Drummondones A and B: Unique Abscisic Acid Catabolites Incorporating a Bicyclo[2.2.2]octane Ring System

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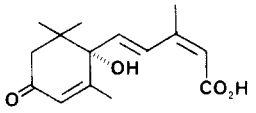
Structures of drummondone A and drummondone B have been determined and are shown to incorporate a unique bicyclo[2.2.2]octane ring system. Both compounds were isolated from *Sesbania drummondii* (Fabaceae) seed, along with drummondol and vomifoliol, and all of these closely related compounds appear to be catabolites of the important plant growth regulator abscisic acid. Structures were established by extensive ^1H and ^{13}C NMR studies and by mass spectrometry.

The isolation and characterization of abscisic acid (**1**), an important plant growth regulator,[1] was followed by the discovery of some of its catabolites—phaseic acid (**2**),[1,2] vomifoliol (**3**),[1] and drummondol (**4**).[3a] Compound **4** was found in close association with the sesbanimide series of antitumor alkaloids, isolated from seed of *Sesbania drummondii* (Rydb.) Cory (Fabaceae).[3] Along with vomifoliol (**3**) and drummondol (**4**), two related compounds were isolated whose structures were not apparent at the time of our paper reporting the structure of **4**.[3a] We now report the novel structures of these abscisic acid catabolites, drummondone A (**5**) and drummondone B (**6**).



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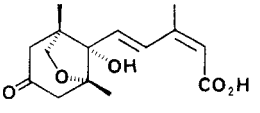

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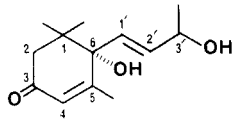

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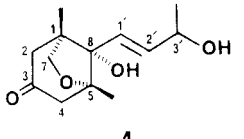

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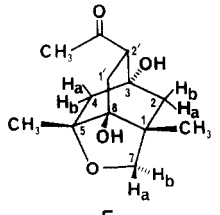

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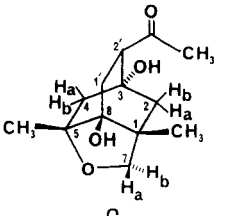

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Table I. NMR Chemical Shifts (δ) and Proton Couplings (J, Hz) for Drummondol (4), Drummondone A (5), and Drummondone B (6)[a]

proton assign	4	proton assign	5	6
		Chemical Shifts		
2a	2.60 dd	2a	1.87 dd	1.61 ddd
2b	2.35 d	2b	1.75 dddd	2.03 ddd
4a	2.61 s	4a	2.10 dd	1.85 ddd
4b	2.61 s	4b	1.69 dd	2.00 d
7a	3.88 dd	7a	3.67 ddd	3.70 ddd
7b	3.72 d	7b	3.51 dd	3.56 dd
1′	5.86 dd	1′a	1.96 dd	2.16 dd
		1′b	1.95 ddd	1.84 ddd
2′	6.20 dd	2′	2.84 dddd	2.76 dddd
3′	4.45 m			
1-CH_3	1.00 s	1-CH_3	1.06 s	1.05 s
5-CH_3	1.20 s	5-CH_3	1.21 s	1.26 s
3′-CH_3	1.33 d	3′-CH_3	2.20 s	2.23 s
		Proton Couplings		
2a,2b	18	2a,2b	13.0	13.0
2a,7a	2	2a,7a	0.5	0.5
		2a,2′		2.2
		2b,4a	3.2	3.2
		2b,7a	1.1	1.1
		2b,2′	0.5	
		4a,4b	13.9	13.9
		4a,2′		0.5
		4b,2′	2.2	
7a,7b	8	7a,7b	7.6	8.1
		7b,1′b	0.5	0.5
1′,2′	15	1′a,1′b	14.0	14.0
1′,3′	1	1′a,2′	14.3	6.5
2′,3′	6	1′b,2′	4.3	12.0
3′,3′-CH_3	6			

[a] Chemical shifts are reported relative to tetramethylsilane. Spectra were recorded at 300 MHz with $CDCl_3$ solutions.

Results and Discussion

After our work reported previously,[3] a new extraction of *S. drummondii* seed was undertaken, using a simplified procedure. The new procedure eliminated steps involving countercurrent distribution, partitioning with aqueous sodium carbonate, and chromatography on alumina. Appropriate applications of column chromatography, HPLC, and TLC gave the known compounds vomifoliol (**3**) and drummondol (**4**) as well as two new compounds, drummondone A (**5**) and drummondone B (**6**). Since the molecular ions of **4**–**6** were identical (m/z 240) and each exhibited 13 signals in the ^{13}C NMR, structural elucidation of **5** and **6** began with the assertion that all three com-

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Table II. ^{13}C NMR (δ) of Drummondol (4), Drummondone A (5), and Drummondone B (6)[a]

C assign	4	5	6
1	47.7 s	44.2 s	44.2 s
2	52.6 t	53.1 t	53.5 t
3	206.9 s	68.7 s	68.6 s
4	53.1 t	46.9 t	46.5 t
5	81.9 s	77.5 s	77.5 s
7	77.3 t	79.0 t	79.5 t
8	85.7 s	83.7 s	83.6 s
1′	124.1 d	26.3 t	25.8 t
2′	140.0 d	55.0 d	55.1 d
3′	68.0 d	210.6 s	210.6 s
1-CH_3	15.4 q	17.6 q	16.9 q
5-CH_3	18.9 q	19.7 q	20.7 q
3′-CH_3	24.2 q	31.3 q	31.1 q

[a] Chemical shifts are expressed relative to tetramethylsilane. Spectra were recorded in deuteriochloroform solution on a Bruker WM-300 spectrometer.

pounds were isomeric. In the ^{13}C NMR spectra of **5** and **6**, all of the chemical shifts are similar and they have the same multiplicities. Moreover, there is a close parallel in ^1H shifts and couplings observed for **5** and **6** although certain significant differences in long-range couplings are discernible (Tables I and II).

As shown in Table II, resonances of C-1, C-2, C-5, C-7, C-8, and methyl groups attached at C-1, C-5, and C-3′ in **4** were comparable to those for **5** and **6**, implying a similar tetrahydrofuran moiety. Compounds **4**–**6** all have one ketone function established by resonances at δ 206.9 for **4** and at δ 210.6 for **5** and **6**. Overall, the ^1H NMR resonances for **5** and **6** are not as closely parallel to those of **4** as the corresponding ^{13}C resonances. However, resonances observed for protons attached at C-7, and for methyl groups at C-1 and C-5, are very similar and further support the presence of a tetrahydrofuran moiety.

On the other hand, there are significant differences between the NMR spectra of **5** and **6** as contrasted with those of **4**. A carbon–carbon double bond in **4** is documented by ^{13}C resonances at δ 124.1 and 140.0 and by ^1H resonances at δ 5.86 and 6.20. All of these resonances are notably absent from the spectra of **5** and **6**. The ^1H spectrum of **4** contains a methyl doublet at δ 1.33 that is replaced by a methyl singlet in **5** and **6** at δ 2.20 and 2.23, respectively. The NMR data are consistent with a carbonyl group at C-3′ in **5** and **6**, in place of the hydroxyl group at that position in **4**. Since the ^{13}C spectra of **5** and **6** each exhibit only one carbonyl resonance, the carbonyl group at C-3 in **4** must be reduced to a hydroxyl in **5** and **6**. The accompanying structures show **5** and **6** with cagelike ring systems that are pseudosymmetrical around a plane passing through C-3, C-8, C-1′, and C-2′ and differ only in the configuration at C-2′. All three isomers (**4**–**6**) probably are formed via a common, as yet unidentified, precursor.

In order to distinguish between isomers **5** and **6**, and to verify the proposed structures, a number of NMR experiments were undertaken. The protons attached at C-7, adjacent to an oxygen, were expected to have the lowest field chemical shifts. Protons at C-2 were more likely than those at C-4 to be coupled to the ones at C-7, and the chemical shifts of protons 2a and 2b were pinpointed by a COSY experiment[6] with drummondone A (Figure 1). It is evident that the proton farthest downfield, designated 7a, is coupled to protons 2a and 2b as well as to 7b. In addition to the expected vicinal couplings with the protons at 1′, proton 2′ is coupled to 4b, the methylene proton at

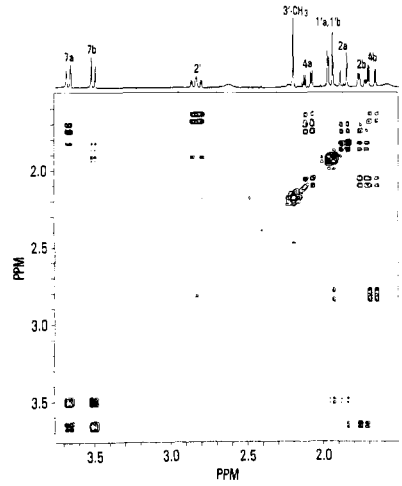

Figure 1. COSY proton shift correlation experiment for drummondone A (**5**).

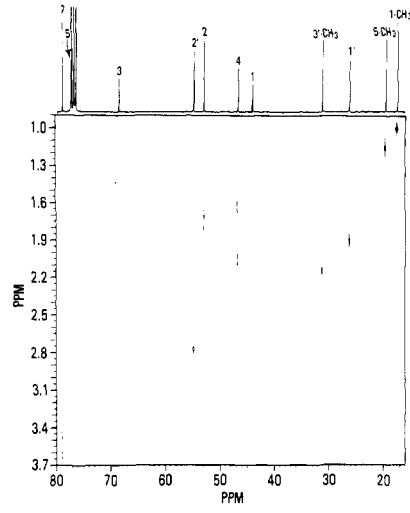

Figure 2. 2D heteronuclear shift correlation experiment for drummondone A (**5**).

highest field. With 4b located, its connection with 4a was readily apparent, and all the proton chemical shifts for **5** were established; similarly those of **6** were assigned by a separate COSY experiment. In both **5** and **6**, protons 2b and 4a have the coupling J = 3.2 Hz and, therefore, they must occur in a planar, zigzag (W) orientation.[4,5] Further stereochemical corroboration was provided by difference NOE experiments. When the C-1 methyl resonance of **5** was irradiated, signals for protons 7a and 2b were enhanced; this observation indicated that these three centers are in close proximity with a syn relationship. Similarly, irradiation at the C-5 methyl signal enhanced the 4a proton signal but not those of 7a or 7b.

Established ^1H shifts then were used in a 2D heteronuclear $^1H/^{13}C$ shift correlation experiment[7] to assign and confirm ambiguous ^{13}C shifts (Figure 2). Using **5**, the resonances for protons 2a and 2b (δ 1.87 and 1.75, respectively) correlate with the ^{13}C resonance at δ 53.1; protons 4a and 4b (δ 2.10 and 1.69, respectively) correlate with the ^{13}C peak at δ 46.9. Corresponding assignments for **6** were then made by analogy.

With all of the resonances assigned for **5**, the differences between **5** and **6** were readily apparent. In **5**, there is a coupling of 2.2 Hz between protons 4b and 2′ but none between protons 2a and 2′. These results require a planar,

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zigzag relationship between protons 4b and 2′ with the acetyl group syn to C-4. In contrast, **6** has corresponding couplings appropriate for a zigzag relationship between protons 2a and 2′, thus placing the acetyl group syn to C-2.

Our stereochemical representation of drummondones A (**5**) and B (**6**) is based on the assumption that their chiral centers, and those of drummondol (**4**), have the same configuration as comparable chiral centers in phaseic acid (**2**),[2] although we have no evidence that this is the case. Attempts to prepare crystals suitable for X-ray crystallographic studies have been unsuccessful.

The bicyclo[2.2.2]octane ring system, which the drummondones exhibit, is of great interest to synthetic and mechanistic chemists but is seldom found in natural products. To our knowledge, the only previous examples of natural products containing this ring system are the atisine group of diterpenoid alkaloids[8,9] and the related atiserene hydrocarbons.[9,10]

At present, we have no bioassay results for **5** and **6** owing to insufficient availability of the compounds.

Experimental Section

Melting points are uncorrected; analytical and preparative TLC was accomplished on silica gel 60 F-254 plates, 0.25 mm thick developed with CH_2Cl_2–MeOH (9:1) unless specified otherwise. HPLC was performed on an instrument equipped with an RI detector. IR spectra were recorded in 1% $CHCl_3$ solutions, and optical rotations were determined in MeOH. ^1H NMR (300-MHz) and ^{13}C NMR (75.47-MHz) spectra were determined in $CDCl_3$ solutions. Mass spectra were obtained (70 eV) utilizing a DEP probe or a gas chromatographic inlet. Fractions were analyzed for individual components by selective ion monitoring and MS–MS techniques.

Extraction Procedure. *S. drummondii* (560 kg of seed plus pod) was ground to pass a 3-mm screen and placed in a 5580-L tank with 2200 L of MeOH. The MeOH was pumped from the bottom of the tank and sprayed over the top of the plant material for 8 h/day over 4 days. Solvent was then drained and concentrated to half-volume in a wiped film evaporator. The percolation and concentration procedures were repeated three more times using additional volumes of MeOH (1040, 1250, and 1250 L). Virtually all of the MeOH-soluble material had been removed by the end of the third extraction. The MeOH concentrates were combined (1874 L) and split into nine batches, and each was diluted with two volumes of water. The resultant solution was defatted by partitioning three times against hexane with a hexane to aqueous MeOH ratio of 1:6.6. Aqueous methanol layers from the hexane partitions were combined into three batches of 1874 L each and partitioned with $CHCl_2$ in a solubles to $CHCl_2$ ratio of 3:1. Each was vigorously stirred for 15–20 min with 3 L of saturated NaCl to retard emulsion, and the mixtures were allowed to separate overnight. Each CH_2Cl_2 extract was concentrated by using a wiped film evaporator followed by a 20-L rotary evaporator; a total of 11 kg of crude extract comprising a thin black syrup, 17.45% solids, was thus obtained.

Chromatographic Separations. Two 15 cm × 3.05 m stainless-steel columns in series were slurry packed with 62 kg of silica gel (200–400 mesh) in MeOH–CH_2Cl_2 (1:19) and conditioned with 151 L of MeOH–CH_2Cl_2 (1:39). The concentrated syrup (11 kg) was pumped onto the column. A stepwise gradient of the following solvents was then passed through the column at a rate of 49–68 L/h and at a pressure of 150–190 psi: MeOH–CH_2Cl_2 (1:39), 151 L; MeOH–CH_2Cl_2 (1:19), 221 L; MeOH–CH_2Cl_2 (1:9), 220 L; MeOH–CH_2Cl_2 (1:1), 151 L; MeOH, 170 L. Fifty fractions of 19 L each were collected, and like fractions (TLC monitoring) were combined and evaporated to dryness. Examination by MS demonstrated that drummondone A (**5**) and drummondone B (**6**) were concentrated in fractions 36–38 (39.4 g), drummondol (**4**) in fractions 34 and 35 (31.0 g), vomifoliol (**3**) in fractions 32 and 33 (57.9 g), and sesbanimide A[3c] in fractions 26–30 (80.0 g).

Further concentration of compounds **3–6** was accomplished by HPLC of the appropriate fraction (3–4 g/injection) on a 2.1 cm × 122 column packed with EM silica gel 60 and eluted with MeOH–CH_2Cl_2 (1:19), followed by reversed-phase HPLC (1–2 g/injection) on a 2.1 cm × 30.5 cm column packed with C_{18} Porasil and eluted with MeOH–H_2O (1:5). Fractions enriched in the desired components were identified by GC–MS.

Vomifoliol 3. Isolation of **3** [90 mg (1.6×10^{-5} % yield); mp 108–110 °C (CH_2Cl_2)] was accomplished by HPLC on a 30 cm × 3.9 mm Porasil column eluted with CH_2Cl_2–MeOH (98.5:1.5). ^1H NMR gave good agreement with literature values:[11] ^{13}C NMR, δ 198.7 (s, C-3), 163.9 (s, C-5), 135.8 (d), 129.2 (d), 126.7 (d), 78.9 (s, C-6), 67.8 (d, C-3′), 49.8 (t, C-2), 41.2 (s, C-1), 24.0 (q), 23.5 (q), 22.9 (q), 18.9 (q); chemical ionization mass spectrum (70 eV) m/z (relative intensity) 225 (MH^+, 18), 207 (100).

Drummondol (4). Final isolation of **4** [90 mg (1.6×10^{-5} % yield)] as a colorless oil was accomplished by reversed-phase HPLC on a C_{18} column eluted with MeOH–H_2O (1:5). This material was identical in all respects (NMR, MS) with known drummondol.[3a]

Drummondone A (5). Compounds **5** and **6** were very similar in retention characteristics on both silica and reversed-phase HPLC columns and were first isolated as an approximate 1:1 mixture (155 mg) as determined by NMR. Chromatography of the mixture on a C_{18} column with MeOH–H_2O (1:5), splitting the peak in order to concentrate the faster and slower eluting components and repeating the procedure several times, ultimately yielded **5** [42 mg (7.5×10^{-6} % yield)] and **6** [63 mg (1.1×10^{-5} % yield)]. Compound **5** is the faster eluting compound under these conditions. Drummondone A (**5**) was recrystallized from ether: mp 170–172 °C; IR ($CHCl_3$) 3700, 1710 cm^{-1}; ^1H and ^{13}C NMR, Tables I and II; $[\alpha]^{23}_D$ +98° (*c* 0.20, MeOH); mass spectrum (70 eV) m/z (relative intensity) 240 (M^+, 2), 222 (4), 170 (15), 139 (18), 125 (18), 113 (13), 85 (18), 71 (13), 69 (15), 55 (36), 43 (100); chemical ionization mass spectrum (CH_4), m/z (relative intensity) 241 (MH^+, 87), 223 (100); found for MH^+, m/z 241.1453, $C_{13}H_{21}O_4$ requires m/z 241.1440.

Drummondone B (6). Upon recrystallization from ether, **6** gave the following results: mp 185–187 °C; IR ($CHCl_3$) 3700, 1710 cm^{-1}; ^1H and ^{13}C NMR, Tables I and II; $[\alpha]^{23}_D$ −63° (*c* 0.12, MeOH); mass spectrum (70 eV) m/z (relative intensity) 240 (M^+, 3), 222 (6), 170 (32), 139 (34), 125 (40), 113 (17), 97 (20), 85 (26), 71 (23), 69 (30), 55 (53), 43 (100); chemical ionization mass spectrum (CH_4), m/z (relative intensity) 241 (MH^+, 66), 223 (100); found for MH^+, m/z 241.1449 $C_{13}H_{21}O_4$ requires m/z 241.1440.

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Registry No. 1, 21293-29-8; 3, 23526-45-6; 4, 77162-65-3; 5, 100655-65-0; 6, 100760-76-7.

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