MICROWAVE IRRADIATION OF EMBELIN AND EVALUATION OF ANTIBACTERIAL ACTIVITY

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Abstract: Under Microwave irradiation Embelin 1 undergone dimerization and resulted in the formation of furanylidene benzofuranone 2, 1, 4-dibenzo furandione 3 and a mixture of unidentified compounds 4. The structure of compounds 2 and 3 were determined by the study of spectral data. Compounds 1-4 were tested for their antimicrobial activity.

A number of herbal medicines used as decoctions in traditional treatments are usually prepared according to well established prescriptions and traditional procedures. During such processes, some of the constituents originally present may possibly undergo chemical changes (1).

Embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone) 1, a major constituent of *Embelia ribes* BURM (*Myrsinaceae*) having anthelmintic activity (2, 3) was transformed into different types of compounds through dimerization during preparation of the decoction (4). In an effort to make more bioactive derivatives of natural products (5-6), here we report the microwave irradiation of embelin and evaluation of antibacterial activity.

Results and Discussion: Embelin 1 when irradiated under microwave oven for 6 min. yielded furanylidene benzofuranone 2, 1,4-dibenzo furandione 3 and a mixture of unidentified compounds 4.

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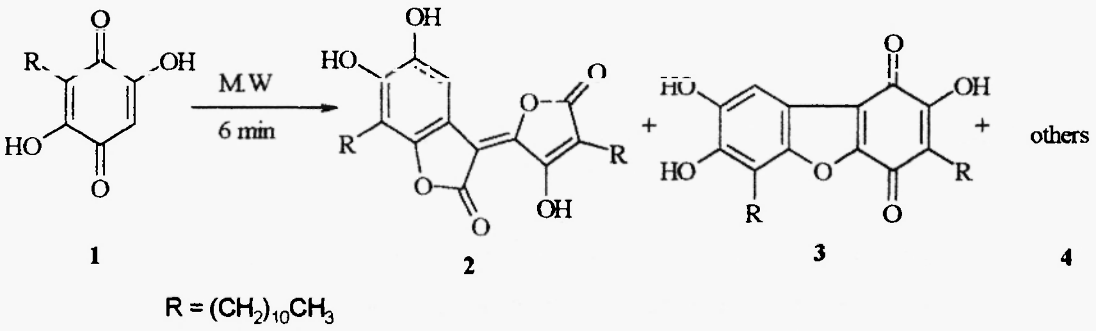

Compound 2 was obtained as red needles m.p. 166-168 °C. The ^{1}H NMR spectrum showed signals for two terminal methyls at δ 0.87 (3H, t, J = 7 Hz) and 0.88 (3H, t, J = 7 Hz), two methylene groups at δ 2.36 (2H, t, J – 7.5 Hz) and 2.69 (2H, t, J = 7.5 Hz), polymethylene chain at δ 1.2-1.6 (36H, br s), an olefinic proton at δ 7.32 (1H, s) and three hydroxy groups at δ 6.14 (1H, br s), 6.84 (1H, br s) and 11.75 (1H, br s) (disappeared on addition of D_2O). The presence of three hydroxy groups further confirmed by acetylation, which yielded corresponding triacetate derivative 2a. The foregoing spectral data were identical with those of furanylidene benzofuranone formed on embelin boiling with water (4).

Compound 3 was obtained as black-red crystals, m.p. 166-167 °C. The ^{1}H NMR spectrum showed signals for two terminal methyls at δ 0.87 (3H x 2, t, J = 7 Hz), two methylene groups at δ 2.52 (2H, t, J = 7.5 Hz) and 2.92 (2H, t, J = 7.5 Hz), polymethylene chain at δ 1.2-1.6 (36H, br s) and one olefinic proton at δ 7.30 (1H, s). In addition to this it showed three hydroxy signals at δ 5.77 (1H, br s), 5.83 (1H, br s) and 7.06 (1H, br s) (disappeared on addition of D_2O). The foregoing spectral data were identical with the compound 1,4-dibenzofurandione formed on embelin boiling with water (4). In addition to the above compounds two more compounds are obtained as a mixture 4.

Antibacterial activity of compounds 1-4 was assayed by disk susceptibility tests according to the NCCLS (8) against gram negative bacteria *Escherichia coli* (ATCC # 25922) and *Pseudomonas aeruginosa* (ATCC # 25619) and gram positive bacteria *Bacillus subtilis* (ATCC # 6051) and *Staphylococcus aureus* (ATCC # 9144) at a concentration of 200μg/ disc.

Table 1 Antibacterial activity of compounds 1-4

Compound	Gram negative		Gram positive	
	E. coli	*P.*	*B. subtilis*	*S. aureus*
1	9	9	7	7
2	6	9	-	-
3	6	-	-	-
4	6	-	-	-

Zone of growth inhibition in mm/dia.
Values are the average standard errors of four replicates.

This process is easy and convenient for dimerization of embelin and also for dimerization of 2, 5-dihydroxybenzoquinones. Earlier, the similar reactions were carried out in boiling water (4, 8), that is time consuming and require workup after completion of reaction.

Experimental section: Melting points were obtained on Fisher John's apparatus and are uncorrected. UV spectra were recorded on a Shimadzu-240 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 240-C spectrophotometer. ^{1}H NMR spectra were recorded on Varian Gemini 200 MHz spectrometer using TMS as internal standard and coupling constant (J) are reported in Hz. Mass spectra were recorded on VG Auto Spec-M instrument. Column chromatography was performed on acid-treated silica gel, which was prepared as follows: Acme 100-200 mesh silica gel was immersed in 0.5 N oxalic acid solution for 48 h, filtered, washed almost free from acid and dried. TLC done on acid-treated silica gel plates, were prepared by dipping the plates in 0.5 N oxalic acid solution and G_{254} silica gel.

Microwave Irradiation of Embelin 1: Compound 1 (50 mg) was taken in an Erlenmeyer flask and placed inside a commercial microwave oven (BPL BMO 700 T). The compound 1 was irradiated at 233 Watt for 6 min. The reaction mixture was taken out from the oven and cooled to the room temperature. The reaction mixture was purified by column chromatography over acid treated silica gel. This afforded compounds 1 (5 mg), 2 (25 mg), 3 (10 mg), and 4 (10 mg).

Compound 2: Red needles, m.p. 166-168 °C; UV (EtOH) $\lambda_{max.}$ (log ε) 275 (4.25), 381 (4.15), and 466 (4.07) nm; IR (neat) $\nu_{max.}$ 3320, 1763, 1721, 1651, 1626, 1463 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (3H, t, J = 7 Hz), 0.88 (3H, t, J = 7.5 Hz), 1.2-1.4 (32 H, br s), 1.58 (4H, m), 2.36 (2H, t, J = 7.5 Hz, H-4'), 2.69 (2H, t, J = 7.5 Hz, H-7), 6.14 (1H, br s, 6-OH), 6.84 (1H, br s, 5-OH), 7.32 (1H, s, H-4), 4.75 (1H, s, 5-OH); FABMS: *m/z* 570 [M$^+$], 429, 346, 289, 205.

Acetylation of compound 2: Compound 2 (5 mg) was acetylated with Ac_2O (0.1 ml) and pyridine (0.05 ml) for 70h at room temperature and worked up as usual and purified by column chromatography yielded 2a (4 mg). Compound 2a was obtained as yellow needles, m. p. 91-93 °C, ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (6H, br t, $J = 7$ Hz), 1.2-1.4 (30H, br s), 1.55 (6H, m), 2.30 (3H, s), 2.33 (3H, s), 2.46 (3H, s), 2.40 (2H, t, $J = 7.5$ Hz), 2.56 (2H, t, $J = 7.5$ Hz), 7.63 (1H, s); FABMS (%): *m/z* [M^+], 654, 612, 570.

Compound 3: Black-red Crystals; m.p. 166-167 °C; UV (EtOH): $\lambda_{max.}$ (log ε) 214 (4.56), 259 (4.45), 290 (4.13), 356 (3.82), 551 (3.40); IR (neat): $\nu_{max.}$ 1601, 1639, 1618 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (3H x 2, t, $J = 7$ Hz), 1.2-1.4 (34H, br s), 1.50 (2H, m), 2.52 (2H, t, $J = 7.5$ Hz, H-3), 2.92 (2H, t, $J = 7.5$ Hz, H-6), 5.77 (1H, br s), 5.83 (1H, br s), 7.06 (1H, br s), 7.30 (1H, s, H-9); FABMS: *m/z* 554 [M^+], 414, 273.

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