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Kavalactones from *Piper methysticum*, and their ^{13}C NMR spectroscopic analyses

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Abstract

The kavalactone, 11-methoxy-5,6-dihydroyangonin, and eight previously reported analogs along with four other aromatic compounds were isolated from the root extracts of *Piper methysticum* (Kava Kava). Their structural elucidations were made by ^{1}H and ^{13}C NMR spectroscopic assignments using COSY, HMBC and HMQC experiments.

Keywords: *Piper methysticum*; Piperaceae; Kavalactones; 11-Methoxy-5,6-dihydroyangonin; Flavokawain; ^{1}H and ^{13}C NMR spectroscopy

1. Introduction

Piper methysticum Forst. f. (Kava Kava) belongs to the family Piperaceae, and grows as a perennial shrub in Fiji and other South Pacific islands (Singh and Blumenthal, 1997). The South Pacific islanders have been using a traditionally prepared beverage from kava roots for thousands of years in social, recreational and ceremonial events, and also as a remedy for stress and anxiety (Singh and Blumenthal, 1997). Due to its beneficial health effects, *P. methysticum* has gained popularity recently in western countries as an alternative medicine especially for the treatment of anxiety disorders (Singh and Blumenthal, 1997). Placebo-controlled, double-blind studies have shown Kava extract to be as effective as the standard therapy for the treatment of anxiety disorders with a more favorable toxicity profile and minimal physical and psychological dependence (Singh and Blumenthal, 1997; Volz and Kieser, 1997).

The chemistry of *P. methysticum* has been extensively studied, and so far more than 40 compounds belonging to the classes of kavapyrones, alkaloids, steroids, chalcones, long-chained fatty acids and alcohols have been isolated and identified (Parmar et al., 1997). Among these compounds, kavalactones have been recognized as the constituents responsible for the reported biological activities in Kava (Singh and Blumenthal, 1997). Eighteen different kavalactones have been reported from the root extracts of Kava and desmethoxyyangonin (**1**), yangonin (**2**), dihydrokawain (**3**), kawain (**4**), dihydromethysticin (**5**) and methysticin (**6**) are the most abundant (Ganzera and Khan, 1999).

Our preliminary investigation on anti-anxiolytic properties of Kava root extract and of the six major kavalactones in an animal model showed that the activity for the root extract was higher than those observed for each major individual compound (Smith et al., 2001). This suggested that synergism or other compounds may be responsible for the higher activity of the extract. Prompted by this observation, an isolation and identification of compounds in the Kava root extract were carried out. Chromatographic separations of the methylene chloride extract of Kava root, followed by recrystallization led to the isolation and identification of nine kavalactones (**1–9**), together with three chalcones (**10–12**), 3,4-methylenedioxycinnamylideneacetone (**13**) and stigmasterol. All but, kavalactone, 11-methoxy-5,6-dihydroyangonin (**7**) were previously reported from *P.*

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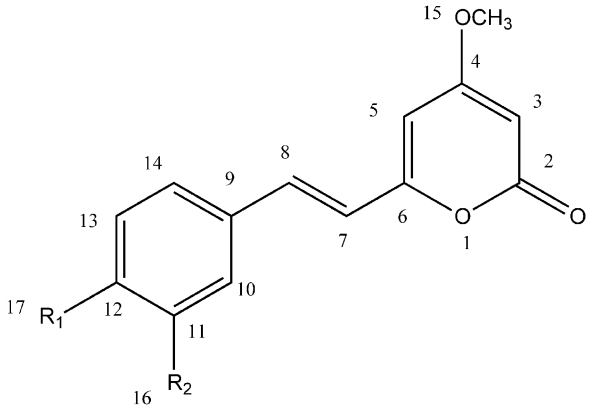

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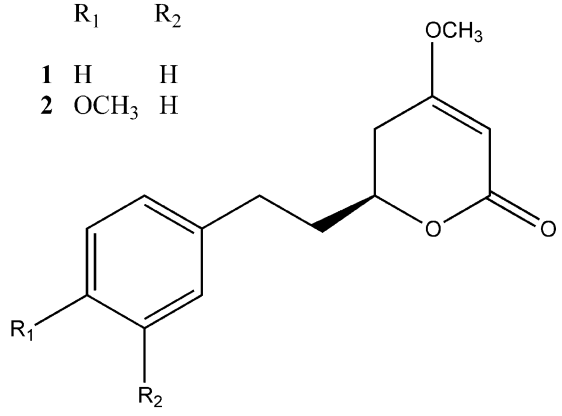
1	H	H
2	OCH_3	H



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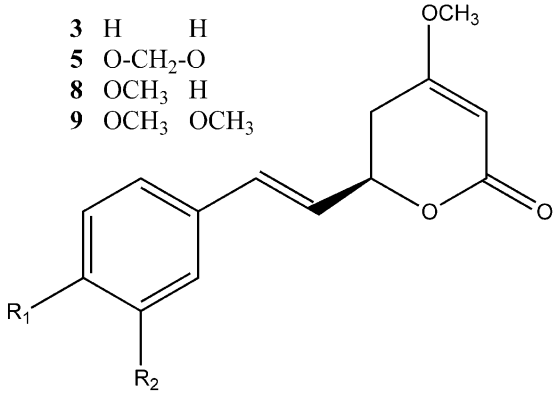
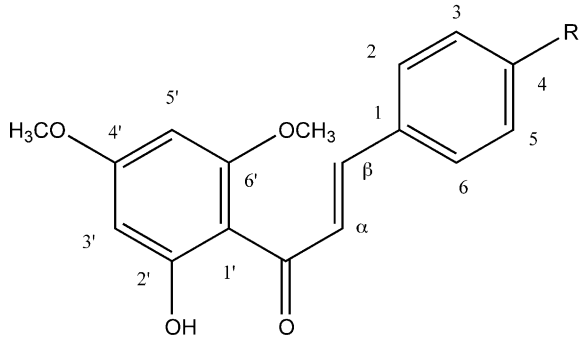
3	H	H
5	O-CH_2-O	
8	OCH_3	H
9	OCH_3	OCH_3



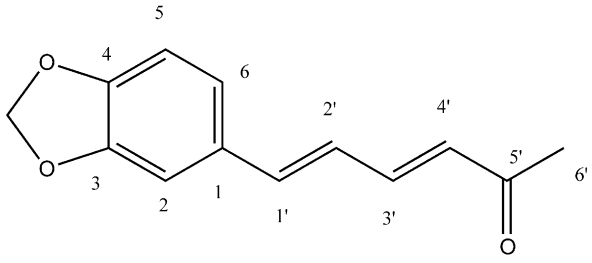
	R_1	R_2
4	H	H
6	O-CH_2-O	
7	OCH_3	OCH_3

methysticum (Parmar et al., 1997). This is the first report of 11-methoxy-5,6-dihydroyangonin (**7**).

Structural elucidation of above compounds was carried out by spectroscopic means and by comparison of the physical spectroscopic data with those in the literature. Though 18 kavalactones have been isolated so far from *P. methysticum* by various research groups (Jössang and Molho, 1967; Achenbach et al., 1973; Dutta et al., 1972; Lopez-Avila and Benedicto, 1997; Parmar et al., 1997; Singh and Blumenthal, 1997; Ganzera and Khan., 1999), complete physical data and ^{13}C and ^{1}H NMR assignments are available (Banerji et al., 1980) only for kawain (**4**), methysticin (**6**) and yangonin (**2**). In this study, the structure elucidation of the new compound, 11-methoxy-5,6-dihydroyangonin and complete ^{13}C and ^{1}H NMR assignments of other kavalactones were carried out using COSY, HMQC and HMBC experiments.



	R_1
10	OCH_3
11	H
12	OH



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2. Results and discussion

Chromatographic separation of the methylene chloride extract of *P. methysticum* roots yielded fourteen compounds. Preliminary spectroscopic analysis indicated that these fourteen compounds comprised of nine kavalactones, three chalcones, 3,4-methylenedioxycinnamylideneacetone and stigmasterol. Detailed NMR spectroscopic analysis and comparison of the physical and spectroscopic data with those reported confirmed the

structures of 3,4-methylenedioxycinnamylideneacetone (**13**) (Jössang and Molho, 1967), stigmasterol and the chalcones as flavokawain A (**10**) (Zhili, 1998), flavokawain B (**11**) (Itokava et al., 1981) and flavokawain C (**12**) (Seeram et al., 1996).

Analysis of the NMR spectra of the kavalactones using HMQC and HMBC experiments permitted the determination of the structures of these compounds and the assignments of ^{13}C signals. These compounds were identified as desmethoxyyangonin (**1**), yangonin (**2**), dihydrokawain (**3**), kawain (**4**), dihydromethysticin (**5**), methysticin (**6**) tetrahydroyangonin (**8**) and 1-methoxytetrahydroyangonin (**9**) (Achenbach et al., 1973), previously isolated and identified from *P. methysticum*.

Analysis of compound **7** by HRMS suggested a molecular formula $C_{16}H_{18}O_5$ ($[M+H]^+ = 291.1154$). The ^1H NMR spectrum of this compound showed three methoxy groups and a typical set of peaks due to the 5,6 -dihydrokavalactone skeleton (see Experimental). Further analysis of the ^1H NMR spectrum of **7** showed an AB doublet for H-13. The^1H NMR signals for H-10 and H-14 appeared close together (δ 6.91 and 6.90, respectively) and a part of H-14 doublet of doublet ($J = 1.6$ and 7.8 Hz) overlapped with the H-10 signal to give a broad signal. These data suggested the 11-methoxy-5,6-dihydroyangonin structure for this compound, which was confirmed by HMQC and HMBC experiments. This is the first report of 11-methoxy-5,6-dihydroyangonin. The (6*R*)-absolute stereochemistry was determined by comparison with the CD data reported for this class of compounds (Snatzke and Hänsel, 1968; Achenbach and Theobald, 1974).

The ^{13}C NMR assignments of nine kavalactones were made using HMQC and HMBC experiments. The assignments for compounds **2**, **4** and **6** were consistent with those previously reported for these compounds (Banerji et al., 1980). The detailed ^1H and ^{13}C NMR spectroscopic data for these compounds are presented in Table 1 and in the Experimental.

Table 1
^{13}C NMR spectroscopic data of kavalactones

Carbon	1	2	3	4	5	6	7	8	9
2	164.2	164.5	167.5	167.0	167.5	167.1	167.2	167.7	167.5
3	89.2	88.7	90.6	90.8	90.5	90.8	90.8	90.7	90.5
4	171.4	171.6	173.2	172.8	172.9	172.8	172.8	173.2	173.2
5	101.8	100.8	33.3	33.5	33.2	33.7	33.7	33.4	33.3
6	158.9	159.4	75.2	76.2	74.8	76.4	76.4	75.2	75.1
7	119.1	116.8	36.6	126.1	36.7	124.1	123.9	36.9	36.8
8	135.8	135.7	31.3	133.3	30.9	133.3	133.4	30.4	30.8
9	135.6	128.4	141.3	136.2	134.8	130.6	129.0	133.2	133.8
10	127.8	129.3	128.8	127.1	109.0	106.2	109.5	129.8	112.2
11	129.2	114.7	128.9	129.0	147.9	148.5	149.5	114.3	149.3
12	129.8	161.1	126.5	128.7	146.1	148.2	149.8	158.4	147.7
13	129.2	114.7	128.9	129.0	108.5	108.7	111.6	114.3	111.8
14	127.8	129.3	128.8	127.1	121.5	122.1	120.5	129.8	120.6
15-OCH_3	56.3	56.2	56.4	56.5	56.2	56.5	56.5	55.6	56.3
16-O–CH_2–O					101.0	101.6			
11-OCH_3							56.2		56.1
12-OCH_3		55.7					56.3	56.4	56.2

3. Experimental

Mps (uncorr.) were recorded on an Electrothermal 9100 instrument. UV spectra were obtained in CD_3OD, using Hewlett Packard 8452A spectrometer and IR spectra were taken on an Ati Mattson (Genesis Series) FTIR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Avance DPX-300 (300 MHz for ^1H NMR and 75.45 MHz for ^{13}C NMR) and Bruker Avance DRX-500 (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) spectrometers in $CDCl_3$ and MeOH-D_4 using TMS as internal standard. High resolution MS (HRMS) were obtained by direct probe using Bruker Bioapex-FTMS with Electro-Spray Ionization. Preparative TLC was carried out using silica gel F 254 plates (thickness 1 mm).

3.1. Extraction and isolation

Piper methysticum (Kava Kava) root extract (1 kg) (Nutratech, Passaic Avenue, Fairfield, NJ 07004, USA: Lot No. 40493240) was extracted three times with methylene chloride at room temperature for 1 h under sonication. The combined methylene chloride extract was evaporated to give a dark green viscous semisolid. This semisolid was dissolved in methanol, diluted with diethyl ether and kept at -10 °C for 12 h to give 30 g of a crystalline material. Recrystallization of this material from methanol yielded yangonin (4.5 g) and the mother liquor was subjected to silica gel column chromatography. Elution of the column with increasing amounts of acetone in hexane as the solvent gave fractions with desmethoxyyangonin (**1**), yangonin (**2**) (0.6 g), dihydrokawain (**3**) (1.3 g), kawain (**4**) (1.35 g), dihydromethysticin (**5**) (2.15 g) methysticin (**6**) (1.25 g), stigmasterol (1.2 g) and flavokawain A (**10**) (0.4 g) as major compounds and were further purified by crystallization. The mother liquor from the original extract was also subjected to column chromatography on silica gel using the same solvent system to give in addition to the above compounds several fractions with minor constituents. Preparative TLC of these fractions using methanol/dichloromethane or hexane/ethyl acetate mixtures yielded the minor compounds **7**, **8**, **9**, **11**, **12** and **13** in pure form.

3.1.1. Stigmasterol

Semisolid, identity confirmed by ^1H NMR and co-TLC with an authentic sample.

*3.1.2. Desmethoxyyangonin (**1**)*

White amorphous solid (0.21%), mp 138–140 °C (lit 138–140) (Rezende et al., 1971). ^{1}H NMR ($CDCl_3$): δ 7.48 (2H, *m*, H-10 and H-14), 7.44 (1H, *d*, *J* = 15.9 Hz, H-8), 7.35–7.31 (3H, *m*, H-11, H-12 and H-13), 6.54 (1H, *d*, *J* = 16.0 Hz, H-7), 5.90 (1H, s, H-5), 5.44 (1H, *d*, *J* = 1.4 Hz, H-3), 3.76 (3H, *s*, H-15).

*3.1.3. Yangonin (**2**)*

Yellow needles (0.4%), mp 152–155 °C (lit. 153–154) (Dutta et al., 1972). ^{1}H-NMR ($CDCl_3$): δ 7.42 (1H, *d*, *J* = 15.9 Hz, H-8), 7.41 (2H, *d*, *J* = 8.8 Hz, H-10 and H-14), 6.87 (2H, *d*, *J* = 8.8 Hz, H-11 and H-13), 6.42 (1H, *d*, *J* = 16.0 Hz, H-7), 5.86 (1H, *d*, *J* = 2.1 Hz, H-5), 5.43 (1H, *d*, *J* = 2.1 Hz, H-3), 3.78 (3H, *s*, H-17), 3.76 (3H, *s*, 15-H).

*3.1.4. Dihydrokawain (**3**)*

White crystals (1.17%), mp 58–60 °C, (lit. 56–60) (Spino et al., 1996). ^{1}H NMR ($CDCl_3$): δ 7.27 (2H, *m*, H-11 and H-13), 7.18 (3H, *m*, H-10, H-12 and H-14), 5.12 (1H, d, *J* = 1.3 Hz, H3), 4.34 (1H, *m*, H-6), 3.70 (3H, *s*, 4-OCH_3), 2.84 (1H, *m*, H-8), 2.73 (1H, *m*, H-8), 2.48 (1H, *ddd*, *J* = 1.3, 11.9, 17.0 Hz, H-5), 2.28 (1H, *dd*, *J* = 3.9, 17.0 Hz, H-5), 2.11 (1H, *m*, H-7), 1.91 (1H, *m*, H-7).

*3.1.5. Kawain (**4**)*

White crystals (0.53%), mp 108–110 °C, (lit. 106–108) (Dutta et al., 1972). ^{1}H NMR ($CDCl_3$): δ 7.32 (2H, *m*, H-11 and H-13), 7.25 (2H, *m*, H-10 and H-14), 7.20 (1H, *m*, H-12), 6.70 (1H, *d*, *J* = 15.9 Hz, H-8), 6.23 (1H, *dd*, *J* = 6.14, 15.9 Hz, H-7), 5.16 (1H, *s*, H-3), 5.02 (1H, *m*, H-6), 3.72 (3H, *s*, H-15), 2.63 (1H, *dd*, *J* = 17.0, 10.7 Hz, H-5), 2.51 (1H, *dd*, *J* = 17.0, 4.5, Hz, H-5).

*3.1.6. Dihydromethysticin (**5**)*

White crystals (0.54%), mp 117–118 °C (lit. 117–118) (Anon. 1999). ^{1}H NMR ($CDCl_3$): δ 6.70 (1H, *d*, *J* = 7.9 Hz, H-13), 6.66 (1H, *d*, *J* = 1.2 Hz, H-10), 6.63 (1H, *dd*, *J* = 7.9, 1.2 Hz, H-14), 5.89 (2H, *s*, O–CH_2–O), 5.11 (1H, *d*, *J* = 1.2 Hz, H-3), 4.33 (1H, *m*, H-6), 3.70 (3H, *s*, H-12), 2.77 (1H, *m*, H-8), 2.67 (1H, *m*, H-8), 2.40 (1H, *ddd*, *J* = 17.0, 12.0, 1.2 Hz, H-5), 2.28 (1H, *dd*, *J* = 7.0, 3.8 Hz, H-5), 2.04 (1H, *m*, H-7), 1.85 (1H, *m*, H-7).

*3.1.7. Methysticin (**6**)*

White crystals (0.44%), mp 134–136 °C (lit. 134–136) (Dutta et al., 1972). ^{1}H NMR ($CDCl_3$): δ 6.88 (1H, *d*, *J* = 1.4 Hz, H-10), 6.79 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-14), 6.72 (1H, *d*, *J* = 8.0 Hz, H-13), 6.59 (1H, *d*, *J* = 15.8 Hz, H-8), 6.06 (1H, *dd*, *J* = 6.4, 15.9 Hz, H-7), 5.92 (2H, s, O–CH_2–O), 5.15 (1H, *d*, *J* = 0.6 Hz, H-3), 4.98 (1H, *m*, H-6), 3.72 (3H, *s*, H-12), 2.61 (1H, *ddd*, *J* = 17.2, 10.8, 0.7 Hz, H-5), 2.48 (1H, *dd*, *J* = 17.1, 4.4 Hz, H-5).

*3.1.8. 11-Methoxy-5,6-dihydroyangonin (**7**)*

Yellow semi-solid (0.01%), $[\alpha]_D^{25}$ +70.0° ($CHCl_3$; *c* 0.025). CD: (MeOH:*c* 0.01): [Ø] $_{232}$ −2.98, $[Ø]_{240}$ + 6.6, [Ø] $_{246}$ + 6.9. UV: λ_{max}^{MeOH} nm (log ε 228 (4.04), 266 (3.87). IR cm^{-1} ν_{max} (neat): 2924, 1707, 1623, 1514, 1462, 1222, 1025, 822. HRMS 291.1154 ($[M+H]^+$) (calculated for $C_{16}H_{18}O_5$, 291.1154). ^{1}H NMR ($CDCl_3$): δ 6.91 (1H, *s*, H-10), 6.90 (1H, *d*, *J* = 7.8, 1.6 Hz, H-14), 6.79 (1H, *d*, *J* = 8.0 Hz, H-13), 6.62 (1H, *d*, *J* = 15.9 Hz, 8-H), 6.13 (1H, *dd*, *J* = 15.9, 6.6 Hz, H-7), 5.15 (1H, *d*, *J* = 0.7 Hz, H-3), 5.00 (1H, *m*, H-6), 3.89 (3H, *s*, H-17), 3.87 (3H, *s*, H-16), 3.76 (3H, *s*, H-15), 2.66 (1H, *ddd*, *J* = 17.1, 11.0, 0.8 Hz, H-5), 2.54 (1H, *dd*, *J* = 17.0, 4.2 Hz, H-5).

*3.1.9. Tetrahydroyangonin (**8**) (Franca et al., 1973)*

Yellow semi-solid (0.015%), ^{1}H NMR ($CDCl_3$): δ 7.12 (2H, *d*, *J* = 8.5, H-10 and H-14), 6.83 (2H, *d*, *J* = 8.4 Hz, H-11 and H-13), 5.14 (1H, *s*, H-3), 4.35 (1H, *m*, H-6), 3.78 (3H, *s*, H-17), 3.72 (3H, *s*, H-15), 2.81 (1H, *m*, H-8), 2.72 (1H, *m*, H-8), 2.50 (1H, *dd*, *J* = 16.9, 12.2 Hz, H-5), 2.29 (1H, *dd*, *J* = 17.0, 3.7 Hz, H-5), 2.09 (1H, *m*, H-7), 1.89 (1H, *m*, H-7).

*3.1.10. 11-Methoxytetrahydroyangonin (**9**)*

White solid (0.011%), mp 121–123 °C (lit. 121–123) (Franca et al., 1973). $[\alpha]_D^{25}$: +27.7° ($CHCl_3$; *c* 0.011). ^{1}H NMR ($CDCl_3$): δ 6.76 (1H, *d*, *J* = 8.6 Hz, H-13), 6.71 (1H, *s*, H-10), 6.70 (1H, *d*, *J* = 8.6 Hz, H-14), 5.09 (1H, *d*, *J* = 0.7 Hz, H-3), 4.31(1H, *m*, H-6), 3.82 (3H, *s*, H-16), 3.81 (3H, *s*, H-17), 3.68 (3H, *s*, H-15), 2.78 (1H, *m*, H-8), 2.68 (1H, *m*, H-8), 2.46 (1H, *dd*, *J* = 17.1, 12.2 Hz, H-5), 2.26 (1H, *dd*, *J* = 17.0, 3.7 Hz, H-5), 2.06 (1H, *m*, H-7), 1.87 (1H, *m*, H-7).

*3.1.11. Flavokawain A (**10**)*

Yellow crystalline compound (0.46%), mp 111–115 °C (lit. 116) (Jössang and Molho, 1967). ^{1}H-NMR ($CDCl_3$): δ 14.40 (1H, *s*, HO-2′), 7.79 (2H, *s*, H-α and H-β), 7.56 (2H, *d*, *J* = 8.8 Hz, H-2 and H-6), 6.92 (2H, *d*, *J* = 8.7 Hz, H-3 and H-5), 6.09 (1H, *d*, *J* = 2.3 Hz, H-3′), 5.95 (1H, *d*, *J* = 2.3 Hz, H-5′), 3.91 (3H, s, 6′-OCH_3), 3.84 (3H, *s*,-OCH_3), 3.82 (3H, *s*, 4′-OCH_3)

*3.1.12. Flavokawain B (**11**)*

Yellow crystalline compound (0.015%), mp 96–98 °C (lit. 92) (Jössang and Molho, 1967). ^{1}H NMR ($CDCl_3$): δ 14.29 (1H, s, HO-2′), 7.90 (1H, *d*, *J* = 15.6 Hz, H-β), 7.78 (1H, *d*, *J* = 15.6 Hz, H-α), 7.62–7.59 (2H, *m*, H-2 and H-6), 7.44–7.39 (3H, *m*, H-2, H-3 and H-5), 6.11 (1H, *J* = 2.3 Hz, H-3′), 5.96 (1H, *d*, *J* = 2.3 Hz, H-5′), 3.91 (3H, *s*, 6′-OCH_3), 3.80 (3H, *s*, 4′-OCH_3)

*3.1.13. Flavokawain C (**12**)*

Yellow crystalline compound (0.012%), mp 185–188 °C (lit. 194–195) (Seeram et al., 1996). ^{1}H NMR (CD_3OD):δ 7.72 (1H, *d*, *J* = 16.2 Hz, H-β), 7.65 (1H, *d*,

J = 15.9 Hz, H-α), 7.44 (2H, *d*, J = 8.6 Hz, H-2 and H-6), 6.82 (2H, *d*,J = 8.6 Hz, H-3 and H-5), 6.02 (1H, *d*, J = 2.3 Hz, H-3′), 5.94 (1H, *d*, J = 2.2 Hz, H-5′), 3.86 (3H, *s*, 6′-OCH_3), 3.77 (3H, *s*, 4′-OMe)

*3.1.14. 3,4-Methylenedioxycinnamylideneacetone (**13**) (Jössang and Molho, 1967)*

Semi solid (0.018%). ^{1}H NMR ($CDCl_3$):δ 7.18 (1H, *dd*, J = 15.4, 10.8 Hz, H-3′), 6.92 (1H, *d*, J = 1.7 Hz, H-2), 6.85 (1H, *dd*, J = 8.0, 1.4 Hz, H-6), 6.77 (1H, *d*, J = 15.5 Hz, H-1′), 6.72 (1H, *d*, J = 8.0 Hz, H-5), 6.62 (1H, *dd*, J = 15.5, 10.8 Hz, H-2′), 6.13 (1H, *d*, J = 15.5 Hz, H-4′), 5.91 (2H, *s*, O–CH_2–O), 2.23 (3H, *s*, H-6′). ^{13}H NMR ($CDCl_3$): δ 27.6 (C-6′), 101.8 (O–CH_2–O), 106.2 (C-2), 108.9 (C-5), 123.5 (C-6), 125.2 (C-2′), 130.1 (C-4′), 130.9 (C-1), 141.3(C-1′), 143.9 (C-3′), 148.7 (C-3), 149.1 (C-4), 198.5 (C-5′).

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