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Chemical Constituents of *Desmos dunalii* (Hk. f. et. Th.) Safford

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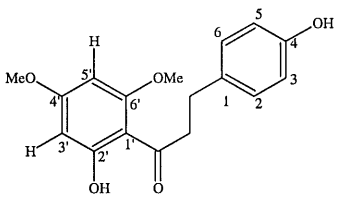
ABSTRACT The leaves of *Desmos dunalii* were investigated for their chemical constituents. Four dihydrochalcones were isolated, namely, 2', 4-dihydroxy-4', 6'-dimethoxydihydrochalcone **1**, 2', 4-dihydroxy-3', 6'-dimethyl-4', 5'-dimethoxydihydrochalcone **2**, 2', 4-dihydroxy-4', 5', 6'-trimethoxydihydrochalcone **3** and 2', 4'-dihydroxy-5'-methyl-4', 6'-dimethoxydihydrochalcone **4**. The structures of these compounds were determined by spectral analysis: ^{1}H NMR, ^{13}C NMR, 2D NMR and MS.

INTRODUCTION

The phytochemical studies on the chemical constituents of tropical plants remain the main interest to many organic chemists in order to discover new compounds and study the relationship between the chemical constituents. Despite the discovery of interesting molecules, more and more new entities are studied for their biological activities and the search for new and potential drugs is still active. Scientifically, some researches have been done on several *Desmos* spp. containing biologically active compounds.

From *Desmos chinensis*, an active substances known as 8-formyl-2,5,7-trihydroxyl-6-methylflavanone, **5** was found to be the tyrosine kinase inhibitor [1]. Also, *Desmos londiflorus* showed antifungal and antibacterial properties [2]. While from *Desmos cochichinensis*, a cytotoxic fatty acid, **6** (known as desmosic acid) had been isolated [3]. These showed that some *Desmos* spp. contain active substances and further research need to be done on them.



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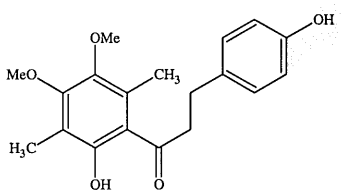

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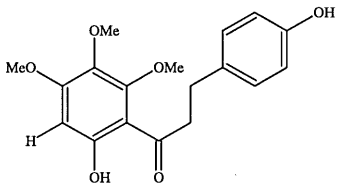

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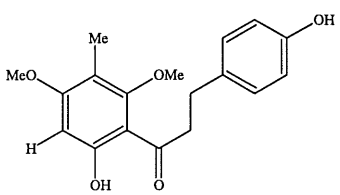

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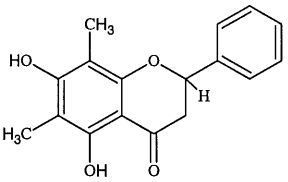

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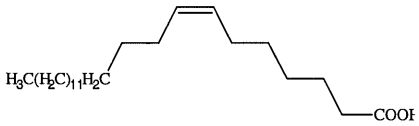

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EXPERIMENTAL

Plant material

The leaves of *Desmos dunalli* (KL 4668) was collected from Segari, Pantai Remis, Perak was studied with herbarium number KL 4668 by the Phytochemical group of Chemistry Department, University of Malaya and at Forest Research Institute of Malaysia (FRIM), Kepong, Malaysia.

Extraction

1.6 kg dried ground leaves of the plant was defatted with petroleum ether for 24 hours. The leaves were then dried under room temperature, wetted with 10% ammonia and left overnight. The leaves were then exhaustively reextracted with dichloromethane successively using the Soxhlet extractor for about 17 hours. The crude extract was then concentrated under reduced pressure using the rotary-evaporator to a volume of about 500 ml.

The concentrated crude extract was repeatedly extracted with 5% HCl. The dichloromethane layer was then evaporated under pressure to dryness. The crude extract (neutral part) thus obtained was a dark gummy residue.

Isolation

5 g of crude extract was subjected to column chromatography over silica gel in dichloromethane with increasing amounts of methanol. The collected fractions were then purified using extensive column chromatography and monitored by thin layer chromatography (TLC).

RESULT AND DISCUSSIONS

2',4-dihydroxy-4',6'-dimethoxydihydro-chalcone

2',4-dihydroxy-4',6'-dimethoxydihydrochalcone was obtained as a yellow amorphous mass and was identified as dihydrochalcone from the comparison and analysis data. The ^{1}H NMR spectrum of compound **1** showed two triplets at δ 2.83 and δ 3.18, which are typical of a dihydrochalcone moiety. It also showed two doublets δ 6.69 (J=8.3 Hz, 2H) and δ 7.01 (J=8.5 Hz, 2H) of a *p*-disubstituted aromatic ring and two doublet at δ 5.99 and δ 5.84 with 2.5 Hz coupling constant due to the meta coupling of the benzene ring. The ^{1}H NMR spectrum also showed the presence of two methoxy groups at δ 3.72 and δ 3.74. One singlet appeared at the low field region and was identified to be a hydroxyl peak from the HMQC spectrum. The COSY spectrum, showed the correlation between H3/5 and H2/6. Furthermore, a correlation between Hα and Hβ was also displayed.

The mass spectral analysis gave a molecular mass of *m/z* 302 which corresponds to $C_{17}H_{18}O_5$. The number of carbons was then confirmed *via* ^{13}C NMR. The ^{13}C NMR spectrum showed four methine carbons at δ 90.9, δ 93.6 (ring A) and δ 115.2 (2xCH) and δ 129.4 (2xCH) (ring B). This identification has been confirmed by 2D NMR (DEPT, HMBC and HMQC). The spectrum also showed a carbonyl carbon at δ 204.8 and two carbons for methoxy groups at δ 55.5 and δ 55.6 respectively.

The placement of methoxy groups and hydroxyl group to ring A and ring B were confirmed by the mass spectral fragmentation. The positions of the methoxy groups and one of the hydroxyl groups in ring A was confirmed through the intense peak at *m/z* 181, the fragmentation of the ring B with the hydroxyl group located at C-4 and analysis of mass spectra which give a peak at *m/z* 107. The analysis were supported by the data from NOESY and HMBC spectrum.

As a result, this compound are confirmed to be 2',4-dihydroxyl-4',6'-dimethoxydihydrochalcone [4, 5, 6]. Table 1 summarized the data from ^{1}H NMR and ^{13}C NMR experiments of compound **1**.

2',4-dihydroxy-4',5'-dimethoxy-3',6'-dimethyldihydrochalcone

2',4-dihydroxy-4',5'-dimethoxy-3',6'-dimethyl-dihydrochalcone was isolated as an amorphous mass. From the ^{1}H NMR spectrum, the signals exhibited significant peaks for the dihydrochalcone skeleton at δ 3.37 (2H, t) and δ 2.93 (2H, t). The spectrum also showed two doublets at δ 7.07 (2H, J = 8.1 Hz) and δ 6.74 (2H, J = 8.6 Hz) indicating a *p*-disubstituted aromatic ring. In the high field region, peaks for four methyls [δ 2.12 (6H, s)] and δ 3.66 (3H, s) and δ 3.72 (3H, s)] were observed. In addition, in the low field region, one singlet displayed at δ 13.04 indicated a hydroxyl at C-2'.

The placement of methyl and methoxy group in the structure was confirmed by using 2D NMR (HMBC and HMQC) and supported by mass spectral fragmentation. From the mass spectrum, the base peak at *m/z* 313 agreed with the molecular formula $C_{19}H_{21}O_4$. The spectrum also showed an intense peak at *m/z* 209 and *m/z* 107 which corresponding to ring A and ring B fragmentation respectively.

In the ^{13}C NMR spectrum, nineteen carbons were observed where two methine carbons [δ 115.25 and δ 129.5] overlapped because of *p*-substituted aromatic ring. The spectrum also showed one peak in the low field region at δ 206.29 which corresponded to the carbonyl carbon. Two methylenes (δ 29.3 and δ 44.9), two methoxys (61.7 and 60.0), and two methyls (δ 8.62 and δ 9.11) were also observed. The data given was supported by the DEPT experiment.

Finally the NOESY spectrum was used to determine the structure of the compound. There was observation of the CH_3O (4') and CH_3O (5') displaying contour with the H3/5 and H2/6. The spectrum also showed nOe contours between H-2/H-6, H-3/H-5 and H-α, H-β and methyl protons. Observation on H-α displayed contours with the H-β. Spectra also showed the contours between H2/6 and H3/5. These data led to the structure of the new compound named as 2', 4-dihydroxyl-4',5'-dimethoxy-3',6'-dimethyldihydrochalcone. Table 1 summarized the data from ^{1}H NMR and ^{13}C NMR experiments of compound **2**.

2,4-dihydroxy-4',5',6-trimethoxydihydrochalcone

The ^{1}H NMR spectrum displayed methylene protons signals centered at δ 2.91 (2H, t) and δ 3.29 (2H, t), one proton singlet at δ 6.22, three aromatic methoxy groups at δ 3.83, 3.76 and 3.93. It also showed four aromatic protons appeared as a pair of doublet at δ 6.76 and 7.07 (each 2H, d, J = 8.3 Hz) representing an A_2B_2 system of *p*-disubstituted benzene derivative.

The spectrum also displayed a sharp singlet proton δ 13.48 attributable to a hydrogen bonded phenolic, OH. The identification of H-3 in ring A was determined by nOe-difference spectra. Irradiation of H-3' signal showed a strong enhancement of the methoxy group and hydroxyl group, thus indicating that there are the methoxy and hydroxyl group next to the H-3'.

The distribution of the hydroxyl and methoxy groups in the two benzene rings were confirmed by the mass spectral fragmentation. From the mass spectrum, the base peak at *m/z* 332 indicate a molecular formula, $C_{18}H_{20}O_6$. The spectrum also showed an intense peak at *m/z* 211 and *m/z* 107 due to the fragmentation at C-α, supporting the presence of methoxy and hydroxyl groups in ring A and the presence of hydroxyl in ring B.

The ^{13}C NMR data indicated 18 signals for carbon which was consistent with five oxygenated carbons at δ 134.8, 159.8, 155.0, 154.0 and 161.6. The spectrum also showed five aromatic protons, one in ring A (δ 96.1) and another four in ring B (δ 129.4) (2xCH) and δ 115.3 (2xCH). The presence of two methylene carbons and a carbonyl carbon at δ 29.6, 45.0 and 205.1 respectively which was correlated with each other in COSY and HMBC suggested this compound is to be a dihydrochalcone. The structure of this compound was deduced from the correlation of COSY, HMBC and HMQC. Detailed analysis of the spectral data obtained led to the conclusion that compound is 2,4-dihydroxyl-4', 5', 6-trimethoxydihydrochalcone. Table 1 summarized the data from ^{1}H NMR and ^{13}C NMR experiments of compound **3**.

2', 4-dihydroxy-5'-methyl-4', 6'-dimethoxydihydrochalcone

2',4-dihydroxy-5'-methyl-4',6'-dimethoxydihydrochalcone was identified as a dihydrochalcone from the analysis of ^{1}H NMR, ^{13}C NMR and 2D NMR spectral.

The ^{1}H NMR spectrum gave three peaks for aromatic protons typical of *p*-substituted aromatic ring [δ 6.22 (1H, s), δ 7.08 (2H, d) and δ 6.75 (2H, d)], two methoxy groups [δ 3.81 (3H, s)] and δ 3.66 (3H, s)] and one phenolic hydroxyl group in ring A appear at δ 13.36, (1H, s). The spectrum also showed the presence of a methyl group at δ 2.03 (3H, s) and methylene protons at δ 3.34 (2H, t, J = 7.3 Hz), δ 2.93 (2H, t, J = 7.6 Hz). Another phenolic hydroxyl group is present at ring B and was confirmed by mass spectral.

The ^{13}C NMR spectrum showed resonances for ketone (C=O) at δ 205.1, two methoxy groups at δ 55.8 and δ 61.6 and two coupled deshielded methylene groups at δ C-α = 44.5 and δ C-β = 29.9. Moreover, the spectrum also displayed twelve aromatic carbons where five of them were unsubstituted [δ_c 108.7 (C-1'), 164.2 (C-2'), 95.8 (C-3'), 164.5 (C-4'), 160.5 (C-5'), 111.5 (C-6'), 129.5 (C-2/C-6), 115.3 (C-3/C-5), 153.9 (C-4- attached to the phenolic hydroxyl group) and δ 133.5 (C-1).

The substitution pattern of ring A was established by the nOe-diff and HMBC spectra, especially by the HMBC correlations of 2-OH and C-1/C-2/ C-3. 'An irradiation of H3 at δ 6.22 enhanced the methoxy group at C-4' and irradiation of the methyl group at δ 2.03 (C-6') enhanced another methoxy group at C-5'. Moreover, the irradiation of H-2/H-6 at ring B, give an enhancement of H-3/H-5, providing evidence of the *p*-substituted type ring B.

The placement of the methoxy groups, methyl and hydroxyl groups were confirmed using the data from mass spectral analysis. From the mass spectrum of this compound, the base peak was observed at *m/z* 316 which was consistent with a molecular formula $C_{18}H_{20}O_5$.

The spectrum also showed an intense ion at *m/z* 195 ($C_{10}H_{11}O_4$) and *m/z* 107 (C_7H_7O) due to the ring A and ring B respectively. In addition, the spectrum also showed the presence of small peak at m/z 121 for C_8H_9O ion. Detailed analysis of the spectral data obtained led to the conclusion that this compound is new and named as 2', 4-dihydroxy-5'-methyl-4', 6'-dimethoxydihydrochalcone. Table 1 summarized the data from ^{1}H NMR and ^{13}C NMR experiments of compound **4**.

Table 1. ^{1}H NMR and ^{13}C NMR data for compound 2', 4-dihydroxy-4, 6-dimethoxydihydrochalcone **1**, 2', 4-dihydroxy-4', 5'-dimethoxy-3', 6'-dimethyldihydrochalcone **2**, 2, 4-dihydroxy-4', 5', 6-trimethoxydihydrochalcone **3** and 2', 4-dihydroxy-4', 5', 6'-trimethoxydihydrochalcone **4** ($CDCl_3$, 400 MHz)

Position	δ_H (ppm) (*J in Hz*)	δ_C (ppm)	δ_H (ppm) (*J in Hz*)	δ_C (ppm)	δ_H (pm) (*J in Hz*)	δ_C (ppm)	7,5	δ_C (ppm)
1'	-	105.7	-	11.5	-	108.1	-	108.7
2'	-	167.54	13.04, s (OH)	160.9	13.48, s (OH)	161.6	13.36, s	164.2
3'	5.99, d (2.2)	93.6	-	115.6	6.22, 1H, s	96.1	6.22, 1H, s	95.8
4'	-	166	-	158.8	-	134.6	-	164.5
5'	5.84, d (2.5)	90.9	-	163.4	-	159.8	-	111.5
6'	-	153.9	-	115.4	-	155	-	160.5
1	-	133.5	-	133.2	-	133	-	133.5
6-Feb	7.01, 2H, d (8.3)	129.4	7.07, 2H, d (8.1 Hz)	129.5	7.07, 2H, d (8.3)	129.4	7.08, 2H, d (8.3)	129.5
5-Mar	6.69, 2H, d, (8.5)	115.3	6.74, 2H, d (8.6 Hz)	115.3	6.76, 2H, d (8.3)	115.3	6.75, 2H, d (8.3)	115.3
4	-	153.9	-	154	-	154	-	153.9
□	3.18, 2H, t	46	3.37, 2H, t	44.9	3.29, 2H, t	45	3.34, 2H, t	44.5
□	2.83, 2H, t	29.9	2.93, 2H, t	29.3	2.91, 2H, t	29.6	2.93, 2H, t	29.9
C=O	-	204.8	-	206.3	-	205.1	-	205.1
OCH_3	3.72 and 3.74 (each give 3H, s)	55.5 and 55.6	3.66, 3H, s; 3.72, 3H, s	61.7, 60.0	3.83, 3.76 and 3.93 (each give 3H, s)	55.9, 60.9 and 61.1	3.81 and 3.66 (each showed 3H, s)	55.8 and 61.6
CH_3			2.12, 6H, s	8.6 and 9.1			2.03, 3H, s	8.57

CONCLUSION

The leaves of *Desmos dunalli* (Hk. f. et. Th.) Safford were fully studied for their chemical constituents. Four dihydrochalcone were isolated: 2',4-dihydroxy-4',6'-dimethoxydihydrochalcone, 2',4-dihydroxy-3',6'-dimethyl-4',5'-dimethoxy-dihydrochalcone, 2',4-dihydroxy-4',5',6'-trimethoxydihydrochalcone and 2',4'-dihydroxy-5'–methyl-4',6'-dimethoxy-dihydrochalcone.

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