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**COMMUNICATION**

# DDQ-promoted dehydrogenation from natural rigid polycyclic acids or flexible alkyl acids to generate lactones by a radical ion mechanism†

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**A novel and facile DDQ-mediated dehydrogenation from natural rigid polycyclic acids or flexible alkyl acids to generate lactones is described. The formation of lactones proceeds by a radical ion mechanism, which has been established by DPPH•-mediated chemical identification, ESR spectroscopy and an enol intermediate trapping.**

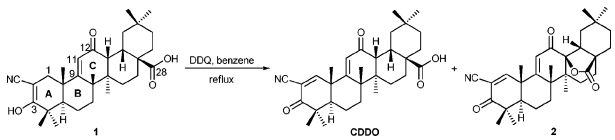
Olean-28,13β-olide with a lactone moiety between C13 and C28 is a member of the highly-functionalized natural oleanane products that have attracted much attention because of their potential anticancer activities.[1–3] Unfortunately, the low natural abundance and highly challenging chemical synthesis of olean-28,13β-olides have limited the medicinal development of these compounds. To the best of our knowledge, the only known method for the synthesis of olean-28,13β-olides is to use peroxide oxidation.[4] However, this method is severely hindered by the incompatibility of peroxide with functional groups such as alkenes, alcohols, and amines, thus limiting its broad use in the field of olean-28,13β-olide synthesis.

In continuation of our studies on the synthesis and biological activities of oleanane derivatives,[5–7] we have developed an effective approach to generate olean-28,13β-olides while investigating an improved preparation of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oate (CDDO) as a potential anticancer drug.[8,9]

To our surprise, in the last step of the improved synthesis, olean-28,13β-olide **2** rather than CDDO was primarily obtained when using two equivalents of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as dehydrogenation reagent (Table 1). The absolute structure of **2** was established by spectroscopy and X-ray crystallography (see the ESI†, S17). To investigate this unexpected transformation, we treated **1** with various amounts of DDQ in refluxing benzene (Table 1). Notably, the reactions of **1** with one, or less than one, equivalent of DDQ generated only a trace amount of **2** even after prolonged reflux (Table 1, entries 1–5). However, when **1** was treated with more than one equivalent of DDQ, **2** was obtained in a DDQ amount- and reaction time-dependent manner (Table 1, entries 6–9). These observations indicated that the DDQ-mediated dehydrogenation of ring A occurred prior to the formation of olean-28,13β-olide. As DDQ-promoted dehydrogenation mainly involves an ionic mechanism,[10,11] we postulate a mechanism for the conversion of **1** to CDDO in Scheme 1. However, subsequent transformation of CDDO into olean-28,13β-olide **2** might proceed *via* a distinct mechanism.

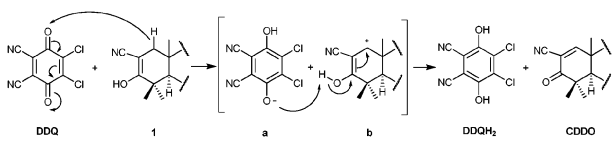
In order to gain insights into the mechanism, CDDO was allowed to react with DDQ or 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) that is known to be a stable nitrogen centered radical and has been used for the detection of free radicals.[12]

**Table 1** Reactions of **1** with various amounts of DDQ[a]




| Entry | DDQ (equiv.) | Time/h | Yield of CDDO[b] (%) | Yield of **2**[b] (%) |
|---|---|---|---|---|
| 1 | 0.8 | 0.5 | 40 | — |
| 2 | 0.8 | 6.0 | 44 | Trace |
| 3 | 1.0 | 0.5 | 91 | — |
| 4 | 1.0 | 6.0 | 90 | <3 |
| 5 | 1.0 | 12 | 87 | <3 |
| 6 | 1.5 | 0.5 | 78 | 11 |
| 7 | 1.5 | 12 | 68 | 23 |
| 8 | 2.0 | 12 | 30 | 57 |
| 9 | 2.0 | 24 | 28 | 58 |

[a] All reactions were performed with DDQ in refluxing benzene. [b] Yield of the isolated product.




**Scheme 1** A plausible mechanism for the DDQ-promoted formation of CDDO.

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† Electronic supplementary information (ESI) available: Experimental procedure, crystallographic data, ESR spectra, enol intermediate trapping experiment, cytotoxicity assay. CCDC 793908. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1cc11633a

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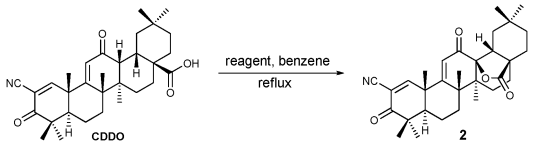
**Table 2** Formation of **2** from CDDO under different conditions




| Entry | Reagent | Time/h | Temp./°C | Yield of **2**[a] (%) | Recovery of CDDO[a] (%) |
|---|---|---|---|---|---|
| 1 | DDQ[b] | 48 | 25 | 0 | 95 |
| 2 | DDQ[b] | 48 | 80 | 55 | 31 |
| 3 | DDQ[c] | 168 | 80 | 58 | 27 |
| 4 | DPPH•[d] | 48 | 80 | 83 | — |

[a] Yield of the isolated product. [b] One equiv. of DDQ employed. [c] Four equiv. of DDQ employed. [d] Two equiv. of DPPH• employed.

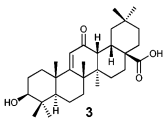
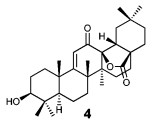
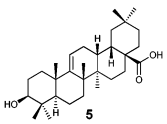
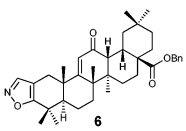
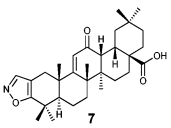
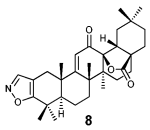
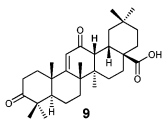
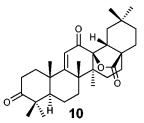
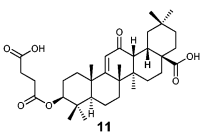
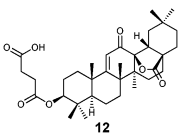
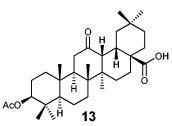
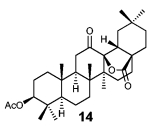
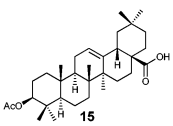
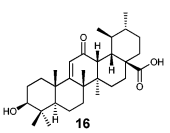
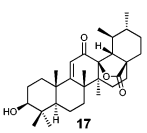
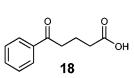
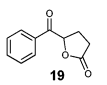
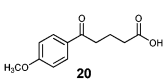
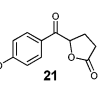
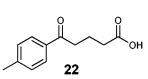
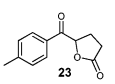
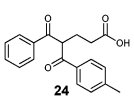
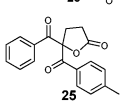
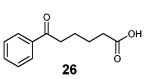
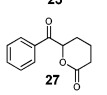
It was observed that treatment of CDDO with DDQ in benzene at room temperature did not produce **2** (Table 2, entry 1), whereas **2** was formed in moderate yield under refluxing conditions (Table 2, entries 2 and 3), suggesting that temperature could be crucial for this cyclization. Interestingly, treatment of CDDO with two equivalents of DPPH• in refluxing benzene for 48 h furnished **2** (83%) and H•-trapping product DPPH-H (80%) which were identified by spectroscopy (Table 2, entry 4).

Given that both DDQ and DPPH• have an ability to convert CDDO to olean-28,13β-olide **2** (Table 2, entries 2 and 4) under the same conditions, it is likely that DDQ undergoes the same radical mechanism as DPPH•. To obtain convincing evidence for the radical mechanism, an electron spin resonance (ESR) technique was employed. It was found that a solution of CDDO or DDQ alone in refluxing benzene did not generate any ESR signal. In contrast, a solution of CDDO and DDQ under the same conditions showed a weak single ESR signal ($g$ = 2.0057), which was expectedly magnified and split by subsequent addition of the spin trapping reagent *N*-*tert*-butyl-α-phenyl nitrone (PBN) ($g_1$ = 2.0058; $g_2$ = 2.0074, $a^N$ = 8.14 Gs; rationalization and interpretation of the signals are provided in the ESI†, S29). These data indicate that a radical mechanism is likely involved in the DDQ-mediated formation of **2**.

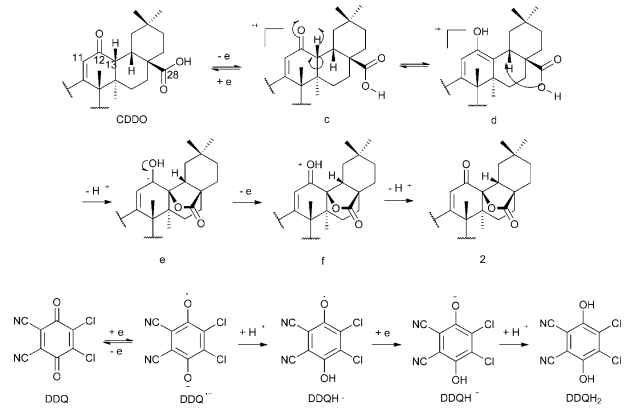
In view of the fact that the α-position of the ketone is usually involved in the reaction, we postulate that the C12-carbonyl may play an important role in the formation of olean-28,13β-olide. It was observed that ketone compound **3** could undergo DDQ-promoted cyclization to form olean-28,13β-olide **4**, whereas **5**, which has the same structural feature as **3** with the exception of the C12-carbonyl moiety, could not do so (Table 3, entries 1 and 2), suggesting that the C12-carbonyl may be an essential group for the formation of the corresponding lactone, probably *via* an enolization process of the ketone which involves both C12-carbonyl and C13-H.

To find more convincing evidence for an enol as a transient intermediate in the formation of lactone, the reaction of excess acetyl chloride with a solution of DDQ and **6** was carried out (**6** is a C28-carboxyl protected derivative of **7**, and **7** can be converted to olean-28,13β-olide **8**, Table 3, entries 3 and 4). Fortunately, a small amount of enol-acetylated product **31** was successfully isolated and identified (see the ESI†, S31), indicating that heating- and DDQ-promoted

**Table 3** DDQ-promoted synthesis of olean-28,13β-olides, urs-28,13β-olide and arylketone lactones[a]

| Entry | Substrate | Product | Yield[b] (%) |
|---|---|---|---|
| 1 |  |  | 82[c] |
| 2 |  | — | —[c] |
| 3 |  | — | —[c] |
| 4 |  |  | 89[c] |
| 5 |  |  | 84[c] |
| 6 |  |  | 78[c] |
| 7 |  |  | 61[c] |
| 8 |  | — | — |
| 9 |  |  | 85[c] |
| 10 |  |  | 54[d] |
| 11 |  |  | 51[d] |
| 12 |  |  | 58[d] |
| 13 |  |  | 36[d] |
| 14 |  |  | 62[d] |

[a] All reactions were performed with one equiv. of DDQ in anhydrous benzene. [b] Yield of the isolated product based on the reacted substrate. [c] Reaction time was 48 h. [d] Reaction time was 168 h.




**Scheme 2** A proposed radical ion mechanism for the DDQ-mediated formation of olean-28,13β-olide **2**.

enolization of the ring C ketone could be involved in the formation of lactone.

A battery of mechanistic information has now been acquired that includes: (i) data obtained from DPPH•-mediated reactions; (ii) ESR spectra of the DDQ-promoted reactions; (iii) investigations to determine the role of the C12-carbonyl; (iv) trapping of a C12-enol transient intermediate; and (v) Floreancig *et al.*'s recent reports showing that DDQ can induce a radical cation intermediate on an electron-rich benzene ring or a vinyl moiety before the α-position carbon hydrogen bond is homolytically cleaved.[13,14] Accordingly, based on the above data, we now propose a radical ion mechanism as depicted in Scheme 2, while a similar mechanism for quinone dehydrogenations of hydroaromatic to aromatic hydrocarbons was mentioned by Höfler and Rüchardt.[15] In refluxing benzene, DDQ can trap an electron from the electron-rich enone on ring C of CDDO to produce a radical cation **c** and a radical anion DDQ•⁻. Subsequent homolytic cleavage of the C–H bond at the C13 position of CDDO forms a dienol radical cation **d**. Then the oxygen atom with a pair of electrons on the C28-carboxylic hydroxyl of **d** acts as a nucleophile to attack the C13, followed by deprotonation to form a lactone radical **e** and DDQH•. The intermediate **e** is subsequently oxidized by DDQH• to generate cation **f** and DDQH⁻. Finally, **f** is deprotonated to provide **2** and $DDQH_2$.

To extend the potential applications of this method, we investigated the DDQ-induced lactonization of other oleanane and ursane compounds. As shown in Table 3, all oleanane and ursane compounds can be converted to their corresponding lactones except for the following three compounds: **5** without a C12-carbonyl, **6** without a free C28-carboxyl and **15** without a C13-H (Table 3, entries 2, 3, and 8). Expectedly, compounds with more electron-rich α,β-unsaturation of the C12-ketone gave higher yields (78–89%, Table 3, entries 1, 4, 5, 6, and 9) in comparison with compound **13** without a double bond (61%, Table 3, entry 7). All results are consistent with the proposed radical ion mechanism. Notably, in contrast to the known synthesis of olean-28,13β-olides, this DDQ-mediated approach tolerates several oxidation-sensitive moieties (Table 3, entries 1 and 9).

To examine whether this methodology could be applied not only to natural rigid polycyclic systems but also to flexible compounds, we investigated the reactions of some arylketone-substituted alkyl acids with DDQ. It was found that these alkyl acids could also be converted to lactones using DDQ in refluxing benzene (Table 3, entries 10–14). And the six-membered ring lactone **27** was obtained in higher yield (62%) than five-membered ring lactones (**19**, **21**, **23**, **25**) (36–58%). These racemic arylketone lactones warrant further investigation in the future.

Finally, the antitumor activity of olean-28,13β-olide **2** against human hepatoma BEL-7402 cells, human cervical cancer HeLa cells, and human breast cancer MCF-7 cells was determined using MTT assays[16] that showed $IC_{50}$s of 2.1, 5.3, and 3.7 μM, respectively. These antiproliferative effects are comparable to those of the positive control CDDO–Me[17] (see the ESI†, S33).

In conclusion, a novel and facile DDQ-mediated approach to generate olean-28,13β-olides and other pentacyclic triterpen-28,13β-olides like urs-28,13β-olide was developed, which can be applied not only to natural rigid polycyclic systems but also to flexible compounds. ESR spectroscopy experiments revealed that DDQ-mediated olean-28,13β-olide formation occurred *via* a radical ion mechanism, which is supported by DPPH•-promoted chemical identification. More importantly, we obtained both direct and indirect evidence for an enol transient intermediate in the formation of olean-28,13β-olide that occurs *via* an enolization of the C12-ketone. A preliminary biological study showed that **2** exhibits comparable *in vitro* antitumor activity to CDDO–Me. Further pharmaceutical investigations of **2** and other olean-28,13β-olides, urs-28,13β-olides are underway, and the results will be reported in due course.

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