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Semisynthesis of miltirone, 1,2-dehydromiltirone, saligerone from Carnosic acid and cytotoxities of their derivatives

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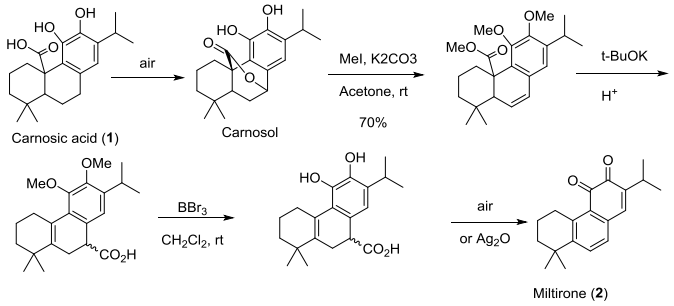
ABSTRACT

Miltirone, 1,2-dehydromiltirone, saligerone have been synthesized form carnosic acid. Among them, one step transformation of carnosic acid into miltirone was realized when decarboxylation/aromatization was promoted by Lewis acid .Moreover, 4 derivatives exhibited potent cytotoxic activities against three human cancer lines in vitro.

Carnosic acid (CA), easily available from widely distributed rosemary, has antioxidant and anti-inflammatory activities. Its resource abundance ensures the use as additive and preservative in food industry. It also exhibits antitumor activity, although generally inactive due to the carboxylic group.[1] Our research interest focuses on the discovery of antitumor molecules, especially the tricyclic terpenoids derivatives [2]. So we chose carnosic acid as starting material to access c-20 norditerpenoid derivatives and thereafter, the synthesized compounds were tested against three human cancer cell lines (i.e., HepG2: human hepatoblastoma (HepG2) cells; MCF7: human breast adenocarcinoma cells; A549: human alveolar basal epithelial cells) by MTT method.

In 1996, Luis group reported an easy hemisynthesis of miltirone, which displayed significant activity in the central benzodiazepine receptor binding assay,[3] from carnosol through an interesting rearrangement triggered by potassium *tert*-butoxide and subsequent decarboxylation.[4] (Scheme 1) Based on the fact that carnosol could be obtained from carnosic acid by air-oxidation,[5] the biomimetic transform from carnosic acid to miltirone in short steps, would be attractive..

In above process, spontaneous decarboxylation at C-7 and aromatization occurred when treated with BBr_3. We recognized that if decarboxylation at C-10 also occur in a Lewis acid condition, the process could be largely simplified. Inspired by a $BiCl_3$ promoted decarboxylation,[6] we began the screening for an ideal Lewis acid to operate in one-step decarboxylation/aromatization sequence.



Scheme 1. Synthetic route to miltirone.

At first, we synthesized carnosol from carnosic acid by Ag_2O oxidation. Considering the structure similarity between carnosol with miltirone, we envisioned that decarboxylation could lead to the formation of miltirone directly because methyl groups were just temporary protecting groups. When BBr_3 was used, miltirone was isolated as minor product (20%). Encouraged by this result, we then applied the same strategy directly from carnosic acid. To our delight, the yield was improved to 35% (Entry, 1, Table 1). Hence, several Lewis acids were investigated and the results were collected in Table 1.

Reaction was carried out at rt in CH_2Cl_2. Boron tribromide promoted the reaction in similar results under Ar or O_2, a little surprise to our speculation that oxidation of catechol to ortho quinone would be easier with O_2. When the amount of BBr_3 was reduced to 2.5 equiv., no desired product was isolated. Weaker

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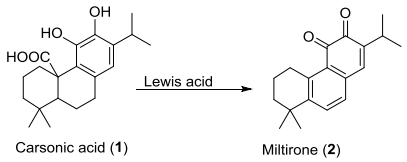
Lewis acid like $BF_3{\cdot}OEt_2$ did not afford the desired product. TMSOTf gave the best result of 70% isolated yield, while TMSI was not effective. Metal containing Lewis acid like $TiCl_4$ destroyed the reaction and strong brønsted acid like CF_3COOH and $MeSO_3H$ did not lead to miltirone.(Scheme 2).



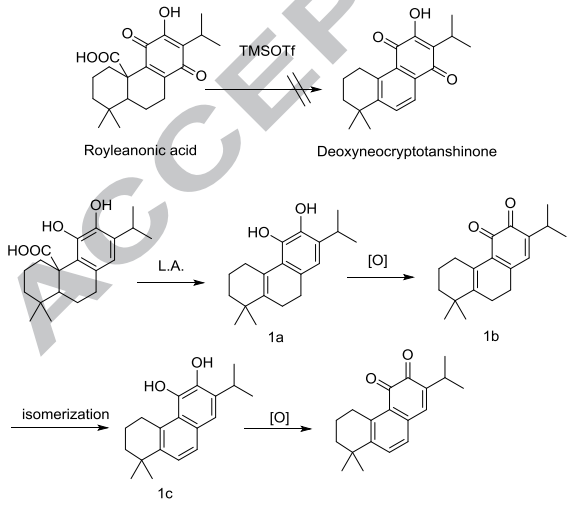
Scheme 2. Synthesis of miltirone from carnosic acid..

Table 1. Optimization of decarboxylation/aromadization.

Entry	Reagent	Conditions	Results
1	BBr_3 (6.0eq)	Ar	35%
2	BBr_3 (6.0eq)	O_2	40%
3	$BF_3.OEt_2$	Ar	NR
4	TMSOTf (2.5eq)	Ar	40%
5	**TMSOTf (5.0 eq)**	**Ar**	**70%**
6	TMSI	Ar	messy
7	$TiCl_4$	Ar	messy
8	CF_3COOH	Ar	NR
9	CH_3SO_3H	Ar	Messy

[a] Reaction was carried out for 12h. [b] NR for no reaction, CA recovered.

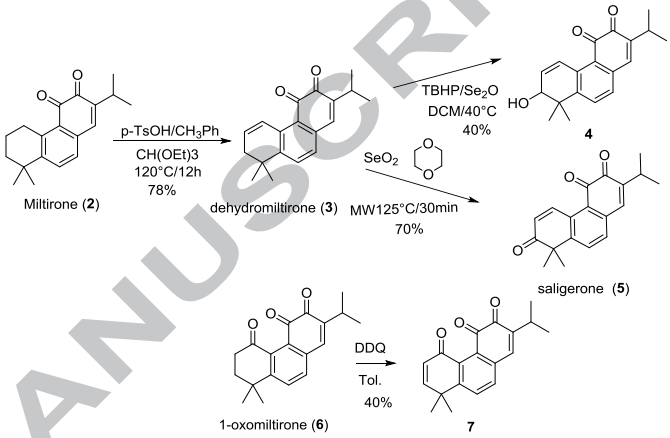
In order to investigate the substrate scope, we conducted this transformation using royleanonic acid as starting material and no desired product was isolated. It implies that the orthobenzoquinone moiety is important for decarboxylation/aromatization. Therefore, the proposed mechanism is depicted below.



Scheme 3. Proposed Mechanism for decarboxylation/aromatization.

Decarboxylation occurred firstly, followed by oxidation of catechol **1a** into ortho benzoquinone **1b** (during workup, because the reaction proceeds effectively even under Ar) and enol isomerization gave **1c**, a second time oxidation of catechol afforded miltirone.

Further transformations of Miltirone were investigated. Since the modification of Tanshinones were mainly concentrated to the C-ring.[7] and the studies on A-ring are rare. Considering the process from **1b** to **1c**, 1,2-dehydromiltirone could be obtained in a similar manner. Finally, treated with *p*-TsOH in Toluene and with the aid of dehydrating agent of triethyl ortho acetate or MS 4Å, dehydromiltirone was isolated in 78%.[8] It is noteworthy that dehydrogenation proceeded very slowly without dehydrator. It was further oxidized into allylic alcohol **4** or enone **5** (Scheme 4). The enone **5**, as a natural product, was isolated in 2003. [9] Its first synthesis was accomplished. Another type of enone of A-ring was synthesized from 1-oxomiltirone with DDQ as oxidative reagent.



Scheme 4. Synthesis of miltirone derivatives.

In vitro inhibitory activities against HepG2, MCF7 and A549 human tumor cell lines of synthesized compounds were tested using the MTT method. The results indicated that 4 tanshinone derivatives exhibited some cytotoxic activities against three human cancer lines in vitro (**Table 2**).

Table 2. Cytotoxicities of compounds against three cancer cell lines (IC_{50} μM)

Compounds[a]	HepG2	MCF-7	A549
2	8.31	6.33	12.06
4	14.69	5.08	12.99
5	**2.40**	**0.83**	**2.24**
7	2.79	1.51	2.43
STS[b]	0.04	0.10	0.0055

[a] Other derivatives with IC_{50} > 40 μM for all the cell lines are not listed. [b] Positive control.

Our researches revealed that, for the first time, carnsonic could be directly transformed into miltirone with Lewis acid and air. In this process, decarboxylation/aromatization occurred concurrently. Our effective transformation provide larger amount of miltirone, comparing with reported total synthesis [10~13] and it would largely boost the bioactivity research of miltirone. From miltirone, *p*-TsOH mediated isomerization afforded 1,2-dehydromiltirone. Subsequent Oxidation completed the first synthesis of saligerone . The synthesized compounds were subjected to antitumor assay towards three human tumor cell line and saligenone showed potent activity and it demonstrated the importance of Michael acceptor.

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Supplementary data

The details of chemical transformation, and biological experimental procedures, MS and NMR spectra of the synthesized compounds associated with this article can be found in the online version at http://dx.doi.org/XXX.

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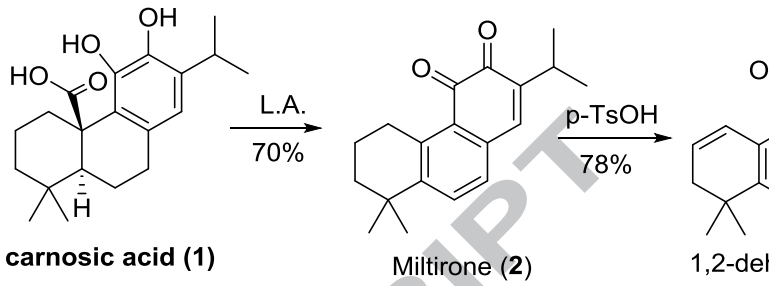

Highlights

- One step synthesis of miltirone was accomplished from carnosic acid
- A-ring modification of c-20 norditerpenoid derivatives have been investigated.
- 4 derivatives have been subjected to antitumor activity test.