Bull. Chem. Soc. Ethiop. **2004**, 18(1), 45-49. ISSN 1011-3924
Printed in Ethiopia

LARVICIDAL BENZOQUINONE FROM *EMBELIA SCHIMPERI*

C.P. Kiprono[1*], J.O. Midiwo[2], P.K. Kipkemboi[1] and Santino Ladogana[3]

[1]Department of Chemistry, Moi University, P.O. Box 1125, Eldoret, Kenya
[2]Department of Chemistry, University of Nairobi, P.O. Box 30197, Nairobi, Kenya
[3]Department of Chemistry and Physics, Southeastern Louisiana University, Hammond, LA, USA

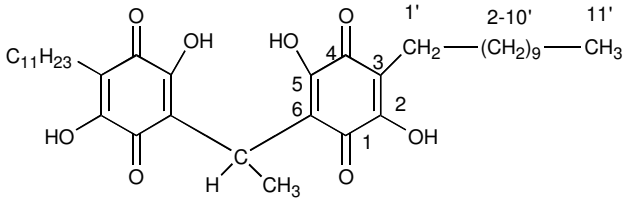
(Received November 28, 2002; revised September 17, 2003)

ABSTRACT. Chromatographic analysis of air-dried berries of *Embelia schimperi* led to the isolation of methyl vilangin (**1**), which was characterized on the basis of physical and spectroscopic data. The compound was found to be lethal against 2nd instar larvae of *Aedes aegypti* (yellow fever vector) by first stopping the process of metamorphosis from the 2nd instar stage to the other stages and finally causing mortality to the larvae.

KEY WORDS: *Embelia schimperi*, *Aedes aegypti*, 2nd instar larvae, Benzoquinones, Methyl vilangin

INTRODUCTION

In spite of many years of research into yellow fever drugs, the disease still remains the leading killer disease mainly in Africa. The yellow fever parasite has lately been found to be resistant to most conventional drugs in the market today. In view of this worrying trend, *Embelia schimperi* – a plant belonging to the family *Myrsinaceae*; known for anti-bacterial and anthelmintic properties [1] – was screened for alkyl benzoquinones that the family is known to accumulate up to 5-16%, w/w, [2]. The screening led to isolation of methyl vilangin (**1**), which was investigated for larvicidal activity against *Aedes aegypti* (yellow fever vector).



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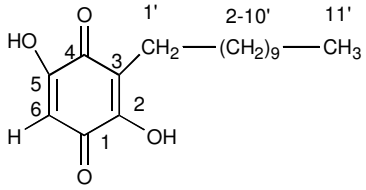

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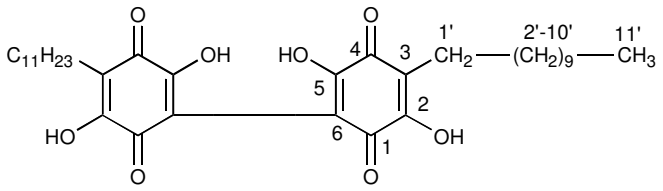
*Corresponding author. E-mail: paulkiprono@yahoo.com



(3)

EXPERIMENTAL

General

Melting point was determined using a Gallenkamp melting point apparatus and was uncorrected. The UV-Vis spectra were obtained using a DU-50 spectrometer. The IR spectra were recorded as KBr pellets on a Perkin-Elmer 720 spectrometer. The MS spectra were obtained on a JEOL – D300 mass spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL GSX-400 with tetramethylsilane as internal standard. Column chromatography was done on deactivated silica gel. Soaking it 3% oxalic acid over night and then drying it in an oven at 110 ^{o}C did de-activation of silica gel. The *Aedes aegypti* eggs were obtained from International Center for Insect Physiology and Ecology (ICIPE). The hatched larvae were fed on commercially available yeast. The *Embelia schimperi* berries were collected from Kericho district, which is 260 km West of Nairobi. A voucher specimen is deposited in the Herbarium of Department of Botany, University of Nairobi.

Solvent extraction and chromatography

The air-dried berries were extracted with cold ethyl acetate and left to stand for 48 hours while mechanically stirring. Filtration was followed by vacuum solvent removal using rotary evaporator. A weight of 55 g of the concentrate was absorbed on dry de-activated silica gel and subjected to column (ϕ = 5.5 x 68 cm) chromatography in a column packed under *n*-hexane with 550 g of the de-activated silica gel. The column was exhaustively eluted with *n*-hexane, dichloromethane and ethyl acetate, respectively. The dichloromethane fraction, the only one found to contain the quinonoid pigments, was subjected to further chromatographic separation. In cases of incomplete separation, preparative thin layer chromatography was employed. Elution of the column with a mixture of *n*-hexane and dichloromethane (1:1, v/v) led to the isolation of orange amorphous crystals (0.06% yield, w/w).

Methyl vilangin (**1**) was obtained as orange crystals (60 mg), m.p. 129-131 ºC, λ_{max} nm (MeOH): 430 (log ε, 2.4) and 290 (log ε, 4.19); IR (KBr) ν_{max} cm^{-1}: 3310 (OH stretch), 1630 (chelated C=O stretch), 3000 (olefinic C-H); MS (70 eV): *m/z* (%) 614 (M^{+}), 348 (2.5), 320 (11), 294 (44.7), 180 (13.8), 154 (100). ^{1}H and ^{13}C NMR data are summarized in Table 1.

Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$) spectral data for methyl vilangin (**1**).

Atom	^{1}H (ppm)	^{13}C (ppm)
1	-	181.00*
2	-	150.20
3	-	116.35
4	-	180.20*
5	-	149.60
6	-	116.09
H-C-CH_3 (bridging carbon)	4,39 (q, J = 7.50 Hz)	28.20
H-C-CH_3 (Me on bridging carbon)	1.59 (d, J = 7.50 Hz)	16.75
1'	2.40 (t, J = 7.1 Hz)	29.60
2'-10'	1.43 -1.25 (m)	32.13, 29.92, 29.87, 29.84, 29.81, 29.77, 29.55, 28.18, 26.87, 26.87, 22.90, 22.76
11'	0.9 (t, J = 13.2, 6.3 Hz)	14.32
2,5, -OH	7.82 (s, D_2O exchangeable)	

*May be interchanged.

Larvicidal test

The method according to Zebitz [3] was adopted for larvicidal assay. Briefly, into each jar holding 40 mL of 0.09% sodium chloride, twenty 2nd instar mosquito larvae were introduced and immediately treated with 10, 25, 50, 75, and 100 µg/mL of methyl vilangin (**1**) each in triplicate. The control experiment contained the 0.09% sodium chloride solution alone. The experiment was followed for 13 days after which period; the data were processed using a simple computer program (SAS) to estimate LC_{50} values with 95% confidence interval for significant comparison of potencies. The results of this assay on day 13 are summarized in Table 2.

Table 2. Larvicidal effects of methyl vilangin (**1**) on day 13.

Concentration	Population (%) at,				
(µg/mL)	2nd instar	3rd instar	4th instar	Pupae	Adults
100	70	25	5	0	-
75	50	30	15	5	-
50	25	35	30	-	10
25	15	15	10	40	20
10	-	10	15	10	65
Control	100	95	95	95	95

RESULTS AND DISCUSSIONS

Structure elucidation

Chromatographic separation of *E. schimperi* berries led to isolation methyl vilangin (**1**), which was orange in colour. The compound turned pink on exposure to concentrated ammonia vapor. Its UV-Vis spectrum displayed a peak at 430 and 290 nm thus indicating the chromophore of the

compound as a 2,5-dihydroxy-1,4-benzoquinone [4]. The compound showed six signals in the ^{1}H NMR spectrum. There was no signal corresponding to the quinonoid proton, which usually appears at 6.50-5.0 ppm. This suggests that the quinonoid ring is fully substituted. A singlet peak due to phenolic hydroxyl hydrogens hydrogen-bonded to carbonyl group appeared at 7.82 ppm. The spectrum also contained peaks at 4.39 ppm (H, q, J = 7.50 Hz) and 1.59 ppm (3H, d, J = 7.50 Hz), which is unusual for the usual alkyl, substituted 1,4-benzoquinones from *Myrsinaceae*. The peak at 4.39 ppm suggests the presence of a proton sitting on the same carbon atom with a methyl group whereas the doublet peak at 1.59 ppm could be due to a methyl group next to a proton. In fact, a methyl group substituted into the ring displays a sharp singlet at approximately 1.90 ppm whereas the quinonoid proton appears either as a singlet or a multiplet at 6.5-5.0 ppm [5-7]. Irradiation of the peak at 4.39 ppm collapsed the doublet at 1.59 ppm into a singlet suggesting that the proton and the methyl group are adjacent to each other and possibly sitting on the same carbon atom. The triplet at 2.40 (J = 7.1 Hz) ppm was assigned to the benzylic methylene protons. Integration of this peak relative to the set at 4.39 ppm corresponded to four protons calculated for two methylene groups. This therefore suggests that the compound under consideration probably consists of two dihydroxyalkyl-1,4-benzoquinones bridged by a carbon atom holding a proton and a methyl group. A multiplet peak at 1.43-1.25 ppm on integration relative to the peak at 4.39 ppm corresponded to 36 protons calculated for 18 methylene groups. The terminal methyl protons, which appeared at 0.9 ppm similarly on integration relative to the peak at 4.39 ppm, revealed the existence of six protons thus accounting for two methyl groups. The ^{13}C NMR data showed peaks due to C-3,3' and C-6,6' at 116.35 ppm and 116.09 ppm, respectively. They were assigned by comparing ^{13}C NMR data of **1** with that of embelin (**2**) and biembelin (**3**). The latter two compounds have previously been isolated from *Myrsine africana* [8]. The C-6,6' peaks corresponded to quaternary carbon atoms bearing the bridging carbon atom (peak at 28.2 ppm) holding a methyl group and a proton. This seems to confirm the presence of two coupled alkyldihydroxy-1,4-benzoquinones in **1**. Its mass spectrum showed significant fragments at *m/z* 320, 294, 180, 154, 139 and 125. Its molecular ion showed at 614 a.m.u. Another most striking feature of the mass spectrum of this compound was the peak at *m/z* 294 nm that evidenced the existence of embelin (**2**) moiety. Also evident in the mass spectrum were a base peak at *m/z* 154 and a low abundance peak at *m/z* 153. Such fragmentation patterns have been observed in the embelin (**2**), which has a quinonoid proton in the ring. Mass spectral data strongly favored the existence of two embelin (**2**) moieties joined together via a bridging carbon atom holding a methyl group and a proton. From the above data, compound **1,** was identified as methyl vilangin ($C_{36}H_{54}O_8$), which is being encountered for the first time as a natural product.

Larvicidal test

Concentrations ranging from 10–100 μg/mL of methyl vilangin (**1**) were applied into a pond containing twenty 2nd instar larvae of *Aedes aegypti*. The compound was found to be lethal against the larvae by first disrupting the process of metamorphosis and finally causing mortality. In fact, when 100 μg/mL of **1** was applied into the pond containing the 2nd instar mosquito larvae, 70% of them were kept at this stage for a period of 12 days before finally being killed. About 25% and 5% of the larvae underwent the process of metamorphosis to 3rd and 4th instar stages, respectively. None of the larvae attained pupae or adult stages. This biological activity could be used to reduce population of *Aedes aegypti* mosquito and hence reduction in prevalence of yellow fever. The results this assay are summarized in Table 2.

ACKNOWLEDGEMENTS

The authors are grateful to Kenya Agricultural Research Institute (KARI) for funding the project, Prof. Berhanu Abegaz of University of Botswana and Mr. Wanyama of International Centre for Insect Physiology and Ecology (ICIPE) for generating NMR (^{1}H and ^{13}C) and mass spectral data, respectively.

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