Bioactive Compounds from *Psorothamnus junceus*

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During a search for bioactive compounds from *Psorothamnus junceus*, four heterocyclic compounds, psorothamnone A (**1**), psorothamnone B (**2**), dalrubone (**3**), and emorydone (**4**) were isolated from the ethanol extract of the stem bark. Psorothamnones A (**1**) and B (**2**) demonstrated inhibitory activity against protein kinase C (PKC), a key enzyme involved in the signal transduction of cell proliferation and differentiation. Dalrubone (**3**) and emorydone (**4**) showed cytotoxicity against several human tumor cell lines.

Psorothamnus (Fabaceae), also known as *Dalea* or *Parosela,* is a genus of approximately 150 species.[1,2] Plants in this genus are annual or perennial herbs or shrubs. They are characterized by sunken glands on the calyx and the leaves.[1,2] In North America, they are distributed mostly in the desert regions of the southwestern United States and Mexico. Native American tribes in these regions have used *P. polyadenia* and *P. emoryi* to dye deer skins and to color baskets.[3] *P. polyadenia* was also used by Native Americans to treat numerous ailments, such as smallpox, pneumonia, tuberculosis, and influenza.[3] *P. formosa* is reputed to relieve pain and to treat influenza and viral infections.[4] Several species in the genus have been studied chemically.[5−13] Simple coumarins and pigments such as dalrubone, methoxydalrubone, and emorydone have been isolated from *P. emoryi*.[5,6] Similar chemical constituents have also been reported for *P. tinctoria* and *P. polyadenia*.[7]

Psorothamnus junceus Rydb. (syn. *Dalea juncea*) is a shrub that grows in the sandy washes and rocky slopes of the eastern side of the Sierra San Padro Martir, BC, Mexico.[1] It differs from other species by its unequal pilose carlyx-lobes and the straggling, glabrous stems. No phytochemical study of this species was reported prior to our recent investigation of protein kinase C (PKC) inhibitors.[14] In that study, a new heterocyclic compound, psorothamnone A (**1**), was isolated as a novel PKC inhibitor. Its structure was elucidated by NMR spectroscopic methods and X-ray crystallography.[14] This was the first report of this highly conjugated, four-ring heterocyclic structure.

PKC has recently received a lot of attention as a potential antitumor target. It is a calcium-activated, phospholipid-dependent enzyme, involved in cellular signaling pathways that regulate cellular proliferation and differentiation.[15] Pharmacologic activation of PKC results in abnormal cellular growth. It is, therefore, believed that PKC inhibitors may have potential for controlling the growth of cancer cells.[16−19] As part of our search for natural antitumor compounds from higher plants, we now report the isolation of another new PKC inhibitor, psorothamnone B (**2**), and two cytotoxic compounds with primarily known structures, dalrubone (**3**) and emorydone (**4**). (See Chart 1.)

Results and Discussion

A new compound, psorothamnone B (**2**), was isolated from the ethanol extract of the stem bark of *P. junceus* as yellow needles. HRCIMS gave $[M + H]^+$ at *m/z* 295.0923, corresponding to the molecular formula $C_{18}H_{15}O_4$ (calcd for 295.0966). UV absorptions at 452, 427, 333, and 227 nm were characteristic of an extended conjugation system. No hydroxyl functionality was observed in the IR spectrum, although absorptions at 1677, 1656, 1615, 1574, and 1569 cm^{-1} suggested the presence of an aromatic ketone system. EIMS showed the peaks at *m/z* 251 and 223 for the consecutive loss of two carbonyl groups from the M-15 peak (*m/z* 279), suggestive of the presence of two carbonyl groups.

All above spectral data indicated that compound **2** was a closely related analogue of psorothamnone A (**1**). The most distinct difference in the ^{13}C NMR spectra of psorothamnones A and B is the absence of the acetyl signals (δ_c 194.6 and 32.9) in the spectrum of psorothamnone B (**2**). This structural difference is corroborated by the absence of an acetyl proton peak (δ 2.82) and the appearance of a new aromatic proton peak at δ 6.87 (singlet) (Table 2). To determine the location of this aromatic proton, both COSY and NOESY experiments were performed. The NOESY and COSY spectra showed the correlation between this aromatic proton and H-5. A long-range, five-bond coupling between H-4 and H-8 was also observed. Therefore, psorothamnone B was determined as 4-deacetylpsorothamnone A (**2**).

In addition to psorothamnones A (**1**) and B (**2**), compounds **3** and **4** were also isolated by tracing cytotoxic activity against A-549 (lung), MCF-7 (breast), and HT-29 (colon) human tumor cell lines. The ^1H NMR and ^{13}C NMR spectra of **3** were similar to those of dalrubone (**3**), but several variations in the chemical shift analysis have been noticed.[5] To elucidate the structure and assign the chemical shifts of dalrubone (**3**) unequivocally, a NOESY experiment was performed. The doublet at δ 7.67 correlated with the doublet at δ 7.16, and the later doublet also correlated with the H-5 aromatic proton at δ 7.30. The two doublets at δ 7.67 and 7.16 were, therefore, assigned as H-3 and H-4, respectively. The coupling between H-4 and H-5 was very small or undetectable in the COSY spectrum because of their four-bond syn-relationship. In addition, the NOESY spectrum showed no correlation between H-3 and the methoxyl group. However, a distinct NOE cross-peak was observed between H-8 and the methoxyl group. The downfield chemical shift of H-3 (δ 7.67) may be induced by an anisotropic effect of the C-2′ carbonyl group nearby. These spectral data supported the structure originally deduced by Dreyer's group,[5] and an alternative structure (**3a**) for dalrubone can thus be excluded. Detailed HMBC spectral analysis further verified the long-range structural correla-

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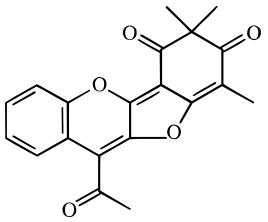
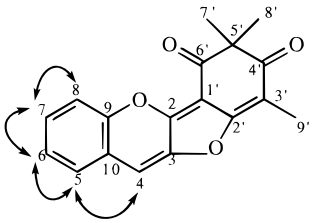
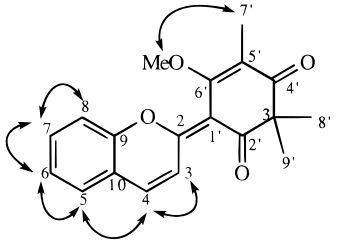
Chart 1



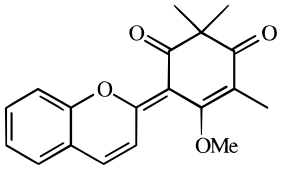
Psorothamnone A (**1**)



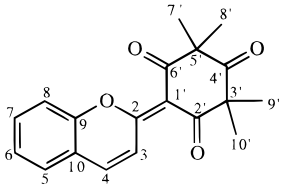
Psorothamnone B (**2**)
(arrows indicate NOE interactions)



Dalrubone (**3**)
(arrows indicate NOE interactions)



3a



Emorydone (**4**)

Table 1. Bioactivities of Psorothamnones A (**1**) and B (**2**), Dalrubone (**3**), and Emorydone (**4**)

compound	IC_{50}[a] (μg/mL)	GI_{50}[d] (μg/mL)				
	PKC[b]	A-549	MCF-7	HT-29	SK-MEL-5	Malme-3M
1	14	4×10^1	6×10^1	5×10^1	2×10^1	6×10^1
2	12	3×10^1	4×10^1	3×10^1	4×10^1	4×10^1
3	NT[c]	2×10^{-1}	1×10^0	2×10^{-2}	4×10^{-1}	2×10^0
4	NT[c]	3×10^0	3×10^0	4×10^0	3×10^0	1×10^0
adriamycin		4×10^{-2}	2×10^{-1}	6×10^{-2}	3×10^{-2}	1×10^{-1}

[a] IC_{50} of standard inhibitors: W-7·HCl [N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride]: 60 ± 30 μg/mL; staurosporin: 5 ± 3 ng/mL. [b] Protein kinase C. [c] Not tested. [d] Concentration for 50% of growth inhibition.

tions (Table 3). The COSY, NOESY, HMQC, and HMBC data analyses allowed us to complete the spectral assignments of the ^{1}H and ^{13}C NMR data of **3**.

The spectral properties (UV, IR, MS, and NMR) of **4** were consistent with those reported for emorydone.[6,22] However, the ^{1}H NMR spectrum was not fully interpreted in the literature. With the aid of ^{13}C NMR, COSY, HMQC, and HMBC experiments, the proton spectral data were thus analyzed unambiguously (Table 4).

Compounds **1**−**4** were tested for their cytotoxicity against five human tumor cell lines (Table 1). Dalrubone (**3**) exhibited significant cytotoxicity against A-549 (lung), HT-29 (colon), and SK-MEL-5 (melanoma, metastasis of the axillary node) and marginal activity against MCF-7 (breast) and Malme-3M (melanoma, metastasis to lung). In addition, dalrubone (**3**) also showed moderate selective cytotoxicity against HT-29 (colon), with a GI_{50} value comparable to that of Adriamycin. Emorydone (**4**) demonstrated only marginal cytotoxicity. Psorothamnones A (**1**) and B (**2**) were inactive against cell lines tested. However, they demonstrated moderate inhibitory activity against protein kinase C. These heterocyclic natural products may serve as lead compounds for synthesis of more potent PKC inhibitors.

All compounds appear to be closely related to flavonoids that have a $C_6-C_3-C_6$ skeleton. Dreyer et al.[5] postulated a pathway for the biogenetic formation of dalrubone (**3**) and emorydone (**4**). The cyclohexadione/cyclohexatrione moiety originates from the polyketide portion. We propose an alternative biogenetic pathway, in which the cyclohexadione/cyclohexatrione moiety of the flavonoid pigments derives from the phenyl ring of phenylalanine (Scheme 1). The metabolism of 2,4′-dihydroxychalcone could lead to 4′-hydroxyflavanone, which is then converted to 4′-hydroxyflavone. The B ring of the latter could be further hydroxylated, and the 4-keto group reduced and dehydrated, resulting in a flavylium salt (**5**). This part of the biosynthetic pathway has been previously established for the

Table 2. NMR Data of Psorothamnones A (**1**) and B (**2**) in $CDCl_3$[a]

	1	**2**	
position	δ_C	δ_C J (Hz)	δ_H J (Hz)
2	151.9	152.7 d (5.7)	
3	150.0	148.8 d (4.4)	
4	113.4	106.2 dd (172.0, 5.8)	6.87 s
5	127.0	127.9 ddd (163.0, 8.3, 4.3)	7.44 ddd (8.1, 1.7, 0.7)
6	126.4	126.1 dd (165.0, 7.9)	7.33 ddd (8.1, 7.3, 1.4)
7	129.8	129.6 dd (165.0, 8.9)	7.45 ddd (8.2, 7.3, 1.7)
8	117.5	117.4 dd (164.3, 7.9)	7.53 ddd (8.2, 1.4, 0.7)
9	150.0	150.1 m	
10	117.8	120.8 dd (8.7, 4.6)	
1′	103.0	101.6 s	
2′	165.8	167.1 q (4.8)	
3′	107.2	104.5 q (6.5)	
4′	199.3	199.3 m	
5′	56.8	56.5 h (3.8)	
6′	193.9	194.6 m	
7′	23.7	23.9 qq (130.7, 4.9)	1.39 s
8′	23.7	23.9 qq (130.7, 4.9)	1.39 s
9′	8.6	8.1 q (129.4)	1.99 s
Ac (C=O)	194.6		
Ac (CH_3)	32.9		

[a] Chemical shifts and coupling constants for H-5−H-8 were determined by spin simulation.

Table 3. NMR Data of Dalrubone (**3**) in $CDCl_3$

position	δ_C	δ_C (lit.[5])	δ_H J (Hz)	HMBC correlations (J = 8 Hz)
2	156.5	166.4		
3	119.7	116.0	7.67 d (10)	C-2, C-10
4	134.1	133.7	7.16 d (10)	C-2,C-5,C-9,C-10
5	127.7	119.1	7.30 dd (7.6, 1.6)	C-4,C-7,C-9
6	125.2	124.7	7.20 ddd (7.6, 7.5, 1.1)	C-8,C-10
7	131.9	127.2	7.42 ddd (8.4, 7.5, 1.6)	C-5,C-8,C-9
8	116.6	131.4	7.24 dd (8.4, 1.1)	C-6,C-9,C-10
9	152.5	152.5		
10	121.1	117.8		
1′	105.3	120.6		
2′	202.0	201.7 (199.4)		
3′	57.4	57.4		
4′	199.4	199.4 (201.7)		
5′	118.5	105.2		
6′	166.3	156.6		
7′	8.9	9.0	1.93 s	C-4′,C-5′,C-6′
8′	22.9	22.9	1.26 s	C-2′,C-3′,C-4′,C-9′
9′	22.9	22.9	1.26 s	C-2′,C-3′,C-4′,C-8′
OCH_3	59.4	59.4	3.80 s	C-6′

biosynthesis of flavonoids and anthocyanins.[20,21] The next important intermediate derived from the flavylium salt is a quinone methide (**6**). A series of *O*-methylation and *C*-methylation on this quinoid may eventually lead to the formation of dalrubone (**3**) and emorydone (**4**). The chemical precedence of this biogenetic pathway has been elegantly illustrated by Roitman et al.[6] in the total synthesis of dalrubone (**3**) and emorydone (**4**) from the flavylium salt **5**, which was prepared from phloroglucinol and coumarin. Epoxidation of one of the quinoid intermediates (**7**, an isomer of desmethyldalrubone), followed by ring closure and dehydration may give rise to psorothamnone B (**2**), which could be converted to psorothamnone A (**1**) by further acetylation. Further biosynthetic experiments are needed to substantiate the above biogenetic proposition.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. UV−vis spectra were taken in methanol or ethanol on a Beckman DU-7 spectrophotometer. IR spectra were obtained in KBr on a Perkin-Elmer 1600 FT-IR spectrometer. NMR spectra were recorded on a Varian VXR-500S spectrometer. HRMS data were collected on a Kratos MS-50 spectrometer. LREIMS spectra were measured on a Finnigan 4000 spectrometer. Si gel 60 (Merck) and reversed-phase C_{18} Si gel (LRP-2, Whatman) were used for column chromatography.

Table 4. NMR Data of Emorydone (**4**) in $CDCl_3$

position	δ_C	δ_H J (Hz)	HMBC correlations (J = 7 Hz)
2	167.1		
3	119.8	8.33 d (9.9)	C-2,C-10
4	140.3	7.71 d (9.9)	C-2,C-5,C-8,C-9
5	127.8	7.51 dd (8.1, 1.4)	C-4,C-7,C-9
6	126.1	7.38 ddd (8.1, 6.8, 1.4)	C-8,C-10
7	133.2	7.63 ddd (8.2, 6.8, 1.4)	C-5,C-9
8	118.0	7.59 dd (8.2, 1.4)	C-6,C-9,C-10
9	152.7		
10	121.0		
1′	108.7		
2′	198.0[a]		
3′	58.3[b]		
4′	211.0		
5′	58.4[b]		
6′	198.1[a]		
7′	23.3	1.37 s	C-4′,C-5′,C-6′,C-8′
8′	23.3	1.37 s	C-4′,C-5′,C-6′,C-7′
9′	23.3	1.37 s	C-2′,C-3′,C-4′,C-10′
10′	23.3	1.37 s	C-2′,C-3′,C-4′,C-9′

[a,b] Chemical shifts may be exchanged.

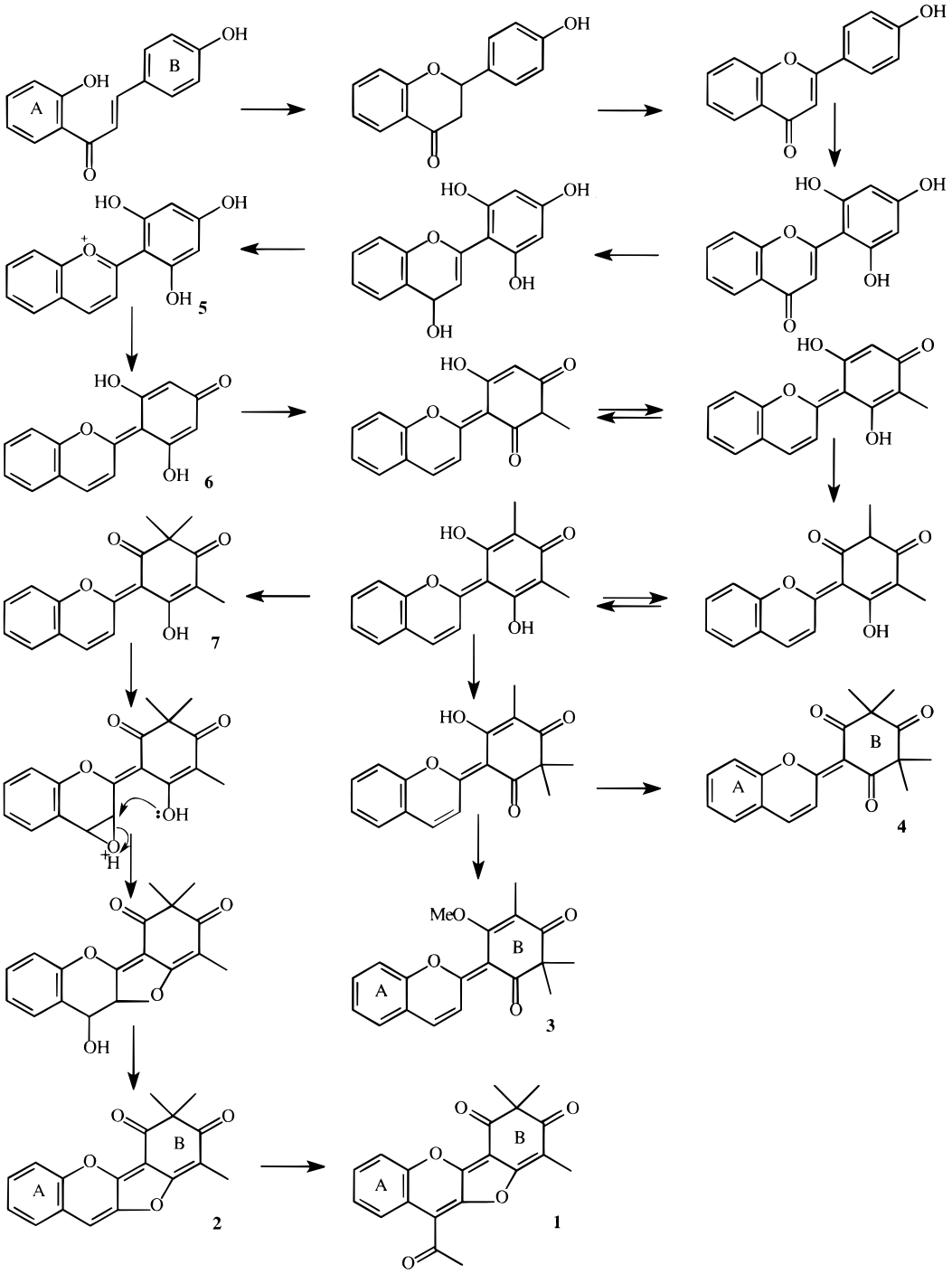
Plant Material. The stem bark of *P. junceus* was collected in the gulf coast desert between San Ignacio and Santa Rosalia, Baja California Sur, Mexico, in April 1990, by Drs. Richard Spjut and Richard Marion, World Botanical Associates (Laurel, MD). The herbarium specimen (WBA-01058) was deposited at the Smithsonian Institution.

Cytotoxicity Assays. Cytotoxicity tests against human cancer cells were performed at the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard MTT protocols with Adriamycin as a positive standard control.[23] The A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), HT-29 (human colon adenocarcinoma), SK-MEL-5 (human melanoma, metastasis to the axillary node), and Malme-3M (human melanoma, metastasis to lung) tumor cell lines were used in this bioassay. Generally, cytotoxicity GI_{50} values less than 10 μg/mL for crude extracts and less than 1 μg/mL for pure compounds are considered active.

PKC Assays. PKC proteins used in this assay were a mixture of PKCα and PKCβ_2, which are recombinant products isolated from insect cell lines.[24] Samples were dissolved in DMSO, and 2 μL of each solution were delivered to the wells. PKC was added in 30 μL of a solution that contained Tris buffer (20 mM, pH 7.4), phosphatidylserine (5 μg), 12-*O*-tetradecanoylphorbol 13-acetate (0.3085 μg), bovine serum album (10 μg), leupeptin (0.5 μg), $CaCl_2$ (5 nmol), and $MgCl_2$ (0.2 μmol). The reactions were started by the addition of 20 μL of a mixture containing $MgCl_2$ (0.3 μmol), lysine-rich histone (12 μg), *p*-nitrophenyl phosphate (0.11 mg), ATP (1.0 nmol), and ATP [γ-^{32}P] (0.5 μCi). After a 30-min incubation at room temperature, the reactions were terminated by placing a 5-μL aliquot of the reaction mixture on to a piece of P81 phosphocellulose paper. These were washed in water, dried, and counted in a liquid scintillation counter.

Extraction and Isolation. Pulverized stem bark of *P. junceus* (2.6 kg) was extracted with ethanol (500 mL each) three times. The ethanol extract (91.8 g) was partitioned between CH_2Cl_2 and water to give two fractions. The CH_2Cl_2 portion (70.0 g) was further partitioned between hexane and 90% methanol (1:1) to give 90% methanol (47.0 g) and hexane portions (19.1 g). Further separations were guided by cytotoxicity against A-549, MCF-7, and HT-29 human tumor cell lines. Active fractions were pooled and subjected to further chromatography. The methanol residue was subjected to Si

Scheme 1. Proposed Biogenetic Pathway for the Biosynthesis of Psorothamnones A (**1**) and B (**2**), Dalrubone (**3**), and Emorydone (**4**)



gel column (600 g) chromatography, eluted sequentially with CH_2Cl_2–hexane (10:100), CH_2Cl_2–hexane (20:100), CH_2Cl_2, CH_2Cl_2–MeOH (100:5), CH_2Cl_2–MeOH (100:10), CH_2Cl_2–MeOH (100:20), CH_2Cl_2–MeOH (100:50), and MeOH to yield eight fractions (F1–F8). F2 (2.5 g) was separated using C_{18} Si gel (25 g) column chromatography eluted with MeOH–H_2O (2:1) under low pressure to generate four fractions. The third fraction (1.5 g) was further chromatographed with a Si gel column (150 g) eluted with hexane–ethyl acetate (6:1) to afford **3** as red crystals (1 g) and **4** as yellow crystals (5 mg). F3 (990 mg) was subjected to Si gel (9 g) column chromatographic separation and eluted with benzene–ethyl acetate (9:1) to yield four fractions. The third fraction (129 mg) yielded **1** as orange crystals (4 mg), after crystallization from ethyl acetate. The fourth fraction (480 mg) yielded **2** as yellow crystals (21 mg), after crystallization from ethyl acetate and hexane.

Psorothamnone A (1): orange crystals, mp 247–248 °C; ^{1}H NMR, HMQC, and HMBC data, see Li et al.[14] and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 2.

Psorothamnone B (2): yellow crystals, mp 252–254 °C; UV (MeOH) λ_{max} (log ϵ) 452 (4.43), 427 (4.41), 333 (4.04), 227 (4. 31) nm; IR (KBr) ν_{max} 1656 (w), 1677 (w), 1615 (vs), 1574 (s), 1569 (m) cm^{-1}; ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 2; EIMS *m/z* 294 [M$^+$] (35), 279 (37), 252 (40), 229 (25), 223 (13), 149 (60), 139 (57), 129 (65), 101 (100); HRCIMS *m/z* 295.0923 (calcd 295.0966 for $C_{18}H_{15}O_4$).

Dalrubone (3): red crystals, mp 98–100 °C (hexane/EtOAc) (lit.[5] mp 100.5–101.5 °C); UV (EtOH) λ_{max} (log ϵ) 426 (4.98) nm; IR (KBr) ν_{max} 1674 (w), 1624 (s), 1579 (s), 1558 (s), 1528 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), ^{13}C NMR ($CDCl_3$, 125 Hz) and HMBC data, see Table 3; EIMS *m/z* 310 [M$^+$] (100), 295 (30), 267 (63), 240 (22), 181 (55), 169 (47), 115 (39).

Emorydone (4): yellow crystals, mp 102 °C (hexane) (lit.[6] mp 88–89 °C); UV (EtOH) λ_{max} (log ϵ) 420 (4.83) nm; IR (KBr)

ν_{max} 1718 (w), 1672 (w), 1631 (s), 1513 (s), 1462 (s), 1441 (m), 1390 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), ^{13}C NMR ($CDCl_3$, 125 Hz) and HMBC data, see Table 4; EIMS *m*/*z* 310 [M^+] (46), 295 (14), 282 (7), 267 (12), 215 (14), 184 (20), 171 (83), 170 (100), 118 (64).

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