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Polyhydroxy Components of Celastraceae Alkaloids. The Structure of Euonyminol, $C_{15}H_{26}O_{10}$*

BY IVAN F. TAYLOR JR AND W. H. WATSON

FASTBIOS Laboratory, Department of Chemistry, Texas Christian University, Fort Worth, Texas 76129, *USA*

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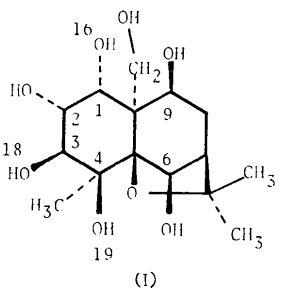
Euonyminol [octahydro-2,5a-bis(hydroxymethyl)-2,9-dimethyl-2*H*-3,9a-methano-1-benzoxepin-4,5,6,7,8,-9,10-heptol] is an alkaline hydrolysis product of the alkaloids isolated from *Tripterygium wilfordii* Hook (Celastraceae). In the parent alkaloids the polyhydroxy nucleus has eight esterified hydroxyl groups, one free hydroxyl group and an O atom involved in a cyclic ether linkage. Euonyminol crystallizes in the monoclinic space group *C*2 with cell dimensions a = 16·323 (7), b = 8·314 (7), c = 11·809 (5) Å and β = 96·7 (1)°. Data were collected on an automatic diffractometer and the structure was solved by direct methods. The 25-atom model was refined to an R index of 0·056. The structure consists of two *trans*-fused six-membered rings and a five-membered cyclic ether formed by fusion of two axial substituents. Euonyminol is related to the polyhydroxy nuclei of other Celastraceae alkaloids such as maytoline and evonine, and a best molecular fit program has been used to compare the structures.

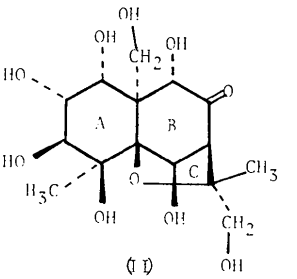
Introduction

The roots of *Tripterygium wilfordii* Hook (Celastraceae), the 'Thunder God Vine', have been used for centuries by Chinese gardners to protect their crops against chewing insects. The active substances are found in the roots and are nontoxic to warm-blooded animals. Extracts of the roots yield five insecticidal ester alkaloids (Beroza, 1951, 1952, 1953*a*,*b*, 1963). The alkaloids upon alkaline hydrolysis yield 5 mol of acetic acid, 1 mol of benzoic acid or 3-furoic acid, 1 mol of dibasic wilfordic or hydroxywilfordic acid and a polyhydroxy compound formulated as $C_{15}H_{26}O_{10}$. The polyhydroxy compound was reported to have ten hydroxyl groups, but chemical studies, NMR and mass spectrometry failed to provide a satisfactory structural assignment (Waller & Lee, 1971; Lee, 1972). Alkaloids isolated from the seeds of *Euonymus europaea* L. (Celastraceae) also gave upon hydrolysis a polyhydroxy compound of the same formula (Pailer & Libiseller, 1962*a*,*b*).

During the last few years a number of complex alkaloids have been isolated from members of the Celastraceae family and their structures established. These compounds upon hydrolysis yield polyhydroxy nuclei which differ slightly in composition but their structural relationship is evident. Maytoline isolated from *Maytenus ovatus* Loes (Kupchan, Smith & Bryan 1970) has two free OH groups and yields nicotinic acid and four acetates upon hydrolysis. The crystal structure of maytoline methiodide was determined (Bryan & Smith, 1971) and the conformation and relative stereochemistry of the polyhydroxy moiety, $C_{15}H_{26}O_8$ (I), are known.



(I)



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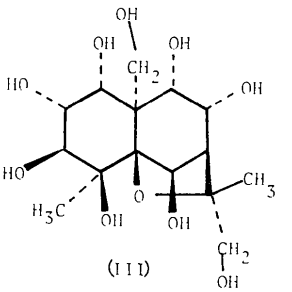

(III)

Evonine, isolated from *Euonymus sieboldiana* Blume, yields upon hydrolysis five acetates, evoninic acid and a polyhydroxy residue, $C_{15}H_{24}O_{10}$. The crystal structure of bromoacetylneoevonine monohydrate has been determined (Sasaki & Hirata, 1972) from which the structure of evonine and the polyhydroxy moiety (II) were deduced.

In 1966 we collected data on the polyhydroxy moiety isolated from *Tripterygium wilfordii* Hook. Early attempts to solve the structure were unsuccessful;

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Table 1. *Crystal and molecular data*

$C_{15}H_{26}O_{10}$, M_r = 366·37, a = 16·323 (7), b = 8·314 (7), c = 11·809 (5) Å, β = 96·7 (1)°; systematic absences hkl, $h + k = 2n + 1$, space group $C2$, Cm or $C2/m$; U = 1591·7 Å^3, Z = 4, D_c = 1·529, D_o (flotation) = 1·528 g cm^{-3}, $F(000)$ = 784, μ = 11·2 cm^{-1}

however, in 1973 application of improved direct-methods programs led to a solution. During the course of the investigation, a correct NMR structural assignment was reported for wilfordine (Shizuri, Yamada & Hirata, 1973) which gave the correct structure for euonyminol (III). Recent interest in these complex biologically active molecules prompted us to refine the structure of euonyminol from the original set of data.

Experimental

A small sample of euonyminol, isolated from *Tripterygium wilfordii*, was supplied by Beroza. The polyhydroxy compound forms colorless crystals when isolated from methanol and has no definite melting point but darkens when heated above 240°C. No optical activity data have been reported. Space group and unit-cell dimensions were determined from precession, oscillation and Weissenberg photographs. Crystal data are given in Table 1.

A crystal of dimensions 0·1 × 0·3 × 0·1 mm was mounted on a Philips Pailred diffractometer with the b axis coincident with the spindle axis. The intensity data, $h0l$ through $h6l$, were collected using equi-inclination Weissenberg geometry and the ω-scan technique. Background counts were taken for 20 s on either side of the scan and a scan range of 3° at 1° min^{-1} was used. Cu $K\alpha$ radiation (λ = 1·54178 Å) and a graphite monochromator were used. Out of the 1280 measured independent reflections 1112 had intensities greater than $3\sigma(I)$. Lorentz and polarization corrections were applied, but no absorption corrections were made. The scattering factors of Cromer & Waber (1965) were used for the C and O atoms while those of Stewart, Davidson & Simpson (1965) were used for H atoms.

Structure determination and refinement

Structure factors were normalized by applying scale and temperature factors obtained from a Wilson plot. The statistical distribution of $|E|$'s fell between the theoretical values expected for centrosymmetric and non-centrosymmetric space groups. Waller & Lee (1971) proposed a structure containing a mirror plane, but attempts to solve the structure in space group $C2/m$ were unsuccessful. The space group was assumed to be $C2$ and subsequent refinement confirmed this choice.

400 reflections with the largest $|E|$ values and 38 reflections with $E = 0·0$ (for PSI ZERO computations) were phased by the direct methods program *MULTAN* (Germain, Main & Woolfson, 1971). The phase set with the lowest PSI ZERO value was selected and an E map revealed a chemically meaningful model containing 19 atoms. Structure factor calculations based on the 19-atom fragment yielded an acceptable R value of 30% where $R = [\Sigma \Delta F_{meas}/\Sigma |F_{meas}|]$.

The remaining six atoms could not be located in subsequent Fourier syntheses. The phases were

Table 2. *Positional parameters* (×10^4; ×10^3 *for* H) *for euonyminol*

	x	*y*	*z*
C(1)	6445 (3)	5992 (–)	861 (4)
C(2)	5898 (4)	7362 (11)	1182 (5)
C(3)	6377 (4)	8444 (11)	2066 (6)
C(4)	6792 (4)	7541 (11)	3122 (5)
C(5)	7318 (3)	6096 (10)	2756 (4)
C(6)	7789 (4)	5100 (10)	3703 (5)
C(7)	8518 (4)	4512 (11)	3095 (5)
C(8)	8192 (4)	3206 (10)	2257 (6)
C(9)	7491 (4)	3823 (10)	1403 (5)
C(10)	6829 (3)	4933 (10)	1874 (4)
C(11)	8775 (4)	6082 (10)	2523 (5)
C(12)	9276 (4)	7236 (11)	3350 (6)
C(13)	9238 (4)	5864 (12)	1479 (6)
C(14)	6155 (4)	7087 (14)	3944 (6)
C(15)	6146 (4)	3926 (10)	2311 (5)
O(16)	5988 (3)	5049 (8)	−12 (4)
O(17)	5154 (3)	6797 (9)	1603 (4)
O(18)	6956 (3)	9333 (8)	1501 (5)
O(19)	7355 (3)	8698 (8)	3708 (4)
O(20)	7975 (2)	6835 (7)	2163 (3)
O(21)	8040 (3)	5980 (8)	4718 (3)
O(22)	7956 (3)	1826 (8)	2883 (5)
O(23)	7105 (3)	2543 (8)	704 (4)
O(24)	10018 (3)	6461 (9)	3847 (4)
O(25)	6398 (4)	2660 (10)	3074 (6)
H(1)*	688	646	45
H(2)	571	802	49
H(3)	602	933	231
H(6)	746	413	388
H(7)	894	404	367
H(8)	863	277	184
H(9)	772	440	76
H(12)	896	760	397
H(12′)	941	825	292
H(13)	894	493	93
H(13′)	978	539	172
H(13″)	927	692	107
H(15)	579	348	165
H(15′)	578	467	268
H(17)	481	622	113
H(18)	747	877	119
H(19)	759	793	439
H(21)	808	527	541
H(24)	1051	740	434

* 19 H atom positions were calculated or located in difference Fourier maps. Three methyl H atoms and four hydroxyl H atoms could not be located. An isotropic temperature factor of magnitude B = 3·0 Å^2 was assigned to each H atom. H atom parameters were not refined.

recycled through the *CONVERGE* section of *MULTAN*. Phases used in the recycling procedure were calculated from the 19-atom fragment and were considered 'known' if $|E| > 1{\cdot}5$ and $|F_c| > 0{\cdot}7\ |F_o|$. One additional general reflection was selected to generate eight more solutions. The original origin- and enantiomorph-defining reflections were retained. Again the phase set with the lowest PSI ZERO value was chosen and the associated *E* map yielded the complete molecular structure plus an additional possible atomic position. The additional atom was rejected in the least-squares refinement. Recycling only through the tangent refinement section of the program did not lead to a solution.

Isotropic least-squares refinement was complete at $R = 0{\cdot}10$. Introduction of anisotropic thermal parameters reduced R to 0·067. Atomic positions for the C(13) methyl H, five hydroxyl H and all methylene H atoms were obtained from a difference Fourier map; however, calculated positions for the methylene H atoms were used in subsequent calculations. 19 of the 26 H atom positions were introduced into a block-diagonal least-squares refinement with fixed positional and thermal parameters. Refinement was terminated at $R = 0{\cdot}056$ and $R_\omega = 0{\cdot}077$ where $R_\omega = [\Sigma\,\omega(\Delta F_{meas})^2/\Sigma\,\omega F^2_{meas}]^{1/2}$. The function minimized was $\Sigma\,\omega(\Delta F_{meas})^2$ where $\omega = 1/(\Delta F)^2$. In the final stages of refinement values of average ΔF *versus* average $|F_o|$ were fitted by least-squares calculations to a straight line yielding $\Delta F = 0{\cdot}6635 + 0{\cdot}0203|F_o|$.

A final difference Fourier map showed no peaks as large as that expected for a H atom. The final atomic positional parameters are given in Table 2.*

Discussion

An *ORTEP* drawing of the euonyminol molecule is shown in Fig. 1. H atoms attached to C(14), O(16), O(22), O(23) and O(25) could not be located in the difference Fourier maps and do not appear in the drawing. Table 3 lists interatomic distances and angles.

Table 4 lists the torsion angles of euonyminol and compares them with those of the polyhydroxy moieties of evonine and maytoline. Ring *B* of euonyminol exists in a distorted chair conformation with distortion arising from fusion of the C(5) and C(7) axial substitutents. The torsion angles in evonine are quite similar to those in euonyminol, but the strain in the *B* ring of maytoline as reflected by the torsion angles appears to be greater. This must be associated with additional repulsive interactions created by the change in stereochemistry at C(9). The C(9) substituents in the euonyminol and evonine molecules are equatorial while the ester group in maytoline is axial. This sets up additional axial–axial interactions with the fused five-membered ring. The five-membered rings in euonyminol and evonine exist in half-chair conformations while the ring in maytoline is close to an envelope conformation. The *A* ring in euonyminol is close to an ideal chair conformation with some distortion around C(3), C(4) and C(5) because of strain transmitted from the fusion of the C(5) and C(7) axial substituents. Because the hydroxyl groups in evonine and maytoline are esterified, the axial interactions are larger and the *A* rings exhibit a flatter conformation. The external angles in all three molecules are similar except for differences created by the change in stereochemistry at C(9) in maytoline and a difference in the solid-state orientation of the O(24) hydroxyl groups in euonyminol and evonine. Numerous inter- and intramolecular hydrogen bonds are possible and some of them are indicated in Table 3.

Identical fragments of the euonyminol, maytoline and evonine molecules were compared using a best molecular fit program (Nyburg, undated, Univ. of Toronto, Canada). The pair-wise fitting of 21 and 22 atoms of the three molecules showed that the conformation of euonyminol is intermediate between the conformations of the polyhydroxy moieties of maytoline and evonine. The sum of the squares of the differences for euonyminol–maytoline and euonyminol–evonine is approximately 0·2 Å^2 while that for the evonine–maytoline pair is 0·4 Å^2.

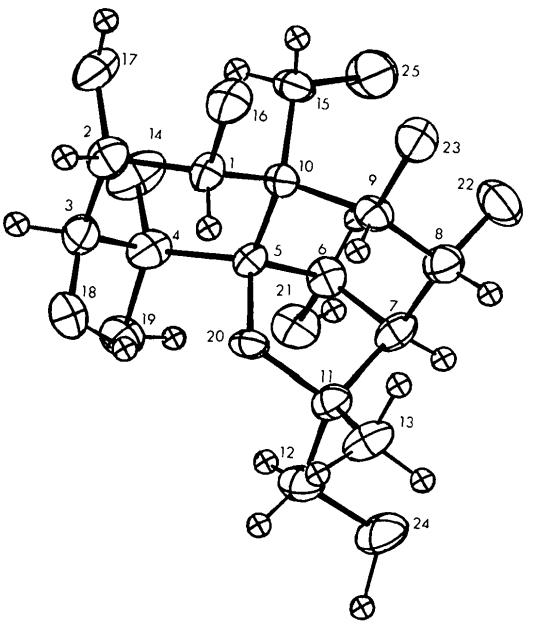

Fig. 1. *ORTEP* drawing of euonyminol. Atoms 16 through 25 are O atoms.

* Lists of structure factors and thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32653 (8 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 3. *Bond distances* (Å) *and bond angles* (°)

C(1)–C(2)	1·522 (8)	C(5)–C(6)	1·525 (9)	C(8)–C(9)	1·524 (9)
C(1)–C(10)	1·556 (8)	C(5)–C(10)	1·569 (9)	C(9)–C(10)	1·571 (9)
C(1)–O(16)	1·434 (7)	C(5)–O(20)	1·481 (7)	C(9)–O(23)	1·446 (9)
C(2)–C(3)	1·523 (11)	C(6)–C(7)	1·539 (8)	C(10)–C(15)	1·532 (9)
C(2)–O(17)	1·444 (8)	C(6)–O(21)	1·423 (8)	C(11)–C(12)	1·535 (10)
C(3)–C(4)	1·543 (10)	C(7)–C(8)	1·523 (11)	C(11)–C(13)	1·531 (9)
C(3)–O(18)	1·425 (9)	C(7)–C(11)	1·551 (11)	C(11)–O(20)	1·466 (7)
C(4)–C(5)	1·567 (10)	C(4)–C(14)	1·550 (9)	C(12)–O(24)	1·435 (8)
C(4)–O(19)	1·449 (9)	C(8)–O(22)	1·441 (10)	C(15)–O(25)	1·415 (11)
Intramolecular distances					
O(18)–O(20)	2·719 (8)	H(18)–O(20)	2·090	O(19)–O(21)	2·732 (8)
H(19)–O(19)	1·064	H(19)–O(21)	1·802	H(18)–O(18)	1·051
O(22)–O(23)	2·841 (7)	O(22)–O(25)	2·670 (7)	O(16)–O(23)	2·832 (8)
O(16)–O(17)	2·865 (7)	O(18)–O(19)	2·665 (7)	O(19)–O(20)	2·680 (7)
Intermolecular distances					
O(24)–O(25)[a]	2·717 (7)	O(19)–O(22)[b]	2·983 (9)	O(18)–O(22)[b]	3·000 (9)
H(24)–O(25)[a]	2·215	O(18)–O(23)[b]	2·850 (9)	O(16)–O(17)[c]	2·881 (7)
H(17)[c]–O(16)	2·001	O(19)–O(21)[d]	2·785 (8)	H(21)[d]–O(19)	1·859
C(1)C(2)C(3)	110·1 (5)	C(2)C(3)O(18)	107·5 (6)	C(7)C(11)C(13)	115·8 (7)
C(2)C(3)C(4)	114·1 (7)	C(4)C(3)O(18)	112·2 (5)	C(12)C(11)C(13)	108·1 (5)
C(3)C(4)C(5)	110·7 (5)	C(3)C(4)C(14)	111·2 (5)	O(20)C(11)C(12)	108·0 (6)
C(4)C(5)C(10)	113·8 (5)	C(3)C(4)O(19)	104·9 (7)	O(20)C(11)C(13)	108·9 (5)
C(5)C(10)C(1)	106·8 (5)	C(5)C(4)O(19)	107·6 (5)	C(8)C(7)C(11)	114·2 (5)
C(10)C(1)C(2)	115·3 (5)	C(5)C(4)C(14)	114·5 (7)	C(10)C(5)O(20)	106·4 (4)
C(5)C(6)C(7)	100·5 (5)	C(14)C(4)O(19)	107·3 (6)	C(7)C(8)O(22)	109·2 (6)
C(6)C(7)C(8)	107·4 (5)	C(4)C(5)O(20)	105·2 (6)	C(9)C(8)O(22)	112·5 (5)
C(7)C(8)C(9)	111·6 (7)	C(4)C(5)C(6)	117·3 (5)	C(8)C(9)O(23)	111·9 (7)
C(8)C(9)C(10)	117·2 (5)	C(6)C(5)O(20)	103·9 (4)	C(10)C(9)O(23)	111·3 (5)
C(9)C(10)C(5)	106·2 (4)	C(5)C(6)O(21)	114·2 (6)	C(9)C(10)C(15)	110·9 (6)
C(10)C(5)C(6)	109·0 (6)	C(7)C(6)O(21)	113·2 (5)	C(9)C(10)C(1)	107·0 (4)
C(10)C(1)O(16)	111·9 (4)	C(6)C(7)C(11)	101·5 (6)	C(5)C(10)C(15)	116·5 (5)
C(2)C(1)O(16)	108·5 (4)	C(5)O(20)C(11)	110·8 (5)	C(1)C(10)C(15)	108·9 (5)
C(1)C(2)O(17)	112·6 (6)	C(7)C(11)O(20)	102·0 (4)	C(10)C(15)O(25)	116·9 (5)
C(3)C(2)O(17)	109·9 (5)	C(7)C(11)C(12)	113·6 (5)		

Symmetry code: (*a*) $\frac{1}{2}+x, \frac{1}{2}+y, z$, (*b*) $x, 1+y, z$, (*c*) $1-x, y, z$, (*d*) $\frac{3}{2}-x, \frac{1}{2}+y, 1-z$.

Table 4. *Comparison of torsion angles for euonyminol* ($R = 5{\cdot}6\%$), *evonine* ($R = 9{\cdot}1\%$) *and maytoline* ($R = 6{\cdot}2\%$)

Ring *A*	Euonyminol	Evonine	Maytoline
C(1)–C(2)	56·6	53·4	56·7
C(2)–C(3)	−52·8	−47·4	−50·8
C(3)–C(4)	51·4	47·0	47·3
C(4)–C(5)	−52·6	−49·8	−47·4
C(5)–C(10)	53·3	52·2	51·8
C(1)–C(10)	−56·1	−55·7	−55·5
Ring *B*			
C(5)–C(6)	77·3	77·5	82·2
C(6)–C(7)	−74·5	−71·5	−77·4
C(7)–C(8)	58·0	57·6	52·8
C(8)–C(9)	−41·9	−41·2	−31·1
C(9)–C(10)	41·0	40·6	33·8
C(5)–C(10)	−59·6	−61·5	−59·5

Ring *C*	Euonyminol	Evonine	Maytoline
C(5)–C(6)	−35·8	−37·9	−29·3
C(6)–C(7)	45·7	43·3	42·1
C(7)–C(11)	−38·3	−34·0	−40·9
C(11)–O(20)	16·3	10·0	23·6
C(5)–O(20)	12·4	18·2	2·7
O(20)C(5)C(6)O(21)	85·6	74·6	92·1
C(9)C(10)C(15)O(25)	50·2	42·6	28·7
O(16)C(1)C(2)O(17)	59·8	59·1	56·3
O(18)C(3)C(4)C(14)	160·4	166·9	155·8
O(18)C(3)C(4)O(19)	44·7	51·3	43·0
O(20)C(11)C(12)O(24)	172·1	−72·4	—
O(22)C(8)C(9)O(23)	−49·1	21·4	—
O(21)C(6)C(7)C(11)	−76·5	−74·3	−78·0
O(18)C(3)C(2)O(17)	−163·1	−169·4	−166·7
O(23)C(9)C(10)C(15)	44·2	38·4	144·2
O(16)C(1)C(10)C(15)	−54·1	−55·3	−57·5
C(1)C(10)C(15)O(25)	167·7	160·7	146·2

No significant biological activity has been reported for the polyhydroxy molecules; however, it has not been determined whether the polyhydroxy moieties might serve as inert carriers of the alkaloid fragment, as active transport agents or as orienting groups for the alkaloid fragment at the active site.

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Fluorescent Probe Conformations: The Crystal and Molecular Structure of Hexaaquamagnesium Bis(8-anilino-1-naphthalenesulfonate) Hexahydrate

BY VIVIAN CODY AND JOHN HAZEL

Medical Foundation of Buffalo Inc., 73 High Street, Buffalo, NY 14203, USA

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The crystal and molecular structure of hexaaquamagnesium bis(8-anilino-1-naphthalenesulfonate) hexahydrate, $[Mg(H_2O)_6][C_{16}H_{12}SO_3]_2.6H_2O$, has been determined. $P\bar{1}$, $Z = 2$, $a = 11{\cdot}1303\ (5)$, $b = 13{\cdot}5383\ (6)$, $c = 6{\cdot}9703\ (3)$Å, $\alpha = 102{\cdot}071\ (5)$, $\beta = 97{\cdot}224\ (6)$, $\gamma = 91{\cdot}009\ (4)°$. Structural analysis shows the geometry at the anilino N to be nearly trigonal, a suggested prerequisite for fluorescence. The overall conformation of the molecule is similar to that of one of the two distinct conformers observed in the structure of the ammonium salt of 8-anilino-1-naphthalenesulfonic acid. The torsion angles between the aromatic rings and the C–N–C plane are C(2′)–C(1′)–N–C(1) = 174° and C(1′)–N–C(1)–C(2) = −54°. The complex is held together by a strong network of ten intermolecular hydrogen bonds. There is also an N–H···O intramolecular hydrogen bond.

1. Introduction

The enhancement of fluorescence properties of a number of polycyclic aromatic compounds as a result of noncovalent interaction with a protein has made them useful as probes of hydrophobic binding sites. They have been used to explore the binding sites of many substrates, enzymes, membranes and proteins (Edelman & McClure, 1968; Brand & Gohlke, 1972; Kolb & Weber, 1975). Generally, the fluorescence of a probe varies with the polarity of the solvent and its fluorescence characteristics are sensitive to its local environment. Probes such as 8-anilino-1-naphthalenesulfonic acid (ANS) (Fig. 1) have been the most widely utilized in contemporary biochemical research for the assessment of hydrophobicity of binding sites on proteins and as a means of monitoring conformational changes in biological macromolecules.

In addition to its important use as a fluorescent probe, ANS has been shown to act as a competitive inhibitor with thyroxine for the binding sites on the thyroid hormone transport proteins (Green, Marshall, Pensky & Stanbury, 1972; Ferguson, Edelhoch, Saroff, Robbins & Cahnmann, 1975).

Recent X-ray data on the protein crystal structure of horse-liver alcohol dehydrogenase (Eklund, Nordstrom, Zeppenzauer, Soderlund, Ohlsson, Boiwe & Branden, 1974) show that there are two binding sites