the Thin Layer Chromatography (TLC) spot of **1** was detected. Therefore, sodium bicarbonate should be important in the modification of **1** into **3**. Other related weak bases (e.g. sodium carbonate, sodium acetate, etc.) could be used as alternatives for sodium bicarbonate.

Although **2** was not obtained in this research, we still obtained a compound (**3**) with higher log P (2.09 +/- 0.42) and lower molecular weight (334.4498 g/mol) than those of **1**. Its low percentage of yield (~19%) suggested that the wavelength of the laser might not be effective enough for the modification of **1** into **3** in the presence of sodium bicarbonate.

Compound **1** was found to have moderate activity against *Plasmodium falciparum* (*P. falciparum*) *in vitro*[9]. We hypothesized that **3**, which is more hydrophobic than **1**, would penetrate the membrane of *P. falciparum* better than **1**, and therefore would have a higher anti-plasmodial activity than **1**. This hypothesis is currently being investigated by our research group.

Materials and Methods

Compound **1** (98.83% pure) was a product of Xi'an App Chem-Bio(Tech), P.R.China. Sodium bicarbonate (99.5% pure) was a product of BDH Limited Poole, England. Hydrochloric acid (37%), silica gel 60 for column chromatography, and silica gel 60 F_{254} for TLC were products of Merck, Germany.

All solvents which were utilized in this research had been distilled before use.

The laser source was a claser diode (CXLaser-B008) with 450 nm (blue light) and 1 W output. One-dimensional NMRs (^{1}H- and ^{13}C-) and two-dimensional NMRs (COSY, HMQC, and HMBC) were measured on a JEOL JNM-ECS400 using deuterated methanol. Log Ps were calculated using ChemSketch 12.01.

Experimental Section

To a solution of **1** (1.7616 g) in methanol (100 mL), sodium bicarbonate (0.4999 g) was added. The solution was stirred moderately and illuminated with

the blue laser. For the illumination, the laser source was maintained at 30 cm above the surface of the solution, perpendicular to it. During the illumination, the temperature of the solution was monitored.

After 6 hours, the effect of the illumination on the solution was evaluated by TLC. The solvent was then removed, and the remaining solid was neutralized with dilute hydrochloric acid and partitioned in ethyl acetate – water (1:1). The ethyl acetate phase was dried and eluted through a silica gel 60 column with ethyl acetate – *n*-hexane (1:1). The final product was precipitated from methanol solution with water (1:1) and identified by NMR. Its log P was then calculated.

Conclusion

Andrographolide (**1**) undergoes modification into **3** after illumination by blue laser for 6 hours in the presence of sodium bicarbonate.

Supplementary Information

Supplementary information is available on the website (http://nopr.niscair.res.in/handle/123456789/60).

Acknowledgments

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