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STRUCTURE OF MAESAQUINONE

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Abstract. Structure of a benzoquinone, maesaquinone, isolated from *Maesa lanceolata* (Myrsinaceae), has been characterized as 2,5-dihydroxy-3-(*Z*)-10'-pentadecenyl-6-methyl-1,4-benzoquinone (**1**) by means of spectroscopic method. It has been found to exhibit *in vitro* cytotoxicity against several solid tumor cells.

In previous papers,[1-3] we reported the isolation, structure and synthesis of a non-specific host defense stimulant, maesanin (**2**) from the fresh fruits of an East African medicinal plant *Maesa lanceolata* (Myrsinaceae).[4] In addition to this host defense stimulation activity, maesanin exhibited various biological activities. Thus, this benzoquinone was found to inhibit 5-lipoxygenase (IC_{90} 3 X 10^{-6} M),[5] and mitochondrial oxidative phosphorylation.[6] Interestingly, maesanin did not show any antimicrobial activity up to 400 μg/ml when tested alone. However, this benzoquinone became active specifically against *Candida utilis* when it was assayed in combination with a sublethal amount of polygodial.[7] Moreover, maesanin has been found to show significant *in vitro* cytotoxicity against several solid tumor cells,[8] and aldose reductase inhibitory activity.[9] These various biological activities of maesanin led us to reinvestigate the same plant searching for its analogues for structure function study. Repeated column chromatography of the mother liquid over silica gel using a solvent gradient with *n*-hexane increasing the amount of EtOAc led to the isolation of another 1,4-benzoquinone analogue designated as maesaquinone.

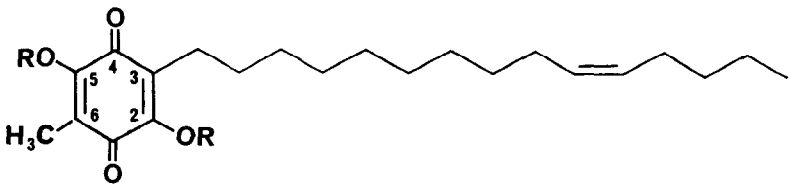
Maesaquinone (**1**), $C_{22}H_{34}O_4$, was obtained as orange yellow needles, m.p. 124-125°C. The positive EI-MS spectrum established the molecular ion $[M]^+$ at *m/z* 362. Its IR spectrum ($CHCl_3$) showed only hydroxyl (3310 cm^{-1}) and a *cis* olefinic bond (1600 and 700 cm^{-1}). The UV spectrum (MeOH) exhibited absorption bands at 206, 292 and 355 nm (log ε 4.162, 4.307 and 3.216) which suggested a dihydroxy substituted *p*-benzoquinone. The presence of a *p*-benzoquinone nuclei having substituted two hydroxyl groups adjacent to quinoid carbonyls, a methyl group and a long alkyl side chain containing a *cis* olefinic bond in maesaquinone was suggested by the inspection of various spectral (UV, IR, MS, ^1H and ^{13}C NMR) data. Maesaquinone was structurally related to maesanin (**2**) except for the presence of a

hydroxyl group in stead of a methoxyl group at C-5 and a methyl group at C-6 in **1**. The ^{1}H NMR spectrum ($CDCl_3$) of maesaquinone (**1**) displayed signals for two enolic protons (δ 7.63, 2H, *br s*, OH-2 and OH-5), one methyl group (δ 1.91, 3H, *s*, CH_3-6) and a (*Z*)-10'-pentadecenyl group[2] (δ 5.32, 2H, *br s*, H-10' and H-11'; 2.40, 2H, *t*, *J*=7.7 Hz, H_2-1'; 1.99, 4H, *br s*, H_2-9' and H_2-12'; 1.44, 2H, *br s*, H_2-2'; 1.29, 16H, *m*, H_2-3' to H_2-8', H_2-13' and H_2-14' and 0.87, 3H, *br s*, H_3-15'). The absence of signals due to proton on C-6 and methoxyl methyl protons on C-5 as in **2**,[2] suggested that maesaquinone could be a 2,5-dihydroxy-3,6-dialkyl tetrasubstituted 1,4-benzoquinone supported by MS spectrum which showed a base peak at *m/z* 168 due to a fragment ion as follows (Figure 1).[10]

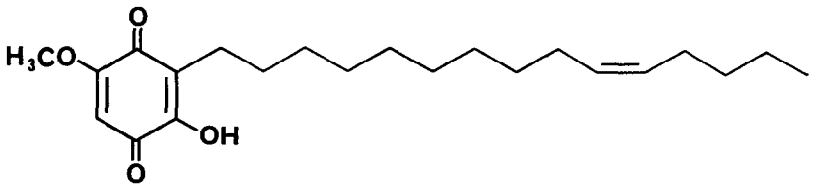
The ^{13}C NMR spectrum of maesaquinone was measured in both d_6-DMSO and $CDCl_3$ at 35°C. Interestingly, only three signals in d_6-DMSO and two signals in $CDCl_3$ due to six ring carbons were observed. This result has been attributed to a rapid interconversion of the two energetically equivalent tautomeric forms (***a*** and ***b***) as illustrated in Figure 2. And hence for this molecule only one averaged structure can be elucidated by ^{13}C NMR studies in solution.[11] However, suppression of the exchange equilibrium was observed in solid state ^{13}C CPMAS NMR[12] and by variable temperature ^{13}C NMR measurements in solution.[11]

The ^{13}C NMR spectrum (in d_6-DMSO) of maesaquinone (**1**) showed only four higher values of chemical shifts appearing at δ 168, 129, 116 and 111. The carbon signal at δ 129 is assigned to C-10' and C-11'. The methyl bearing carbon (C-6) and (*Z*)-10'-pentadecenyl bearing carbon (C-3) are absorbed at 116 and 111 ppm, respectively.[11] Four oxygen bearing ring carbons (C-1, 2, 4 and 5) in **1** appeared as a single broad signal at 168 ppm as in the case of hydroxyperezone.[11] The same spectrum (Figure 3) of **1** when measured in $CDCl_3$ at 35°C, no broad signal corresponding to oxygen bearing ring carbons was observed due to rapid interconversion between the exchange forms (***a*** and ***b***) of maesaquinone. However, maesanin (**2**) showed all the ring carbons in its ^{13}C NMR spectrum,[2] indicating existence of only one structure in the solution. The tautomeric interconversion was absent in **2** due to the presence of a methoxy group at C-5, adjacent to C-4 quinoid carbonyl. The assignment of the side carbon in maesaquinone has been carried out in accordance with previous studies.[12] Interpretation of these spectral data fully corroborated the structural assignment of maesaquinone as 2,5-dihydroxy-3-(*Z*)-10'-pentadecenyl-6-methyl-1,4-benzoquinone.

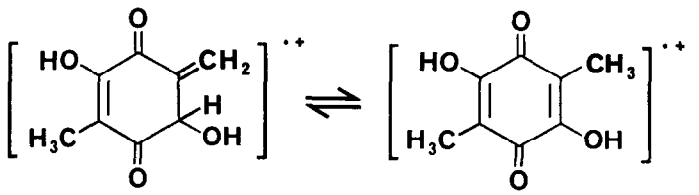
Bioassay with the purified maesaquinone indicated the two benzoquinones (**1**,**2**) differed in their biological activity. Most significantly, maesaquinone (**1**) did not show any host defense stimulation activity. However, both maesanin and maesaquinone exhibited comparative cytotoxicity against HeLa epithelioid cervix carcinoma cells with the IC_{50} of 1.25 and 2.05 μg/ml, and BT-20 breast carcinoma cells with those of 1.80 and 1.58 μg/ml, respectively.[12] The chemical difference between these two benzoquinones is that maesanin can be attacked by a nucleophilic reagent, but maesaquinone cannot be.



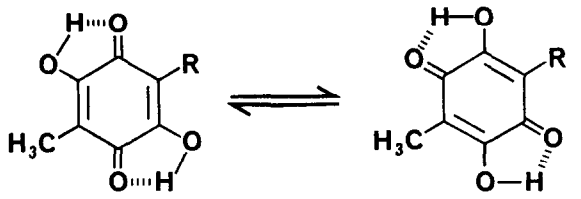
1 R=H
3 R=CH_3



2



m/z 168
Figure 1.



a *b*

cis
R=$(CH_2)_9$-CH=CH-$(CH_2)_3$-CH_3
Figure 2.

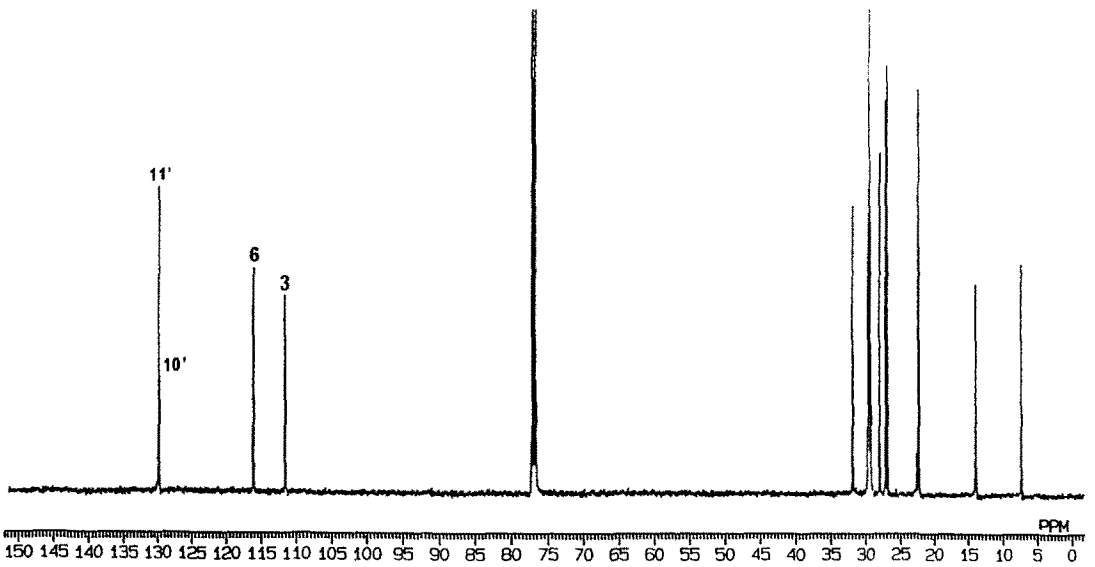


Figure 3. ^{13}C NMR spectrum of maesaquinone (**1**) in $CDCl_3$ at 35°C.

References and Notes.

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7. This combination was also found to be active against *Candida albicans* but not *Saccharomyces cerevisiae*.
8. It was tested against HeLa epithelioid cervix and BT-20 breast carcinoma cells, as well as L1210 lymphocytic leukemia and P388 lymphoid neophoid neoplasm cells.
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13. Cytotoxicity assay was performed as previously reported.[13] We thank Dr. S. Kurozumi for technical assistance for cytotoxicity assay.
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