The above-described method for in situ preparation of oxyfluoro reagents from elemental fluorine promises great versatility and possesses a broad potential in organofluoro chemistry.[16]

References and Notes

(1) D. H. R. Barton, L. S. Godhino, R. H. Hesse, and M. M. Pechet, *Chem. Commun.*, 804 (1968); D. H. R. Barton, L. J. Danks, A. K. Ganguly, R. H. Hesse, G. Tarzia, and M. M. Pechet, *ibid.*, 227 (1969).
(2) In fact, perchloryl fluoride can partly be considered as an electrophilic fluorinating reagent, but its hazardous nature and its serious limitation as a fluorinating reagent are well documented (e.g., see ref 3).
(3) R. H. Hesse, *Isr. J. Chem.*, **17**, 60 (1978), and references therein.
(4) D. H. R. Barton, R. H. Hesse, R. E. Markwell, M. M. Pechet, and S. Rozen, *J. Am. Chem. Soc.*, **98**, 3036 (1976).
(5) J. H. Prager and P. G. Thompson, *J. Am. Chem. Soc.*, **87**, 230 (1965); P. G. Thomson and J. H. Prager, *ibid.*, **89**, 2263 (1967).
(6) The use of CF_3CF_2OF is mentioned in three patents. When irradiated by UV, it served as a fluorine radical source for the following reactions; $C_6H_{12} \rightarrow C_6H_{11}F$; $C_6H_6 \rightarrow C_6H_5F$. See, for example, J. Kollonitsch (to Merck and Co., Inc.), U.S. Patent 4 030 994 (1977).
(7) The oxidation power of the reaction mixture can be determined by titration of the iodine liberated from acidic solution of KI.
(8) G. L. Gard and G. H. Cady, *J. Inorg. Chem.*, **4**, 594 (1965).
(9) This product was also synthetized by one of the authors (S. Rozen) during his work with D. H. R. Barton, R. Hesse, and M. Pechet in the Research Institute for Medicine and Chemistry, Cambridge, Mass. He is very grateful for the opportunity to work in their laboratories.
(10) The stereochemistry of the isomers **4** is evident from their NMR (^1H and ^{19}F) spectra. The erythro isomers possess coupling constants J_{H-H} smaller than those of the threo isomers. See also D. H. R. Barton, R. H. Hesse, G. P. Jackmann, L. Ogunkoya, and M. M. Pechet, *J. Chem. Soc., Perkin Trans. 1*, 739 (1974). In addition in the erythro isomers the fluorine nuclei resonates at higher field than in the threo ones.
(11) While CF_3OF, when reacting with olefins, yields considerable amounts of the undesired difluorides (**5**) (see ref 3, 10), we found that **1** gives only very small amounts of them.
(12) S. Nakanishi and E. V. Jensen, *J. Org. Chem.*, **27**, 702 (1962).
(13) Trifluoroacetyl hypofluorite (**9**) is known; see ref 8. However, as in **1**, the tedious low-yield synthesis have completely prevented its synthetic use.
(14) M. Zupan and A. Pollak, *Tetrahedron*, **33**, 1017 (1977).
(15) G. Aranda, J. Jullien, and J. A. Martin, *Bull. Soc. Chim. Fr.*, 1890 (1965); 2850 (1966).
(16) All new compounds had the correct composition established by microanalysis. Their spectral data (IR, UV, ^{19}F and ^1H NMR, and mass spectra) are in excellent agreement with the assigned structures and stereochemisty.

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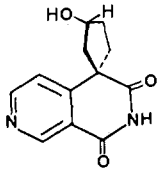
Sesbanine, a Novel Cytotoxic Alkaloid from *Sesbania drummondii*

Sir:

An earlier report from our laboratory disclosed the potent antileukemic activity associated with extracts from seeds of *Sesbania drummondii* (Leguminosae), a native plant with a history of toxicity to livestock.[1] We now report the isolation and structural elucidation of sesbanine (**1**), a cytotoxic compound in the extract. Sesbanine has a previously unreported and highly unusual spirocyclic structure based on the 2,7-naphthyridine nucleus.



1

Sesbanine (~50 mg) was isolated from the ethanol extract of *S. drummondii* seed (450 kg) through a multistage fractionation procedure that included a series of solvent partitioning steps,[2] a 10-stage countercurrent distribution, chromatography on silica, removal of polyphenolics by extraction with aqueous sodium carbonate, chromatography on Sephadex G-10, and finally high-performance liquid chromatography in two stages on reversed-phase columns. This sequence provided crystalline **1**, $C_{12}H_{12}N_2O_3$ (M + 1, *m/e* 233.0907; $C_{12}H_{13}N_2O_3$ requires *m/e* 233.0888):[3] mp 240–243 °C (MeOH); $[\alpha]^{23}_D$ +14.6° (*c* 0.56, MeOH); IR 3510, 3490, 1710, 1690, 1600 cm^{-1} (KBr);[4] UV λ_{max}^{MeOH} 228 nm (ϵ 10 500). The ^1H NMR spectrum in $CDCl_3$–CD_3OD (1:1) showed aromatic protons at δ 9.26 (d, J = 1 Hz, H-8), 8.88 (dd, $J_{6,8}$ = 1, $J_{5,6}$ = 6 Hz, H-6), and 7.94 (d, $J_{5,6}$ = 6 Hz, H-5);[5] the H-10 proton was apparent (δ 4.74, m), but remaining protons appeared in poorly resolved upfield multiplets (1.8–2.5). In Me_2SO-d_6, the ^1H NMR spectrum showed δ 8.5–9.4 (br m, H-8 and H-6), 7.85 (d, $J_{5,6}$ = 6 Hz, H-5), 4.50 (m, H-10), 2.65 (dd, J = 7.14 Hz, B portion of ABM system, one H-9 proton),[6] 1.7–2.8 (br m, remaining H-9, H-11, H-12 protons). ^{13}C NMR spectra of **1** are summarized in Table I. Since the available spectral data did not specify a structure for sesbanine and the limited amount precluded an extensive chemical study, a single-crystal X-ray crystallographic study was undertaken.

Table I. ^{13}C NMR Chemical Shift Assignments for Sesbanine[a]

position	in Me_2SO-d_6	in pyridine-d_5
1	177.2	178.5 (s)
3	163.8	165.0 (s)
4	52.1	53.2 (s)
4a	155.8	156.6 (s)
5	121.7[b]	*c*
6	148.3	*c*
8	153.9	154.5 (d)
8a	121.7[b]	*c*
9	48.5	49.7 (t)
10	72.7	73.9 (d)
11	36.1	37.8 (t)
12	42.4	43.9 (t)

[a] Chemical shifts (δ) are in parts per million from tetramethylsilane. For pyridine-d_5 spectrum, multiplicities of signals were determined by partial decoupling. [b] Overlapping signals. [c] Signal obscured by solvent peaks.

Sesbanine (**1**) crystallized as flat plates in the monoclinic crystal system. Accurate lattice constants, obtained by carefully centering 15 high angle reflections, were a = 8.00 (3), b = 14.665 (5), c = 9.549 (4) Å; β = 70.69 (3)°. The systematic absences and known chirality indicated space group $P2_1$, and the cell volume required *two* molecules of $C_{12}H_{12}N_2O_3$ in the asymmetric unit for a physically reasonable density. The extremely limited amount of sesbanine precluded a density measurement. All available crystals of sesbanine had considerable mosaic spread in their diffraction maxima. Intensity data were collected on a fully automated four-circle diffractometer using graphite monochromated Mo Kα radiation (0.71069 Å) and a variable-speed, 2.5° ω scan. A total of 2306 unique diffraction maxima with $2\theta \leq 50.0°$ were collected in this fashion, and, after correction for Lorentz, polarization, and background effects, 2116 (92%) were judged observed ($F_o \geq 3\sigma(F_o)$).[7] During data collection, it became apparent that, while the crystal must belong to space group $P2_1$ and have two independent molecules, it could be approximately described as being $P2_1/a$ ($h0l$, h = $2n$ + 1, very weak) with only one independent molecule. In view of the limited data at high 2θ values and the size of the structure, we elected to determine an approximate phasing model in the centrosymmetric space group $P2_1/a$. Most of the molecule was revealed by this procedure except for the C(10), C(11), and O(15) fragment. After lowering the symmetry to $P2_1$ and carrying out full-matrix least-squares refinements with anisotropic nonhydrogen atoms and fixed isotropic hydrogens, the conventional crystallographic discrepancy index was 0.059 for the observed data.

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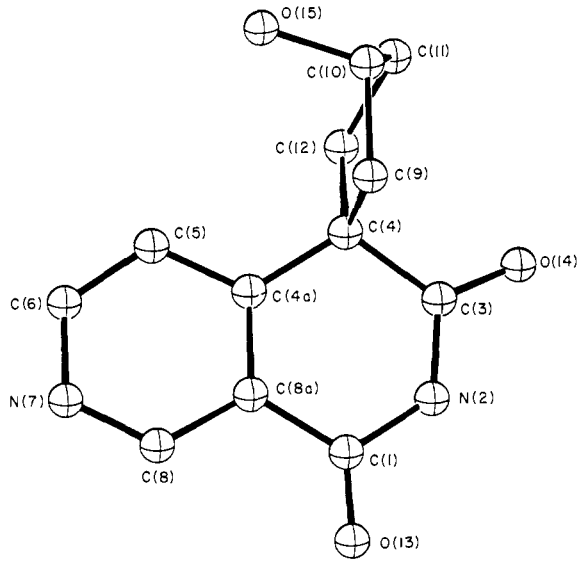

Figure 1. A computer-generated drawing of sesbanine. Hydrogens are omitted for clarity and no absolute configuration is implied.

Both independent molecules have the same configuration and a computer-generated perspective drawing of one of them is shown in Figure 1. During least-squares refinements, there was a marked correlation between pesudosymmetry related coordinates and, with the exception of C(10), C(11), and O(15), all final atomic positions are related within ~0.2 Å by the rule ($x' = \frac{3}{2} - x, y' = 1 - y, z' = 1 - z$). The X-ray experiment defines only the relative configuration of sesbanine as C(4) (R^*) and C(10) (R^*).

Bond lengths in the planar heterocyclic nucleus of **1** are in excellent agreement with generally accepted values. The spirocyclopentane fragment is less well behaved. The cyclopentane ring is best described as having the envelope conformation with C(11) serving as the flap and the three bonds between C(9), C(10) and C(11), and C(12) are all several hundredths of an Å shorter than expected. It is not clear whether this is due to the quality of the diffraction data, the breakdown of the pseudosymmetry in this region of the molecule, or some disordering process in the cyclopentane ring. Curiously, the two independent molecules do not make any close contacts with each other and all hydrogen bonds are formed between a molecule and its symmetry related mates. The hydrogen bond lengths follow: O(15) H-O(13), 2.82; O(15′) H-O(13′), 2.82; N(2) H-N(7), 2.87; N(2′) H-N(7′), 2.91 Å. There are no other abnormally short intermolecular contacts. The supplementary material described at the end of this paper contains further crystallographic details.

We propose the trivial name sesbanine (**1**) for this metabolite.[8] There are no reports of closely related compounds in the literature and further work in our laboratory will explore the chemistry of sesbanine and related metabolites. The isolation of sesbanine was originally guided by both in vivo (P388 leukemia) and in vitro (KB cell culture) bioassays[9] which showed parallel results. The scarcity of material precluded reliance on the in vivo assay in the latter stages of the isolation and pure sesbanine was isolated by the in vitro cell culture.

Acknowledgment. The diffractometer used in this work was purchased with a National Science Foundation equipment grant. We thank David King and Dr. W. K. Rohwedder for chemical ionization mass spectra; Dr. G. J. Jordan, Lederle Laboratories, Pearl River, N.Y., for an FT IR spectrum; and Dr. R. E. Perdue, U.S. Department of Agriculture, Beltsville, Md., for *S. drummondii* seed.

Supplementary Material Available: Tables of fractional coordinates, bond distances, bond angles, and structure factors for sesbanine (12 pages). Ordering information is given on any current masthead page.

References and Notes

(1) R. G. Powell, C. R. Smith, and R. V. Madrigal, *Planta Med.*, **30**, 1 (1976), and references therein.
(2) The initial stages of the isolation were carried out essentially as described in ref 1 except that conditions were manipulated so as to obtain the active principle in fraction D (Scheme I) instead of fraction C.
(3) The high-resolution chemical ionization–mass spectrum of **1** was carried out with a Kratos MS-30 instrument using methane as the reacting gas. 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 firms or similar products not mentioned.
(4) The IR spectrum of homophthalimide shows 3387, 1698, 1714 cm^{-1} (CCl_4): G. Pangon, G. Thullier, and P. Rumpf, *Bull. Soc. Chim. Fr.*, 1991 (1970).
(5) Compare with ^1H NMR spectrum of isoquinoline: P. J. Black and M. L. Heffernan, *Aust. J. Chem.*, **19**, 1287 (1966).
(6) The M portion of this ABM system is at δ 4.5 (verified by irradiation) and the A portion is obscured in upfield multiplets (1.7–2.8).
(7) All crystallographic calculations were done on a Prime 400 computer operated by the Materials Science Center and the Department of Chemistry, Cornell University. The principle programs used were REDUCE and UNIQUE, data reduction programs, M. E. Leonowicz, Cornell University, 1978; BLS, block-diagonal least-squares refinement, K. Hirotsu, Cornell University, 1978; ORFLS (modified), full-matrix least squares, W. R. Busing, K. O. Martin, and H. S. Levy, Oak Ridge, ORNL-TM-305; ORTEP, crystallographic illustration program, C. Johnson, Oak Ridge, ORNL-3794; BOND, structural parameters and errors, K. Hirotsu, Cornell University, 1978; MULTAN-76, direct methods and fast fourier transform, G. Germain, P. Main, and M. Woolfson, University of York.
(8) Dr. Kurt L. Loening of CAS has kindly informed us that the proper IUPAC name is *trans*-3-hydroxyspiro[cyclopentane-1,4′(1′*H*)-[2,7]naphthyridine]-1′,3′(2′H)-dione.
(9) R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, B. J. Abbott, *Cancer Chemother. Rep.*, **3**(3), 17 (1972).

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Stopped-Flow Circular Dichroism (SFCD) Spectroscopy. Implication of Significant Conformational Differences in the Redox Mechanism of Cytochrome *c*

Sir:

Recently we found preliminary evidence that cytochrome *c* (cyt *c*)[1] undergoes a considerable and rapid conformation change associated with the exchange of the sixth ligand of heme *c* during rapid reductions with some inorganic reductants.[2] This conclusion was based principally on the following observations from SFCD spectroscopy: (1) a remarkably enhanced CD absorption appeared at the early stages of the reaction between the alkaline form of cyt c^{III} and the reductant (dithionite), indicating rapid formation of a complex ($\tau < 5$ ms); (2) the subsequent rapid appearance of an intense transient peak ($\tau \sim 15$ ms) was followed by its relatively slow disappearance ($\tau \sim 40$ ms) leading to the final absorption of cyt c^{II}.

This observation indicates that an unstable (transient) cyt c^{II} having a markedly enhanced and distinct positive rotational strength was formed from other stable species; then it converted to native cyt c^{II} relatively slowly with a substantial conformation change ($k_1 = 17\ s^{-1}$, 28 °C). Previously reported SFCD spectroscopy[3] is restricted only to changes (at 222 nm) which do not afford direct information about conformational changes of an active site, but provide some indirect information.

Now we report interesting and significant results of the

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