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# Two New Triterpene Dimers from Celastraceae, Their Partial Synthesis and Antimicrobial Activity

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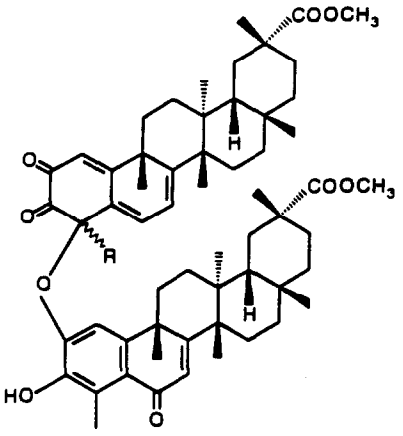
***Abstract***: **Two new triterpene dimers, 7 and 8, were isolated from *Maytenus umbellata* and proved to be oxidized structures of tingenone from which they could also be synthesized. These compounds were screened for antimicrobial activity and compared with related compounds.**

The Celastraceae are widespread throughout the hot and hot-to-temperate regions of the world and have a long history of use at folk level both for medicinal[1,2] and agricultural purposes. For instance, in China they have been traditionally used to protect other plants from the ravages of insects[3].

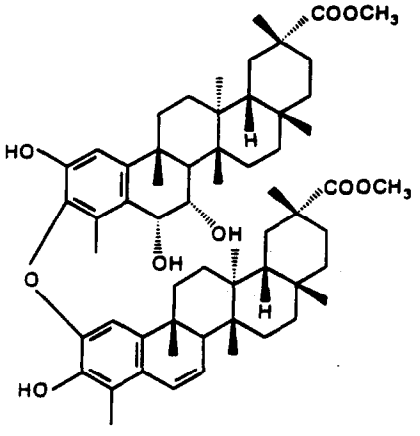
Not long ago[4] we reported the isolation from *Rzedowskia tolantonguensis* (Celastraceae), structural elucidation and synthesis of two new epimeric metabolites, **1** and **2**, whitch proved to be di-triterpene quinone ethers based on two units of pristimerin (**11**). Last year Professor H. Itokawa et al[5] reported the isolation and structural characterization of four new cytotoxic dimeric triterpenes from *Maytenus ilicifolia* (Celastraceae) three of which were based on two units of pristimerin (**11**): cangorosin A (**3**), atropcangorosin A (**4**), dihydro-atropcangorosin A (**5**) while cangorosin B (**6**) has one unit of pristimerin (**11**) and one of tingenone (**10**). To the best of our knowledge, these are the only dimers from the Celastraceae with a methylene triterpene quinone basis which have been published to date.

Two new dimers based on oxidized units of tingenone (**10**) have now been isolated from the Madeiran species, *Maytenus umbellata* (R. Br.) Mabb[6], and partially synthesized from tingenone (**10**). Their antimicrobial activity has been studied and compared with that of other dimeric and monomeric triterpene substances from Celastraceae some of which have been shown in "in vitro" studies to possess considerable antimicrobial activity[7,8,9]. In the case of netzahualcoyone (**12**)[10], for example, this activity involves the inhibition of the respiratory chain. After repeated chromatography on si gel, two products were isolated as pale yellow lacquers from the plant extract and were named umbellatin α (**7**) and umbellatin β (**8**). Neither compound displayed a molecular ion under eims although fragments were observed at m/z 436 (calc. for $C_{28}H_{36}O_4$, 436.2613; found, 436.2601) and 420 (calc. for $C_{28}H_{36}O_3$, 420.2685; found, 420.2675) for each of the two units present in these dimeric ethers.



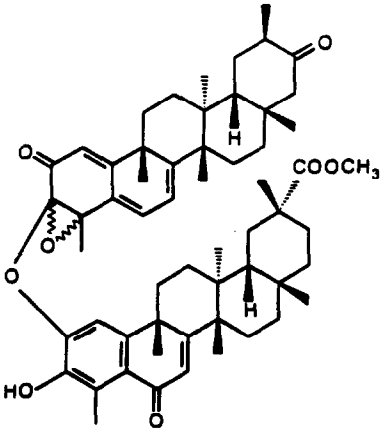
**1** R= α Me

**2** R= β Me

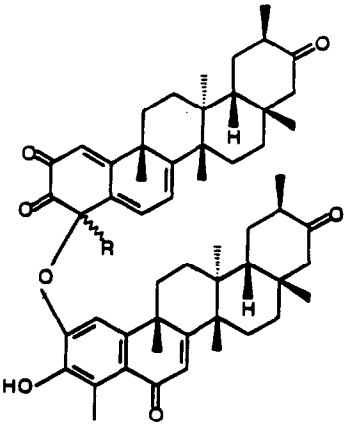


**3**; **4** atropisomer of **3**

**5** 6', 7' dihydro derivative of **3**



**6**



**7** R= α Me

**8** R= β Me

The $^1$H NMR spectra of **7** and **8** are virtually superimposable on those of the known pristimerin-type dimers **1** and **2**, respectively, between δ 4.5 and 7.0. The most notable differences are the lack of signals for methoxy groups in the spectra of **7** and **8** and the presence of the signals corresponding to the E ring of tingenone (**10**). The appearance of a signal for Me-23 at δ 2.75 in the $^1$H NMR spectrum of both **7** and **8** clearly indicates the presence of a methyl on an aromatic ring which is, moreover, strongly shielded by a carbonyl group, as is also the case with dimethyl-6-oxopristimerol (**9**)[11], and indicates the presence of one oxidized unit of tingenone with an aromatic group on the A ring, rings A and B being of the same sort as found in **9**. A similar type of unit also forms part of cangorosin B (**6**)[5] (see Table 1).

**Tabla 1:** $^1H$ NMR (200 MHz) Data (δ, $CDCl_3$) of **7** and **8** (J are given in Hz in the brackets)

| | 7 | | 8 | |
|---|---|---|---|---|
| | **H** | **H'** | **H** | **H'** |
| 1 | 6.10* | 6.24 s | 6.11* | 6.25 s |
| 6 | 6.55 d (7.0) | | 6.28* | |
| 7 | 6.12 d (7.0) | 6.76 s | 5.99 d (6.6) | 6.81 s |

* Superimposable

The $^{13}C$ NMR spectra of **7** and **1** are very much alike, the chemical shifts of C-1 to C-10 in both products being identical[4] and the signals for the carbons of D and E in **7** are similar to those of tingenone (**10**) (see Table 2). These data are borne out by the recent studies of the $^1H$ and $^{13}C$ NMR spectra of methylene triterpene quinones published by A.A.L. Gunatilaka & T. Kikuchi[12] based on $^1H$-$^1H$ and $^1H$-$^{13}C$ two-dimensional techniques and earlier work[11].

**Table 2:** $^{13}C$ NMR (50 MHz) Data (δ, $CDCl_3$) of Umbellatin α (**7**) and Tingenone (**10**)[12] (Chemical shifts are given in ppm)

| | 7 | | 10 | | 7 | | 10 |
|---|---|---|---|---|---|---|---|
| **C** | **C** | **C'** | **C** | **C** | **C** | **C'** | **C** |
| **1** | 110.49 | 115.05 | 119.8 | **15** | 28.95 | 28.52 | 28.5 |
| **2** | 188.40 | 174.00 | 178.4 | **16** | 35.53 | 34.17 | 35.5 |
| **3** | 172.66 | 170.05 | 146.1 | **17** | 38.10 | 38.16 | 38.2 |
| **4** | 91.16 | 123.89 | 117.1 | **18** | 43.60 | 43.60 | 43.6 |
| **5** | 128.59 | 132.24 | 127.8 | **19** | 30.14 | 31.92 | 32.1 |
| **6** | 126.17 | 189.40 | 133.5 | **20** | 41.85 | 41.85 | 41.9 |
| **7** | 115.05 | 128.46 | 118.1 | **21** | NOT RECORDED | NOT RECORDED | 213.5 |
| **8** | 163.50 | 151.15 | 168.6 | **22** | 52.39 | 52.42 | 52.5 |
| **9** | 38.65 | 43.63 | 42.7 | **23** | 13.11 | 23.85 | 10.3 |
| **10** | 137.00 | 151.17 | 164.7 | **25** | 40.20 | 39.82 | 39.1 |
| **11** | 33.16 | 33.16 | 33.8 | **26** | 20.82 | 22.30 | 21.6 |
| **12** | 29.65 | 29.90 | 30.0 | **27** | 19.59 | 19.76 | 19.7 |
| **13** | 41.85 | 39.83 | 40.6 | **28** | 32.58 | 32.58 | 32.6 |
| **14** | 44.30 | 44.01 | 44.7 | **30** | 15.06 | 15.06 | 15.1 |

When tingenone (**10**) was treated with DDQ in dioxan, several products were formed, two of which proved identical to umbellatin α (**7**) and umbellatin β (**8**) thus confirming the proposed structures and also the quite generalized behaviour of triterpene methylene quinones treated with DDQ[4]. The mechanistic aspects of these

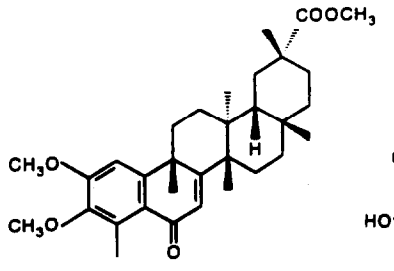
reactions are still under study although interesting earlier instances[13,14] seem to indicate a radical route such as proposed by ourselves elsewhere[4,15].

**Table 3**: MIC ($\mu$g/ml) of **1**, **2**, **7** and **8** Against Gram Negative and Gram Positive Bacteria.

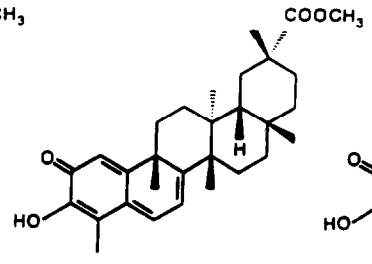
| Bacteria | 1 | 2 | 7 | 8 |
|---|---|---|---|---|
| *S. aureus* | >100 | 70-80 | >45 | >100 |
| *B. subtilis* | 23-20 | 2-1 | 5-2.5 | >100 |
| *B. cereus* | 23-20 | 10-8 | 30-25 | >100 |
| *Salmonella sp.* | - | - | >45 | >100 |
| *E. coli* | >50 | >50 | >45 | >100 |

- Not assayed.
All assays were carried out in triplicate.

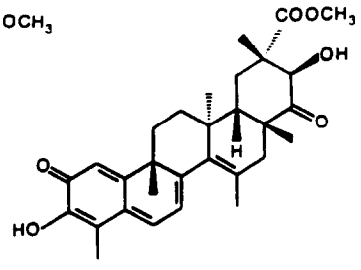
Table 3 shows the MIC (minimal inhibitory concentrations) of the different products tested on Gram positive and Gram negative bacteria, the former proving to be more susceptible to the activity of the compounds with the exception of product **8**, which did not display any activity. *Bacillus* species were more sensitive than *Staphylococcus aureus*. This fact seems to be a general trait of triterpene quinones, as netzahualcoyone[7], tingenone (**10**)[8] and pristimerin (**11**)[9] are all more active on *B. subtilis* than on *S. aureus*. The most active compound was **2**, its MIC on *B. subtilis* (1-2 $\mu$g/ml) being of particular interest.



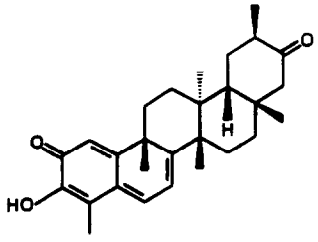

**9**




**11**




**12**




**10**

+ DDQ ⟶ **7** + **8** + not identified mixtures

As products **1** and **2** are pristimerin-type and **7** and **8**, tingenone-type dimers, their activity was compared with that of their respective monomers. Pristimerin (**11**) had an MIC of beween 0.75 and 0.70 μg/ml on *B. subtilis* and of more than 100 μg/ml against *S. aureus*. The MIC of compound **7** was about ten times greater (0.5-0.3 μg/ml) than that of tingenone (**10**) which, however, could also affect *S. aureus* (2.5-2.0 μg/ml) whereas **7** proved inactive against that bacterium. The dimers were active only on *B. subtilis* and even then were less active than the monomers.

## EXPERIMENTAL

$^1$H NMR spectra were recorded on a Bruker WP-200 SY instrument at 200.13 MHz with chemical shifts in δ. $^{13}$C NMR spectra were taken at 50.32 Hz. The mass spectra were run on a VG-Micromass ZAB-2F spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter and concentrations are given in g/100 ml solvent. Ultraviolet spectra were taken on a Perkin-Elmer 550 SE and TLC on pre-coated Schleicher & Schüll 254 silica gel plates, column chromatography on silica gel (Merck, 0.063-0.2 mm) and flash chromatography on silica gel (Merck, 0.04-0.063 mm)[16]. Repeated chromatography of the root extract of *Maytenus umbellata* separated umbellatin α (9 mg) and umbellatin β (7 mg) among other products. *Staphylococcus aureus* ATCC 6538, *Bacilus subtilis* CECT 39, *B. cereus* CECT 496, *Escherichia coli* CECT 90 and *Salmonella sp.* CECT 456 were used. The bacteria were grown and maintained in Nutrient Agar (Oxoid) and cultured in YP medium [composition per litre: yeast extract (Oxoid) 10 g, peptone (Difco) 20 g]. The inocula were prepared by diluting overnight cultures with a sterile saline solution. The minimal inhibitory concentrations (MIC) were determined in liquid medium as follws: Nutrient Broth (Oxoid) (3 ml) was inoculated with diluted culture (1 ml) to $10^5$-$10^6$ cells/ml and the compounds to be tested were added in a solution of dimethylsulfoxide (DMSO). The final volume was raised to 4 ml by adding sterile saline solution. The cultures were incubated at 37ºC in rotatory shaker and growth was determined by viable counting on Nutrient Agar plates. Tubes with the same proportion of DMSO were used as controls.

**Umbellatin α (7).** An amorphous pale yellow lacquer: $[\alpha]_D$ - 187.3 (c, 0.2, $CHCl_3$); EIMS m/z 436 (calc. for $C_{28}H_{36}O_4$ , 436.2613; found, 436.2607), 420 (calc. for $C_{28}H_{36}O_3$ , 420.2685; found, 420.2675); UV $\lambda_{max}$ (EtOH) nm: 217, 224, 243, 290, 305; IR $\nu_{max}$ ($CHCl_3$) cm$^{-1}$: 3400, 2920, 1700, 1650, 1640, 1590, 1580, 1560, 1530, 1450, 1370, 1300, 1200, 1150, 1080, 1060, 1050, 1010, 1000, 860, 850; $^1$H NMR δ ($CDCl_3$): 0.97 (6H, s), 0.99 (9H, s), 1.25 (6H, s), 1.35, 1.40, 1.54, 1.60, 2.75 (each 3H, s), 5.18 (1H, s), 6.10 (1H, s), 6.12 (1H, d, J= 7.0 Hz), 6.24 (1H, s), 6.55 (1H, d, J= 7.0 Hz), 6.76 (1H, s). $^{13}$C NMR δ: see table 2.

**Umbellatin β (8).** An amorphous pale yellow lacquer. $[\alpha]_D$ + 196.6 (c, 0.3, $CHCl_3$); EIMS m/z 436 (calc. for $C_{28}H_{36}O_4$ , 436.2612; found , 436.2601), 420 (calc. for $C_{28}H_{36}O_3$ , 420.2686; found, 420.2678) UV $\lambda_{max}$ (EtOH) nm: 215, 222, 254, 290; IR$\nu_{max}$ ($CHCl_3$) cm$^{-1}$: 3400, 2920, 1700, 1660, 1630, 1590, 1450, 1370, 1300, 1200, 1140, 1080, 1050, 1040, 1000, 860; $^1$H NMR δ ($CDCl_3$): 0.97 (6H, s), 0.99 (9H, s), 1.25 (6H, s), 1.36, 1.49, 1.55, 1.60, 2.75 (each 3H, s), see table 1 for rest of signals.

**Treatment of Tingenone (10).** Two drops of $H_2O$ were added to a solution of tingenone (225 mg, 0.53 mmol) in freshly-distilled 1.4 dioxan (100 ml) at room temperature while stirring, followed by a solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (150 mg) in dioxan (2 ml). After 7 min, TLC indicated the disappearence of part of the starting material and its transformation to at least four products, the major

compounds being the two more polar products which were identified with umbellatin α (**7**) and umbellatin β (**8**). Hydroquinone (100 mg) was thereupon added and stirred for 10 min after which the solvent was evaporated off at reduced presure and $CHCl_3$ was added to the resulting solid, part of which was dissolved and then chromatographed various times on silica gel to yield the starting material, tingenone (**10**) (20 mg), umbellatin α (**7**) (16 mg, 3% yield based on starting material), and umbellatin β (14 mg, 28% yield) as well as two other products which have not yet been purified.

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