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SESBANIMIDE A AND RELATED TUMOR INHIBITORS FROM *SESBANIA DRUMMONDII*: STRUCTURE AND CHEMISTRY

RICHARD G. POWELL,* CECIL R. SMITH, JR. and DAVID WEISLEDER

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604, U.S.A.

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Key Word Index—*Sesbania drummondii*; Fabaceae; rattlebox; antitumor activity; cytotoxins; sesbanimides; glutarimide derivatives.

Abstract—Extracts of *Sesbania drummondii* seed have yielded three new glutarimide tumor inhibitors which are markedly active in the PS leukemia (*in vivo*) system and in the KB cell culture system. Structures of sesbanimide A, sesbanimide B, sesbanimide C, acetylation products of the sesbanimides and five compounds obtained by hydrogenating sesbanimide A under mild conditions have now been established using high-field ^{1}H NMR, ^{13}C NMR and mass spectral correlations. Treatment of sesbanimide A diacetate with sodium bicarbonate in aqueous methanol gives rapid elimination of acetic acid and two subsequent products derived via solvolysis of the glutarimide ring.

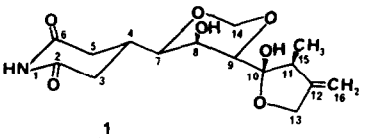
INTRODUCTION

Sesbania drummondii (Rydb.) Cory is one of three closely related legumes native to the Gulf Coastal Plains of the U.S.A. which have a long history of toxicity to livestock [1]. Alcoholic extracts of the seeds of *S. drummondii*, (otherwise known as *Daubentonia longifolia* or *D. drummondii*) are markedly cytotoxic against KB cells *in vitro* and show significant inhibitory activity against PS leukemia (*in vivo*); extracts of *S. vesicaria* (= *Glottidium vesicarium*) and *Sesbania punicea* (= *Daubentonia punicea*) exhibit similar activity [2, 3]. Our efforts directed towards identification of the antileukemic principles of *S. drummondii* seed culminated in the isolation and characterization of three active compounds—sesbanimide A, sesbanimide B and sesbanimide C. In a preliminary communication [4], we disclosed the structure of sesbanimide A.† We now present full details of the isolation and characterization of sesbanimides A, B and C, and some of the chemistry of these unusual multifunctional compounds.

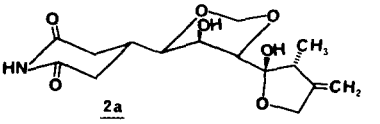
RESULTS AND DISCUSSION

An ethanol extract of *S. drummondii* seed was defatted and partitioned between butanol and water; the butanol solubles were then separated into ten fractions by countercurrent distribution with the solvent system water–ethyl acetate–methanol (2:2:1). Fractionation was guided by assay against KB cell culture and against PS leukemia‡ in mice [3]. Purified active materials, sesbanimides A (**1**), B (**2**) and C (**3**) were successfully isolated from countercurrent fraction 3 after chromatography on alumina and silica columns, followed by repeated HPLC on silica, preparative TLC on silica and HPLC on C_{18} reversed-phase columns. Treatment of countercurrent fraction 7 in a similar manner gave additional **1**. Sesbanimide A (**1**) was isolated as a crystalline solid, mp 158–159°, $[\alpha]_D$ −5.6° (MeOH), and combustion analysis was consistent with the empirical formula $C_{15}H_{21}NO_7$.

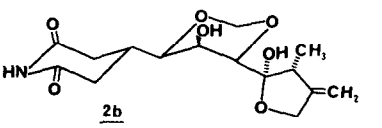
The ^{1}H NMR spectrum of **1** indicated the presence of 21 protons, three of which were readily exchanged with



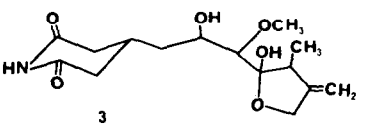
1



2a



2b



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*To whom correspondence should be addressed.

†Sesbanimide A was simply called sesbanimide in our preliminary communication.

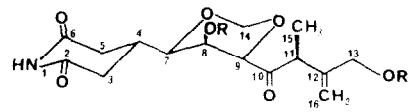
‡Cytotoxic and antitumor activities were assayed under the auspices of the National Cancer Institute by the procedures described by Geran *et al.* [5]. Sesbanimide A (**1**) gave *T/C* values of 140–181 in the 0.008–0.032 mg/kg range against PS leukemia and an ED_{50} of 7.7×10^{-3} μg/ml against KB cells *in vitro*. Compounds **2** and **3** showed similar activity in limited testing, but at approximately ten times these dose levels.

deuterium oxide and the ^{13}C NMR spectrum of **1** contained 15 carbon signals. High-resolution mass spectrometry yielded an $[M-H_2O]^+$ ion at m/z 309.1246 ($C_{15}H_{19}NO_6$). Since the available data did not readily distinguish among several possibilities, the structure of **1** was determined by a single-crystal X-ray crystallographic study [4]. Sesbanimide A (**1**) was shown to have an unprecedented structure consisting of three rings linked by single bonds. Since the X-ray experiment defined only the relative stereostructure, the absolute configuration of **1** remains to be determined.

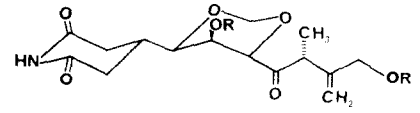
Once the structure and relative stereochemistry of **1** were known, it was possible to assign the ^1H NMR signals unambiguously (Table 1). Equatorial protons of the glutarimide ring were observed at δ2.76, 2.90 (H-3', H-5'); axial protons appeared at δ2.38, 2.47 (H-3, H-5) and the axial H-4 proton was evident at δ2.63. Irradiation of the signal at δ2.63 demonstrated that the H-4 proton was coupled to all four methylene protons of the glutarimide ring and to a proton which appeared as a slightly broadened doublet at δ3.34 (H-7). Irradiation of H-7 sharpened the signal at δ4.00 (H-8), while irradiation at δ4.00 sharpened both the doublet at δ3.34 and the broad singlet at δ3.58 (H-9). In one high dilution experiment, H-8 appeared as a well-defined doublet (δ4.00) coupled to a similar doublet assigned to the C-8 hydroxyl proton (δ3.56). Coupling constants ($J_{7,8}$ and $J_{8,9}$) were quite low (both 1.3 Hz) as was expected from the observed angles in the X-ray model and the Karplus relationship. A multiplet observed at δ2.60 (H-11) was coupled to a methyl doublet (H-15, δ1.19) and also exhibited long-range coupling to two vinyl protons (H-16, H-16'; δ4.96, 5.01). In addition, both vinyl protons showed long-range coupling to protons of an oxygenated methylene group (H-13, H-13'; δ4.47, 4.55). The remaining two protons, in a methylenedioxy group (H-14, H-14'), appeared as two doublets at δ4.78 and 5.22.

Assignments for the 15 carbons of **1** (Table 2) are as follows: carbonyls (C-2, C-6), δ172.1 and 171.6; olefinic (C-12, C-16), δ150.3 and 104.3; acetal and hemiketal (C-14, C-10), δ105.1 and 93.9; oxygenated methylene (C-13), δ69.5; oxygenated methines (C-7, C-8, C-9), δ81.4, 81.1, and 64.7; non-oxygenated methines (C-11, C-9), δ45.8 and 30.8; non-oxygenated methylenes (C-3, C-5), δ33.9 and 33.2 and methyl (C-15) δ11.9. Multiplicities determined by off-resonance decoupling experiments were confirmed by a DEPT [6] experiment. Well-separated signals for diastereotopic methylene carbons at C-3 and C-5, and for the two carbonyl carbons, have been noted previously for cycloheximide and related glutarimides substituted at C-4 [7].

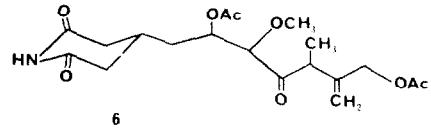
Acetylation of **1** under mild conditions yielded a diacetate **4**, mp 128–129°, corresponding to the ring-opened γ-hydroxyketone form of **1** (**4a**). This conclusion was evident from the ^{13}C NMR spectrum, in which the hemiketal carbon of **1** (C-10, δ105.1) was replaced by a ketone carbon in **4** (δ205.6). In comparing the ^1H spectra of **1** and **4**, marked downfield shifts were noted for H-8 due to acetylation and for H-9 and H-11, protons adjacent



4, R=Ac
4a, R=H



5, R=Ac
5a, R=H



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to the C-10 carbonyl (Table 1). Signals for H-13 and H-13' shifted downfield only slightly in **4**. The remainder of the ^1H spectra of **1** and **4** was nearly identical except for the appearance of two acetate methyl signals in **4** (δ2.06, 2.07). Chemical ionization mass spectrometry (CIMS) of diacetate **4** gave an $[MH]^+$ peak at m/z 412 consistent with a MW of 411 ($C_{19}H_{25}NO_9$).

Sesbanimide B (**2**) was isolated as a 7:3 mixture of two compounds (sesbanimides B-1 and B-2); from NMR spectra, we concluded that these are anomers differing only in their configuration at C-10.* In the ^1H NMR spectrum of the mixture, two sets of signals were observed for most of the protons, particularly H-7, H-9, H-13, H-13', H-14, H-14', H-15, H-16 and H-16'. Repeated attempts to purify the predominant anomer by HPLC and TLC were of no avail since isolated fractions invariably gave solution spectra indicating the same two compounds in about the same proportions. Apparently a very mobile equilibrium between anomers B-1 and B-2 (**2a** and **2b**) exists in solution, probably mediated by **5a**. A similar situation must exist in the case of sesbanimide A (**1**) except that steric factors favor the predominance of only one of the two possible anomers of **1** in chloroform.

Compound **2** gave an apparent $[M]^+$ peak at m/z 327 in the mass spectrum and the ^1H NMR spectra of **1** and **2a** were very similar; however, the C-15 methyl signals of **2a** and **2b** both appeared slightly upfield from that of **1**. The ^{13}C NMR spectra of **1** and **2a** were also quite similar except for significant shift differences in signals assigned to C-11 and to carbons α and β to C-11. Acetylation of the mixture (**2a** and **2b**) gave a single diacetate (**5**); $[MH]^+$, m/z 412, formed via γ-hydroxyketone (**5a**). The ^1H NMR and ^{13}C NMR spectra of **4** and **5** (the diacetates of sesbanimides A and B, respectively) fully support the conclusion that these two compounds are diastereomers differing only in configuration at C-11 (Tables 1 and 2).

Sesbanimide C (**3**) gave an $[MH-(H_2O+MeOH)]^+$ ion in the positive ion CI mass spectrum. The negative ion CI mass spectrum was more informative, however, in that the molecular anion was observed at m/z 313, corresponding to $C_{15}H_{23}NO_6$, along with peaks at m/z 295 $[M-H_2O]^-$ and m/z 281 $[M-MeOH]^-$. The ^1H NMR spectrum of **3** differed from those of **1** and **2** in the following respects: it contained a sharp *O*-methyl singlet

*For convenience we refer to the predominant anomer as sesbanimide B-1 (**2a**) and the lesser anomer as sesbanimide B-2 (**2b**). Structures **2a** and **2b** are intended to emphasize relative stereochemistry at C-10, but it has not been determined which is the predominant anomer.

Table 1. ^{1}H NMR chemical shifts and coupling constants (in parentheses) for sesbanimide derivatives*

Proton assignment	1	2a†	3‡	4	5	6	7	8	8a	9	9a	10	10a	11	11a	12	13	14	15†
3	2.38 *dd* (17.0, 9.9)			2.43 *dd* (17.2, 9.5)	2.41 *dd* (17.3, 9.2)	2.29 *dd*	2.28 *dd* (16.8, 9.6)	2.33 *dd* (16.4, 9.8)	2.46 *dd* (17.2, 9.8)	2.42 *dd*	2.46 *dd*	2.42 *dd*	2.37 *dd*	2.35 *dd* (16.6, 9.5)	2.41 *dd*	2.47 *dd* (17.7, 9.0)	2.58 *dd*	2.27 *dd* (15.4, 7.5)	
3′	2.76 *dd* (17.0, 4.4)			2.76 *dd* (17.4, 4.6)	2.76 *dd* (17.4, 4.5)	2.70 *dd* (17.6, 3.9)	2.66 *dd* (16.8, 4.5)	2.67 *dd*	2.78 *dd* (17.2, 4.5)	2.71 *dd*	2.68 *dd*	2.73 *dd*	2.68 *dd*	2.75 *dd* (16.6, 4.5)	2.76 *dd*	2.78 *dd* (17.3, 4.4)	2.63 *dd*	2.35 *dd* (15.4, 5.4)	2.42 *m*
5	2.47 *dd* (17.4, 9.9)	~2.60 *m*	~2.60 *m*	2.48 *dd* (17.4, 9.1)	2.52 *dd* (17.3, 9.2)	2.34 *dd*	2.36 *dd* (17.1, 9.6)	2.40 *dd* (17.3, 9.8)	2.52 *dd* (17.0, 9.0)	2.51 *dd* (17.1, 9.6)	2.56 *dd*	2.52 *dd* (17.4, 8.6)	2.51 *dd*	2.47 *dd* (16.9, 9.5)	2.49 *dd*	2.53 *dd* (17.7, 9.0)	2.63 *dd*	2.50 *dd* (16.1, 6.4)	
5′	2.90 *dd* (17.4, 4.4)			2.85 *dd* (17.2, 4.5)	2.81 *dd* (17.4, 4.5)	2.82 *dd* (16.7, 3.9)	2.81 *dd* (17.1, 4.2)	2.85 *dd*	2.88 *dd* (17.0, 4.5)	2.90 *dd* (—, 4.2)	2.90 *dd*	2.92 *dd* (17.2, 4.2)	2.85 *dd*	2.92 *dd* (16.9, 4.5)	2.84 *dd*	2.88 *dd* (17.3, 4.4)	2.74 *dd*	2.60 *dd* (16.1, 6.8)	
4	2.63 *m*			2.27 *m*	2.33 *m*	2.18 *m*	2.54 *m*	2.58 *m*	2.28 *m*	2.70 *m*	2.40 *m*	2.68 *m*	2.36 *m*	2.62 *m*	2.31 *m*	2.28 *m*	2.40 *m*	2.66 *m*	2.65 *m*
7	3.34 *dd* (8.8, 1.3)	3.32 *m* (7.2)	~1.60 *m*	3.51 *dd* (8.6, 1.1)	3.53 *dd* (8.2, 1.3)	1.67 *m* 1.80 *m*	3.23 *dd* (8.6, 1.2)	3.35 *d*	3.50 *dd* (8.6, 1.0)	3.47 *dd* (8.3, 1.0)	3.63 *dd* (7.9, 1.4)	3.30 *d* (8.3)	3.48 *d*	3.35 *dd* (8.2, 1.0)	3.50 *dd* (9.4, 1.0)	3.52 *dd* (8.6, 1.0)	4.50 *dd* (3.8, 2.1)	4.64 *dd* (3.9, 2.2)	4.64 *dd* (3.9, 2.2)
8	4.00 *br s*	3.96 *m*	—	5.41 *m* (1.8)	5.36 *m* (1.6)	5.20 *m* (4.3)	3.91 *m* (1.2)	3.91 *m*	5.45 *m* (1.7)	3.72 *m* (1.1)	5.10 *m* (1.5)	3.68 *m* (1.9)	5.06 *m*	3.51 *m*	5.07 *m*	5.46 *m*	5.87 *d* (2.1)	5.98 *d* (2.2)	5.98 *d* (2.2)
9	3.58 *br s*	3.60 *d* (1.2)	3.40 *d*	4.29 *d* (1.8)	4.40 *d* (1.6)	3.73 *d* (4.3)	3.31 *d* (1.2)	3.96 *s*	4.10 *d* (1.7)	4.71 *d* (1.1)	4.80 *d* (1.5)	3.46 *m* (1.9)	3.55 *m*	3.62 *dd* (7.3, 0.8)	3.73 *dd* (8.1, 1.1)	4.20 *d* (1.8)	—	—	—
11	2.60 *m* (6.8)	~2.60	~2.60	3.69 *q* (6.9)	3.62 *q* (6.9)	3.58 *q* (6.9)	2.28 *m* (6.9)	2.87 *m* (6.8)	2.96 *m* (6.8)	—	—	2.40 *m*	2.23 *m*	2.14	2.06	3.11 *m*	3.65 *q* (6.9)	3.67 *q* (6.9)	3.67 *q* (6.9)
13§	4.47 *dd* (12.9)	4.51 *m*	4.50 *m*	4.58 *m* (14.0)	4.56 *m* (13.6)	4.60 *s*	3.60 *dd* (8.3, 5.8)	0.79 *d* (6.8)	0.82 *d* (6.8)	7.14 *m*	7.08 *m*	3.53 *dd* (8.2, 4.4)	3.39 *dd* (8.3, 6.2)	3.48 *dd* (8.1, 9.8)	3.44 *dd* (8.2, 10.1)	3.84 *dd* (11.1, 6.1)	4.59 *m* (13.7)	4.59 *m*	4.59 *m*
13′	4.55 *dd* (12.9)	4.53 *m*	—	—	4.60 *m* (13.6)	—	3.88 *dd* (8.3, 6.9)	—	—	—	—	3.98 *dd* (8.2, 5.7)	3.97 *dd* (8.3, 6.4)	3.98 *dd* (8.1, 8.1)	3.96 *dd* (8.2, 8.3)	4.04 *dd* (11.1, 6.0)	—	—	—

Table 1. (*contd.*)

Proton assignment	1	2a†	3‡	4	5	6	7	8	8a	9	9a	10	10a	11	11a	12	13	14	15†
14	4.78 *d*	4.77 *d*	—	4.79 *d*	4.80 *d*	—	4.67 *d*	4.71 *d*	4.81 *d*	4.86 *d*	4.91 *d*	4.72 *d*	4.78 *d*	4.76 *d*	4.84 *d*	4.82 *d*	4.90 *d*	4.91 *d*	4.91 *d*
	(6.2)	(6.2)		(6.4)	(6.4)		(6.2)	(6.2)	(6.4)	(6.4)	(6.4)	(6.3)	(6.3)	(6.4)	(6.4)	(6.4)	(6.6)	(6.7)	(5.6)
14′	5.22 *d*	5.20 *d*	—	5.25 *d*	5.28 *d*	—	5.10 *d*	5.17 *d*	5.30 *d*	5.21 *d*	5.30 *d*	5.19 *d*	5.27 *d*	5.15 *d*	5.25 *d*	5.30 *d*	5.22 *d*	5.20 *d*	5.19 *d*
	(6.2)	(6.2)		(6.4)	(6.4)		(6.2)	(6.2)	(6.4)	(6.4)	(6.4)	(6.3)	(6.3)	(6.4)	(6.4)	(6.4)	(6.6)	(6.7)	(5.6)
15	1.19 *d*	1.13 *d*	1.30 *d*	1.16 *d*	1.22 *d*	1.20 *d*	0.95 *d*	0.94 *d*	0.95 *d*	1.98 *s*	1.97 *s*	0.93 *d*	0.90 *d*	0.83 *d*	0.84 *d*	1.01 *d*	1.25 *d*	1.25 *d*	1.25 *d*
	(6.8)	(6.9)	(6.9)	(6.9)	(6.9)	(6.9)	(6.9)	(6.9)	(7.0)			(7.0)	(6.8)	(7.3)	(7.1)	(7.0)	(6.9)	(6.9)	(6.9)
16‖	4.96 *dd*	4.99 *m*	5.00 *m*	4.93 *m*	5.05 *m*	4.96 *m*	0.96 *d*	0.86 *d*	0.89 *d*	1.92 *d*	1.89 *d*	0.98 *d*	0.91 *d*	0.98 *d*	0.96 *d*	0.95 *d*	4.90 *m*	4.96 *m*	4.94 *m*
16′	5.01 *dd*	5.06 *m*	—	5.14 *m*	5.23 *m*	5.19 *m*	(7.0)	(6.8)	(6.7)	(1.3)	(1.2)	(6.9)	(6.7)	(6.9)	(6.9)	(6.9)	5.14 *m*	5.16 *m*	5.14 *m*
NH	8.12 *br s*	8.11 *br s*	8.35 *br s*	8.27 *br s*	7.88 *br s*	7.78 *br s*	9.23 *br s*	—	7.95 *br s*	8.25 *br s*	7.86 *br s*	7.78 *br s*	7.78 *br s*	7.89 *br s*	7.71 *br s*	7.90 *br s*	7.93 *br s*	—	—
OH	3.56 *br s*	3.83 *br s*	—	—	—	—	—	—	—	—	—	4.22 *br s*	—	3.53 *br s*	—	—	—	—	—
OH	4.22 *br s*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MeC=O	—	—	—	2.07 *s*	2.07 *s*	2.07 *s*	—	—	2.06 *s*	—	2.10 *s*	—	2.17 *s*	—	2.18 *s*	2.01 *s*	2.07 *s*	2.08 *s*	2.07 *s*
MeC=O	—	—	—	2.06 *s*	2.08 *s*	2.10 *s*	—	—	—	—	—	—	—	—	—	2.06 *s*	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	3.76 *m* (7.3, 3.1)	3.55 *m*	4.09 *m* (7.3, 4.7)	3.85 *m* (8.0, 4.1)	—	—	—	—
12	—	—	—	—	—	—	2.15 *m*	1.94 *m*	1.97 *m*	—	—	2.30 *m*	2.21 *m*	2.39 *m*	2.39 *m*	2.28 *m*	—	—	—
OMe	—	—	3.70 *s*	—	—	3.44 *s*	—	—	—	—	—	—	—	—	—	—	—	3.67 *s*	3.66 *s*

*Chemical shifts (δ) are expressed in ppm from internal TMS, and coupling constants (J) are expressed in Hz. Extensive decoupling was used to verify assignments. Spectra were recorded in $CDCl_3$ on Nicolet NT-470 or Bruker WM-300 spectrometers. Signals for protons assigned to C-3 may be interchanged with those assigned to C-5 in all cases.

†Chemical shifts for several protons of **2b** (minor anomer) are readily distinguished from the corresponding signals of **2a** and appear as follows: H-7 (δ3.38), H-9 (δ3.58), H-14 (δ4.77), H-14′ (δ5.17) and H-15 (δ1.07). In **15**, protons 3, 3′, 5 and 5′ appear as an unresolved broad multiplet centered at δ2.42.

‡Chemical shifts for **3** are approximate values obtained from a 90 MHz spectrum.

§Since C-13 is a methyl group in compounds **8** and **8a**, all protons are equivalent. Only one proton is attached to C-13 in **9** and **9a**.

‖Since C-16 is a methyl group in compounds **7**–**12**, all protons are equivalent.

Table 2. ^{13}C NMR spectral data of sesbanimides A, B, and selected derivatives*

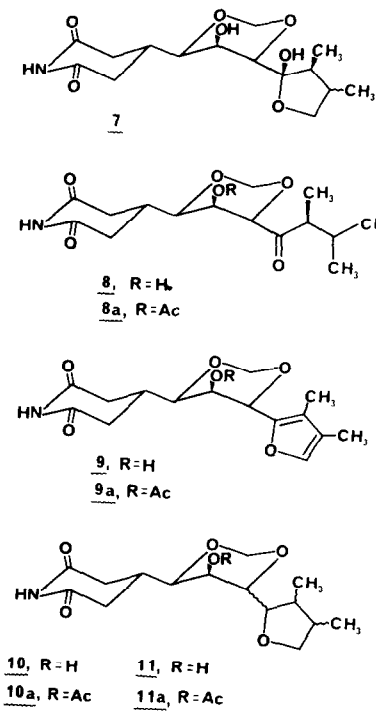
Carbon assignment	1	2a	4	5	13
2†	172.1 (*s*)	172.0	171.1	170.8	171.2 (*s*)
3‡	33.9 (*t*)	33.9	33.9	34.0	34.3 (*t*)
4	30.8 (*d*)	31.1	31.2	31.5	34.6 (*d*)
5‡	33.2 (*t*)	33.4	33.3	33.5	31.6 (*t*)
6†	171.6 (*s*)	171.9	170.7	170.3	171.1 (*s*)
7§	81.4 (*d*)	78.5	81.1	81.4	74.6 (*d*)
8§	81.1 (*d*)	81.4	80.1	80.2	108.4 (*s*)
9§	64.7 (d)	63.7	66.0‖	65.8‖	151.1 (*s*)
10	105.1 (*s*)	106.0	205.6	203.6	194.4 (*s*)
11	45.8 (*d*)	42.0	44.8	45.7	43.8 (*d*)
12	150.3 (*s*)	148.5	142.9	142.9	143.3 (*s*)
13	69.5 (*t*)	69.2	66.2‖	65.6‖	65.9 (*t*)
14	93.9 (*t*)	93.8	93.4	93.6	90.3 (*t*)
15	11.9 (*q*)	10.7	15.3	15.8	15.6 (*q*)
16	104.3 (*t*)	105.3	114.6	115.6	114.2 (*t*)
C=O	—	—	170.3	170.2	170.3 (*s*)
C=O	—	—	169.8	170.2	—
Me	—	—	20.6	20.5	20.8 (*q*)
Me	—	—	20.8	20.7	—

*Chemical shifts (δ) are expressed in ppm from internal TMS. Proton-decoupled and off-resonance decoupled spectra were recorded in $CDCl_3$ solution on a Bruker WM-300 spectrometer. Multiplicities assigned to carbons of **1** and **13** were confirmed in DEPT experiments. Satellite signals were observed for most carbons in the anomeric mixture of **2a** and **2b**; only signals which could readily be assigned to the major anomer (**2a**) are reported.

†, ‡, §, ‖Assignments with the same symbol, in any column, may be interchanged. In **13**, assignments for carbons 7, 8 and 9 are unambiguous.

(δ3.70), lacked the two doublets characteristic of a methylenedioxy group (H-14, H-14′ of **1** and **2**), and exhibited an additional complex multiplet near δ1.60 (H-7). Acetylation of **3** gave diacetate **6**, MW 397. All 27 protons in the ^1H NMR spectrum of **6** were unequivocally assigned (Table 1) from extensive decoupling experiments and by correlation with the spectra of **1**–**4** and streptimidone.† The stereochemistry of the various chiral centers of **3** and **6** remain to be determined.

Treatment of **1** with 10% palladium–charcoal under hydrogenation conditions gave a complex mixture of products, of which five (**7**–**11**) were isolated in quantities sufficient for characterization. The glutarimide and 1,3-dioxy rings of **1** remained intact in all of these products as demonstrated by their ^1H NMR spectra (Table 1). Rapid uptake of 1 mol equivalent of hydrogen occurred, and the reaction was stopped after 1.4 mol equivalents had been consumed. Saturated derivative **7** ($[M-H_2O]^+$, 313) was readily characterized by conversion to diacetate **12** and by correlating the ^1H NMR spectra of **7** and **12** with other members of the series. In the spectrum of **7**, vinyl proton signals were absent and had been replaced by an additional methyl doublet at δ0.96; this new methyl doublet was coupled to a new, one-proton multiplet at δ2.15 (H-12). Saturation of the 12,16-double bond also resulted in upfield shifts of the H-11 signal (to δ2.28) and of the H-13 and H-13′ signals (to δ3.60, 3.88); both H-13 and H-13′ were also coupled to H-12. Acetylation of **7** (to **12**) produced marked downfield shifts for H-8 (δ3.91 to 5.45), H-9 (δ3.31 to 4.20), H-11 (δ2.28 to 3.10), and H-13, H-13′ (δ3.60, 3.88 to 3.83, 4.04). Relative stereochemistry at C-12 in **7** remains unspecified.



The hydrogenation–hydrogenolysis product **8** ($[MH]^+$, m/z 314) gave only a monoacetate (**8a**; $[MH]^+$, 356). Vinyl proton signals were absent in the ^1H NMR spectrum of **8** and three methyl doublets were apparent (δ0.79, 0.86, 0.94). Assignments for all other protons of **8** and **8a** are given in Table 1. Upon acetylation (**8** to **8a**), only the H-8 proton signal shifted markedly downfield (δ3.91 to 5.45).

The ^1H NMR spectrum of compound **9** was exceptional in that a low field aromatic singlet was present at δ7.16 (one proton) together with two vinyl methyl signals (singlets at δ1.92 and 1.98). Long-range coupling was noted between the vinyl proton and the more upfield methyl signal. The signal for H-9 was also shifted downfield to δ4.72 indicating that it was adjacent to an unsaturated center. Acetylation of **9** gave a monoacetate (**9a**); $[M]^+$, m/z 351. The signal for H-8, at δ3.72 in **9**, was shifted downfield to δ5.10 in **9a**. Thus, we concluded that **9** was a dehydration–rearrangement product from **1** in which the hemiketal ring had been converted to a trisubstituted furan.

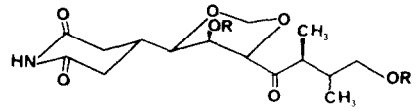
Hydrogenation–hydrogenolysis products **10** and **11** were isomeric ($[MH]^+$ 314), and yielded isomeric monoacetates (**10a** and **11a**). Signals for two methyl groups in the ^1H NMR spectrum of **10a** were coincident doublets at δ0.90, while in the spectrum of **11a** these two methyl signals appeared at δ0.84 and 0.96. Other differences in the ^1H NMR spectra of **10a** and **11a** occur in the chemical shifts and coupling constants of H-9, H-10,

†A pattern of complex signals observed for H-7, H-7′ of **6** (δ1.26–1.47) is identical to that observed for H-7, H-7′ in the spectrum of streptimidone as recorded in our laboratory.

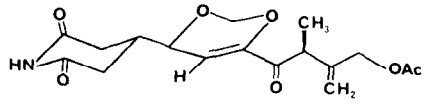
H-11, H-12, and H-13. These differences have not been fully interpreted; however, it is apparent that these cyclic ethers differ in relative stereochemistry at one or more positions (C-10, C-11, or C-12). Formation of these compounds may be envisioned as occurring by hydrogenolysis of **7** at C-10, but, more likely, they originate via hydrogenation of **9**.

Sesbanimide A diacetate (**4**) proved to be quite labile in mild base—1% sodium bicarbonate in methanol–water (3:1). Elimination of acetic acid occurred rapidly under these conditions at room temperature, yielding unsaturated monoacetate **13**; $[MH]^+$, m/z 352. Prominent in the ^{1}H NMR spectrum of **13** was a vinyl proton doublet (δ5.87, H-8) coupled to a doublet of doublets at δ4.50 (H-7). A single acetate methyl signal was apparent at δ2.07. The remainder of the spectrum of **13** was nearly identical to that of **4** except for the absence of any signal which could be attributed to H-9. In the ^{13}C NMR spectrum of **13**, two additional signals (δ108.4, 151.1) in the olefinic carbon region replaced two (δ80.1, 66.0) associated with oxygenated carbons in **4**; the C-10 carbonyl signal was shifted upfield from δ205.6 to 194.4. Thus, **13** was characterized as the 8,9-unsaturated derivative of **4** formed by elimination of acetic acid.

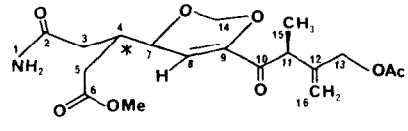
When **4** was reacted with the same methanolic bicarbonate solution for 18 hr, significant amounts of two ester amides (**14** and **15**) were formed in addition to **13**. Compounds **14** and **15**, differing only in configuration at C-4, were separable by TLC and gave ^{1}H NMR spectra which were strikingly different in the δ2.2–2.8 region where the C-3 and C-5 methylenes resonate. The remaining signals of the two spectra were similar, and included singlets for the carbomethoxy groups near δ3.67; other signals resembled those of **13**. Accordingly, we concluded that **14** and **15** are formed by base-catalysed solvolysis of the imide grouping in a non-specific manner to provide two diasteromeric ester amides; the observed differences in the ^{1}H NMR spectra of **14** and **15** must be due to conformational effects. Other more polar products were evident in the reaction mixture, possibly including the free acids corresponding to **14** and **15**; however, these were not characterized.



12, R=Ac



13



14,15 (diastereomers)

EXPERIMENTAL

TLC was accomplished on silica gel 60 F-254 developed with CH_2Cl_2–MeOH (19:1 or 9:1) and visualized under UV light. Silica gel (60–200 mesh) or activity grade III neutral alumina was used for CC. HPLC was performed with an RI detector and operated at a flow rate of 2 ml/min. Satisfactory HPLC analyses were achieved on a C_{18} μ-Bondapak column (30 cm × 7.9 mm) using H_2O–MeOH (4:1) or on a μ-Porasil column (30 cm × 3.9 mm) with CH_2Cl_2–MeOH (98.5:1.5). ^{1}H NMR (300 MHz) and ^{13}C NMR (22.63 MHz) spectra were determined in $CDCl_3$ solutions with TMS as internal standard. EIMS and CIMS (*i*-butane), were obtained (70 eV) using a Finnegan MAT 4535/TSQ instrument equipped with a DEP probe.

Extraction and fractionation. Seed of *Sesbania drummondii* (Rydb.) Cory was harvested from the wild in Texas during November 1975 (PR-44884). The collection was arranged and authenticated by Dr. Robert E. Perdue, USDA, Beltsville, MD. This collection (454 kg) was extracted with 95% EtOH, and the materials of interest were concd by solvent partitioning and countercurrent distribution into ten fractions as described previously [3]. Each stage of fractionation was monitored by bioassay and by TLC.

Countercurrent fraction 3 (675 g) was divided into nine equal portions and each was chromatographed on activity grade III neutral Al_2O_3 using 3 l. $CHCl_3$–MeOH (2:1) as the eluting solvent. Similar fractions from all nine column runs were combined on the basis of TLC similarity to yield 19 g of activity-enriched material. Oily substance (23 g) preceded the active fraction, and 137 g of additional material was eluted after the active fraction. The remaining inactive materials were irreversibly absorbed on the Al_2O_3.

Further concn of active components was achieved by CC on columns packed with 150 g silica gel (2 batches). Eluting solvents for both runs consisted of a stepwise gradient of MeOH in CH_2Cl_2: 750 ml each of CH_2Cl_2, CH_2Cl_2–MeOH (19:1), CH_2Cl_2–MeOH (9:1), CH_2Cl_2–MeOH (3:1), CH_2Cl_2–MeOH (1:1) and MeOH. Similar fractions were again combined on the basis of TLC analysis; the most active fraction (1.6 g) had been eluted with CH_2Cl_2–MeOH (19:1). Additional separations were then made by HPLC on silica with CH_2Cl_2–MeOH (97.5:2.5); repeated prep. TLC with silica gel plates developed with CH_2Cl_2–MeOH (9:1); and repeated HPLC on C_{18} μ-Bondapak with H_2O–MeOH (4:1). The elution order on a C_{18} (reversed phase) column, using the aforementioned solvent system and a flow rate of 2 ml/min, was **2** (22 min), **1** (25 min) and **3** (29 min). Final purification by prep. TLC on silica gave **1**, 16 mg (R_f 0.5); **2**, 22 mg (R_f 0.4) and **3**, 3.6 mg (R_f 0.4). Countercurrent fraction 7 (688 g) was treated in a similar manner, yielding additional **1** (244 mg) but no additional **2** or **3**.

Sesbanimide A (**1**). The combined yield of **1** from countercurrent fractions 3 and 7 was 250 mg (5×10^{-5} % based on seed material). Recrystallization from Et_2O–CH_2Cl_2 gave **1**: mp 158–159°; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3555, 3480, 3380, 2945, 2880, 1700, 1080; UV, end absorption < 220 nm; $[\alpha]_D^{23}$ −5.6° (*c* 0.27; MeOH); ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; EIMS m/z (rel. int.): 309 (5), 279 (1), 179 (1), 125 (100). Found for $[M - H_2O]^+$: m/z 309.1246; $C_{15}H_{19}NO_6$ requires: m/z 309.1212. CIMS m/z (rel. int.): 328 $[MH]^+$ (7), 310 (56), 280 (79), 157 (49), 142 (100), 139 (85). Found: C, 54.94; H, 6.42; N, 4.22; $C_{15}H_{21}NO_7$ requires C, 55.06; H, 6.42; N, 4.28%.

Sesbanimide A acetate (**4**). A portion of **1** (11 mg) was acetylated in 2 ml Ac_2O–pyridine (1:1) for 18 hr at 26°, and the resulting soln was evapd to dryness under N_2. The residue was dissolved in $CHCl_3$ and subjected to HPLC on μ-Porasil using $CHCl_3$–MeOH (98.5:1.5), which gave 12 mg of product.

Recrystallization from Et_2O gave **4**; mp 128–129°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 2935, 2860, 1730, 1710; $[\alpha]_D^{23}$ −86° (*c* 0.38; $CHCl_3$); ^1H NMR and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* (rel. int.): 351 $[M-60]^+$ (15), 256 (67), 113 (95), 85 (78), 67 (53), 60 (100), 43 (89). Found for $[M-60]^+$: *m/z* 351.1313; $C_{17}H_{21}NO_7$ requires: *m/z* 351.1318. CIMS *m/z* (rel. int.) 412 $[MH]^+$ (23), 352 (100), 292 (42).

Sesbanimide B (**2**). Repeated attempts to crystallize **2**, 22 mg, from mixtures of Et_2O–CH_2Cl_2 gave only a colorless glass (5×10^{-6} % yield based on seed material): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 3320, 2940, 2890, 1695, 1080; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* (rel. int.): 309 (0.1), 260 (3), 197 (5), 113 (25), 85 (75), 68 (53), 41 (100). CIMS *m/z* (rel. int.): 328 $[MH]^+$ (1), 310 (51), 280 (68), 139 (100).

Sesbanimide B acetate (**5**). A portion of **2** (10 mg) was acetylated and purified by HPLC, by the procedure described for **1**, yielding **5** (8 mg) as a colorless glass: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 2935, 2860, 1730, 1710; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* (rel. int.): 351 $[M-60]^+$ (2), 256 (25), 113 (51), 85 (46), 67 (29), 60 (29), 43 (100). Found for $[M-60]^+$: *m/z* 351.1321; $C_{17}H_{21}NO_7$ requires: 351.1318. CIMS *m/z* (rel. int.): 412 $[MH]^+$ (12), 352 (100), 292 (33).

Sesbanimide C (**3**). Sesbanimide C, 3.5 mg, was isolated as a colorless glass (7×10^{-7} % yield based on seed material): ^1H NMR see Table 1; CIMS *m/z* (rel. int.): 264 $[MH-(H_2O+MeOH)]^+$ (100); negative ion CIMS *m/z* (rel. int.): 313 $[M]^-$ (3), 295 $[M-H_2O]^-$ (45), 281 $[M-MeOH]^-$ (100), 263 (20), 155 (32).

Sesbanimide C acetate (**6**). A portion of **3** (1.8 mg) was acetylated and purified by HPLC, as described for **1**, yielding **6** (1 mg) as a colorless glass: ^1H NMR, see Table 1; CIMS *m/z* (rel. int.): 398 $[MH]^+$ (63), 338 (20), 306 (98), 264 (94), 113 (100).

Hydrogenation of sesbanimide A (**1**). A 54-mg sample of **1** in 5 ml absolute EtOH was treated with hydrogen, at 26° and atmospheric pressure, and 20 mg 10% Pd on C catalyst. Rapid uptake of hydrogen was noted during the first 10 min (1 mol equiv.), and after 30 min 1.4 mol equiv. H_2 had been consumed. Analytical TLC of the reaction mixture (49 mg) revealed the presence of five major and several minor products. The mixture was separated by HPLC on μ-Porasil yielding, in order of elution: **9**, 12 mg (4.5 min); **10**, 3 mg (5.4 min); **8**, 6 mg (7.2 min); **11**, 4 mg (8.3 min); and **7**, 6 mg (11.0 min).

Characterization of **7** *and its acetate* (**12**). Hydrogenation product **7** (6 mg) gave ^1H NMR data (Table 1) consistent with the structure, but traces of other materials were evident by TLC and NMR; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510, 3400, 2940, 2890, 1700, 910; EIMS *m/z* (rel. int.): 313 $[MH-H_2O]^+$ (100), 294 (18). Acetylation of **7** (4 mg) in the usual manner, followed by prep. TLC, HPLC and recrystallization from Et_2O gave **12**, 3 mg; mp 159–161°; ^1H NMR, see Table 1; CIMS *m/z* (rel. int.): 414 $[MH]^+$ (23), 354 (100), 294 (51).

Characterization of **8** *and its acetate* (**8a**). Hydrogenation product **8** (6 mg) appeared homogeneous by TLC and HPLC, but failed to crystallize: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3380, 2960, 2870, 1700; ^1H NMR, see Table 1; EIMS *m/z* (rel. int.): 314 $[MH]^+$ (7), 197 (17), 141 (13), 112 (25), 99 (96), 85 (91), 71 (100), 55 (37). Acetylation of **8** (5 mg), followed by prep. TLC and HPLC, gave **8a** (4 mg) which also appeared to be homogeneous by all criteria, but failed to crystallize: ^1H NMR, see Table 1; EIMS *m/z* (rel. int.): 356 $[MH]^+$ (1), 99 (81), 85 (83), 71 (86), 55 (32), 43 (100); CIMS *m/z* (rel. int.): 356 $[MH]^+$ (100), 314 (34), 296 (39).

Characterization of **9** *and its acetate* (**9a**). Compound **9** (12 mg) was obtained approximately 90–95% pure, after TLC and HPLC, and gave a ^1H NMR spectrum consistent with the structure shown (Table 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 3390, 2900, 2860, 1700, 910. Acetylation of **9** (10 mg), followed by prep. TLC and HPLC, gave **9a** (2 mg): ^1H NMR, see Table 1; EIMS *m/z* (rel. int.): 351 $[M]^+$ (2), 291 (1), 179 (23), 167 (20), 125 (96), 43 (100); CIMS *m/z* (rel. int.): 352 $[MH]^+$ (100).

Characterization of **10** *and its acetate* (**10a**). Hydrogenation product **10** (3 mg) crystallized from Et_2O: mp 100–105°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2930, 2880, 1700; ^1H NMR, see Table 1; EIMS *m/z* (rel. int.): 314 $[MH]^+$ (16), 154 (34), 142 (31), 112 (38), 99 (100), 85 (24), 70 (39), 55 (38); CIMS *m/z* (rel. int.): 314 $[MH]^+$ (100). Acetylation of **10** (2.5 mg), followed by TLC and HPLC, gave **10a** (0.8 mg) and unreacted **10** (1.2 mg). Acetate **10a** was obtained as a colorless glass: ^1H NMR, see Table 1; EIMS *m/z* (rel. int.) 356 $[MH]^+$ (0.1), 314 (0.1), 99 (100), 55 (18), 43 (88); CIMS *m/z* (rel. int.), 356 $[MH]^+$ (100), 314 (63).

Characterization of **11** *and its acetate* (**11a**). Hydrogenation product **11** (4 mg) was isolated as a colorless glass: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3410, 2930, 2880, 1710; ^1H NMR, see Table 1; EIMS *m/z* (rel. int.): 314 $[MH]^+$ (3), 154 (23), 141 (49), 115 (37), 99 (100), 85 (40), 70 (48), 55 (59). Acetylation of **11** (1.9 mg), followed by TLC and HPLC, gave **11a** (1.0 mg) as a colorless glass: ^1H NMR, see Table 1; EIMS *m/z* (rel. int.): 183 (13), 141 (35), 99 (76), 55 (25), 43 (100); CIMS *m/z* (rel. int.): 356 $[MH]^+$ (100), 314 (31).

Reaction of **4** *with sodium bicarbonate in aq.* MeOH. A 23 mg portion of **4** was dissolved in 2.5 ml 1% $NaHCO_3$ soln in MeOH–H_2O (3:1) at ambient temp., and progress of the reaction was monitored by TLC. After 5 hr, a major spot of higher R_f (**13**) was predominant, although there was some remaining **4**. At 18 hr, substantial **13** remained, two more polar compounds (**14**, **15**) were evident, and **4** was no longer detectable. Products (19 mg) were recovered by diluting the mixture with 2 ml H_2O and extracting them into CH_2Cl_2. Separation of these by prep. TLC then yielded **13** (4 mg), **14** (3 mg) and **15** (4 mg). Compound **13** was obtained as a colorless oil: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 2935, 1740, 1710; UV λ_{max}^{MeOH} nm (ε): 257 (2880); $[\alpha]_D^{23}$ −52° (*c* 0.172; $CHCl_3$); ^1H NMR and ^{13}C NMR, see Tables 1 and 2; EIMS *m/z* (rel. int.): 351 $[M]^+$ (1), 113 (28), 85 (13), 67 (30), 43 (100). Found for $[M]^+$: *m/z* 351.1342; $C_{17}H_{21}NO_7$ requires: *m/z* 351.1318. CIMS *m/z* (rel. int.): 352 $[MH]^+$ (100), 322 (17), 292 (27), 262 (12). Compound **14** was obtained as a colorless oil: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3425, 2940, 1730 (br); ^1H NMR, see Table 1; CIMS *m/z* (rel. int.): 384 (100), 354 (31). Compound **15** was obtained as a colorless oil: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3425, 2940, 1730 (br); ^1H NMR, see Table 1; CIMS *m/z* (rel. int.): 384 (100), 354 (38), 292 (24).

Acknowledgements—Comparison samples of cycloheximide, streptovitacin A and streptimidone were obtained from Dr. Matthew Suffness, National Cancer Institute. We thank Dr. W. K. Rohwedder and Ronald Plattner for mass spectra and Barry Jones and Tim Mueser for technical assistance. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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NOTE ADDED IN PROOF

While this paper was in press, a paper was published by Gorst-Allman, C. P., Steyn, P. S., Vleggaar, R. and Grobbelaar, N. (1984) *J. Chem. Soc. Perkin Trans.* 1, 1311, reporting the isolation of sesbanimide A from *Sesbania punicea* and confirmation of its structure by NMR. These authors found that in solvents more polar than $CHCl_3$ (i.e. MeOH, pyridine and DMSO) sesbanimide exists as an equilibrium mixture of **1** and **4a**.