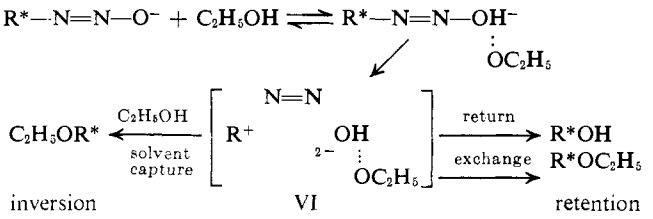
Scheme I



a front-side exchange process. The results parallel $H_2{}^{18}O$ hydrolysis of optically active octane-2-diazotate, in which 2-octanol from OH return showed retention, whereas 2-octanol from solvent capture showed inversion.[9] There are also analogies to diazo ester collapse in carboxylic acids.[4,10] In polar solvents, such esters collapse with return and retention.[4] The esters formed by solvent capture, however, may form with retention or inversion.[4,10] In ethanolysis of I, the high nucleophilicity of the solvent (as compared to a carboxylic acid) ensures that its capture by VI will occur mainly with inversion.

The present results support the generality of ion pair intermediates in deaminative reactions and extend the "counterion hypothesis"[4] to diazo ethers. The persistence of the return with retention, solvolysis with inversion dichotomy[4,9] in the diazotate ethanolysis reaction, is especially noteworthy.

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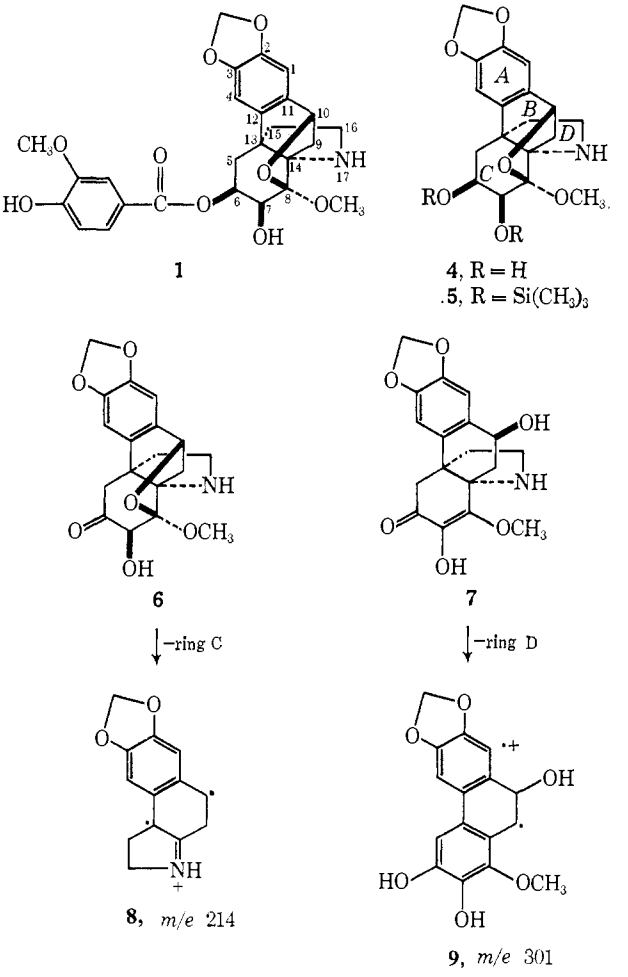
The Structure of Stephavanine, a Novel Hasubanan Ester Alkaloid

Sir:

We wish to report the structure of a new alkaloid isolated from the rhizomes of *Stephania abyssinica* Walp., a creeping plant indigenous to Eastern and Southern Africa which is reputed to possess a variety of medicinal uses.[1,2] The principal alkaloid, stephavanine (**1**), is the most highly oxygenated hasubanan alkaloid reported to date, and the first recognized to contain ester or ketal functions.

Crystallization of the "weak base" fraction[2] from chloroform yielded stephavanine hydrochloride, $C_{26}H_{28}ClNO_9$[3]: mp 217–218° dec; $[\alpha]^{32}D$ +16° (*c* 0.73, MeOH). Treatment with ammonia gave stephavanine (**1**), $C_{26}H_{27}NO_9$ (mp 229–230° dec; $[\alpha]^{32}D$ +30° (*c* 0.90, pyr); *m/e* 497 (M^+)); 7-trimethysilyl ether (**2**), $C_{29}H_{35}NO_9Si$ (mp 201–202° dec); *O,O,N*-triacetyl derivative **3**, $C_{32}H_{33}NO_{12}$ (mp 189–190°); and hydrobromide $C_{26}H_{28}NO_9Br$ (mp 191–192° dec). The mass spectrum of **1** showed one major fragmentation, characteristic of a hasubanan-type alkaloid,[4] to an ion at *m/e* 214. Alkaline hydrolysis of **1** gave vanillic acid; stephine (**4**), $C_{18}H_{21}NO_6$ (mp 224–226° dec); and 6,7-bistrimethylsilyl ether (**5**), $C_{24}H_{37}NO_6Si_2$ (mp 197–199° dec). The nmr spectrum of **5** (Table I) showed singlets for the two aromatic protons, indicative that the methylenedioxy group was attached at C(2) and C(3).

Oxidation of stephine (**4**) with Jones reagent in acetone–HOAc gave 6-dehydrostephine (**6**, $C_{18}H_{19}NO_6$, mp 191–192° dec) after $NaHCO_3$ work-up. After NaOH work-up, or by brief treatment of **6** with NaOH, iso-6-dehydrostephine (**7**, $C_{18}H_{19}NO_6$, mp 128–130°) was obtained. Whereas **6** showed its base peak at *m/e* 214, corresponding to loss of the C ring to give **8**, **7** showed its base peak at *m/e* 301, corresponding to loss of the D ring to give ion **9**. We have also observed loss of the D ring to be the preferred fragmentation in other C-ring enone hasubanan alkaloids.[5] Reduction



of **6** with $NaBH_4$ led to stereospecific conversion to stephine (**4**).

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Table I. Nmr Signals of Stephavanine Derivatives[a]

Compd	C(1), C(4)	C(2), C(3)	C(6)	C(7)	C(10)	OCH_3	Vanillate ester	Other signals
2	3.48 s 3.52 s	4.28 d (1.5) 4.78 d (1.5)	4.83 m	5.67 d (4)	5.12 d (5.5)	6.10 s 6.43 s	2.67 d (1.5) 3.12 d, d (1.5, 8) 3.28 d (8)	$Si(CH_3)_3$ 9.88 s
3	3.40 s 3.47 s	4.22 d (1) 4.55 d (1)	4.62 m	4.73 d (4)	5.03 d (6)	6.15 s 6.50 s	2.57 s 3.12 s (2 H)	$COCH_3$ 7.68 s 7.80 s 7.95 s
5	3.37 s 3.45 s	4.10 s (2 H)	6.13 m	6.00 d (4)	5.20 d (5.5)	6.53 s		$Si(CH_3)_3$ 9.83 s 10.17 s
6	3.32 s 3.48 s	4.07 s (2 H)		5.38 s	5.28 d (6)	6.40 s		
7	3.10 s 3.18 s	4.03 s (2 H)			5.18 t (3)	6.20 s		

[a] τ values (60 MHz) in $CDCl_3$ (TMS).

Of the six asymmetric centers in stephavanine, the relative configurations at C(8), C(10), C(13), and C(14) could readily be assigned on the basis of the constraints of the cage ring system. The axial (β) orientation of the C(6) vanillate ester in **1** was indicated by the unprecedentedly high-field signals and line separation for the methlenedioxy protons of **2** [τ 4.28 and 4.78 (d, J = 1.5 Hz)].[6] Examination of molecular models showed the close proximity of the aromatic ring of the vanillate ester to the methylenedioxy protons solely in the 6β-axial ester. The stereospecific reduction of **6** to **4** was also in good accord with equatorial delivery of hydride to afford β-axial alcohol. Assignment of a 7β-equatorial alcohol configuration was favored on the basis of the selective Jones oxidation of diol **4** to monoketone **6**. Proof of the postulated structure by chemical interrelation with previously known hasubanan alkaloids was deemed impractical because of the unique ring A oxygenation pattern of **1**. Unequivocal proof of the structure, the stereochemistry at C(7), and the absolute configuration was achieved by X-ray crystallographic analysis of the hydrobromide.

Stephavanine hydrobromide was obtained from methanol–ethyl acetate as orthorhombic crystals, of space group $P2_12_12_1$, with cell dimensions a = 7.73, b = 24.82, and c = 16.04 Å. The crystal density is 1.435 g cm^{-3}, whereas the density calculated on the basis of the unit cell containing four molecules of hydrobromide is 1.250 g cm^{-3}, and this difference is accounted for by the presence of solvent molecules in the crystal structure. Because the crystals available to us were extremely fine needles, most of the X-ray reflections were characterized by low and statistically unsatisfactory counting rates when measured on a four-circle diffractometer; accordingly, only the intensities of the relatively strong low-angle reflections were obtained with that instrument and most of the intensity data were derived from long-exposure Weissenberg photographs taken with Cu Kα radiation. The intensities of 1539 independent reflections were employed in the crystal-structure determination.

The initial coordinates of the bromide ion were derived from the Patterson synthesis, and the carbon, nitrogen, and oxygen atoms of the alkaloid cation were then located in three-dimensional electron-density distributions. The atomic coordinates and vibration parameters (anisotropic for the bromide ion, isotropic for the carbon, nitrogen, and oxygen atoms) were subsequently adjusted by least-squares calculations. Corrections for anomalous dispersion by the bromide ion were incorporated in the least-squares program, and the refinement converged to R = 11.8% for the absolute configuration shown in **1** and to R = 12.2% for the mirror image of **1**. The results of the analysis unambiguously establish the structure and absolute stereochemistry of stephavanine to be as shown in **1**.

The electron density associated with the solvent molecules is much more diffuse than the electron density associated with the alkaloid and coordinates could not be obtained for individual atoms, though the site of the solvent has been firmly established. The solvent molecules are packed in an apparently random manner in channels in the crystal structure, and this disordered arrangement has many precedents in other solvated crystals.[7]

The individual bond lengths and angles in the molecule have standard deviations of *ca.* 0.03 Å and 2.5°, respectively. The average lengths of the several types of bonds in the alkaloid are normal: C(sp^3)–C(sp^3), 1.532 Å; C(sp^3)–O, 1.448 Å; benzene C–C, 1.387 Å; phenolic C–O, 1.39 Å; C(sp^3)–N$^+$, 1.50 Å. On the other hand, the valency angles in the bridged ring system show some marked deviations from the tetrahedral value of 109.5°. The distortion is most notable in the C(10)–C(9)–C(14) angle of 95°, and since the analogous angle in 4-demethylhasubanonine brosylate[5] is 112° there is no doubt that formation of the bridge O between C(10) and C(8) is responsible for the contraction in the angle. The valency angles at C(14) range from 102 [C(8)–C(14)–C(9)] to 116° [C(8)–C(14)–C(13)], those at C(8) range from 104 [O(OCH_3)–C(8)–C(14)] to 116° [C(7)–C(8)–C(14)], and those at C(13) range from 104 [C(14)–C(13)–C(15)] to 113° [C(12)–C(13)–C(15)].

The conformation of the five-membered ring N(17)–C(16)–C(15)–C(13)–C(14) approximates to an envelope form in which C(13) is the out-of-plane atom; the

(6) Nmr signals for methylenedioxy protons generally appear as two-proton singlets at τ 4.0 ± 0.15, with line separations of the order of τ 0.15 or less; *cf.* N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "NMR Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962; D. W. Matheson, Ed., "Nuclear Magnetic Resonance for Organic Chemists," Academic Press, New York, N. Y., 1967, p 20.

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torsion angles about the N(17)–C(16), C(16)–C(15), C(15)–C(13), C(13)–C(14), and C(14)–N(17) bonds are −3, −20, 35, −37, and 25°, respectively. The conformation of the other five-membered ring O–C(10)–C(9)–C(14)–C(8) is closer to a half-chair form than an envelope form, the torsion angles about the O–C(10), C(10)–C(9), C(9)–C(14), C(14)–C(8), and C(8)–O bonds being −17, 39, −45, 39, and −13°, respectively. The six-membered ring C(5)–C(6)–C(7)–C(8)–C(14)–C(13) is substantially flattened in comparison with an ideal cyclohexane ring, the deviations of atoms C(5) and C(8) from the plane of the other four atoms being 0.64 and −0.46 Å, and the mean of the valency angles in the ring 113.1°; the torsion angles about the bonds C(5)–C(6), C(6)–C(7), C(7)–C(8), C(8)–C(14), C(14)–C(13), and C(13)–C(5) are −56, 53, −48, 46, −44, and 51°, respectively. Because of the O–C(8) bridge between C(10) and C(14) the six-membered ring C(9)–C(10)–C(11)–C(12)–C(13)–C(14) does not adopt the normal half-chair form of a cyclohexene, and it is best described as an envelope form in which atom C(9) is the out-of-plane atom; in this ring the torsion angles about the bonds are C(9)–C(10) −76°, C(10)–C(11) 41°, C(11)–C(12) 1°, C(12)–C(13) −2°, C(13)–C(14) −41°, and C(14)–C(9) 79°.

The bromide ions in the crystal are associated with the alkaloid cations by hydrogen bonds of the N^+–H···Br (3.22 and 3.24 Å) and O–H···Br (3.27 and 3.30 Å) types.

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The Binding of 4-Hydroxy-3-nitrobenzenesulfonamide, a Reporter Group Labeled Inhibitor, to Carbonic Anhydrases

Sir:

The investigation of inhibitor and substrate binding to metalloenzymes provides valuable insight into the roles of metal ions in biological systems. Carbonic anhydrase is particularly suitable for this kind of investigation since it is a relatively well-studied metalloenzyme for which numerous primary sulfonamides are tightly bound specific inhibitors.[1] Strong evidence exists that the sulfonamide group binds directly to the Zn(II) at the active site replacing the water ligand present in the free enzyme.[2] We report here the results of a kinetic study of the binding to carbonic anhydrase of 4-hydroxy-3-nitrobenzenesulfonamide, a new inhibitor which has recently been prepared in this laboratory.[3] This sulfonamide possesses a chromophoric reporter[4] group whose absorption spectrum in the visible region undergoes a significant shift to longer wavelength on complex formation with carbonic anhydrase at pH 7.6.[3] Thus, the kinetics of binding could be followed directly at 420 mμ using a Durrum–Gibson stopped-flow spectrophotometer. Some previous studies on the kinetics of sulfonamide binding to carbonic anhydrase have been reported,[5,6] but these investigations employed an indirect more complicated method of measurement involving the rates of inhibition by the sulfonamides of the enzymatic hydration of carbon dioxide and the hydrolysis of *p*-nitrophenyl acetate.

In the presence of excess inhibitor a rapid first-order reaction is observed when 4-hydroxy-3-nitrobenzenesulfonamide is mixed with either bovine carbonic anhydrase or human carbonic anhydrase B.[7] The first-order rate constants, k_{obsd}, measured are related to the rate constants of eq 1,[6] where E is the free

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \qquad (1)$$

$$k_{obsd} = k_{-1} + k_1[I]_0 \qquad (2)$$

enzyme, I is the unbound inhibitor, and EI is the enzyme–inhibitor complex, and by eq 2 where $[I]_0$ is the initial sulfonamide concentration. Kinetic data obtained for the bovine enzyme plotted according to eq 2 are shown in Figure 1. The k_1 values found for this species and for human carbonic anhydrase B are given in Table I.

The dissociation constant, K_I, of the 4-hydroxy-3-nitrobenzenesulfonamide–bovine carbonic anhydrase complex was determined by equilibrium dialysis studies and from inhibition experiments. Our equilibrium dialysis data were plotted according to eq 3 where r is the ratio of moles of inhibitor bound/total moles of enzyme.[8] K_I was found to be (4.3 ± 1.7) ×

$$\frac{1}{r} = 1 + \frac{K_I}{[I]_{unbound}} \qquad (3)$$

10^{-6} M at 25.0°, pH 7.6, and μ = 0.25 in the case of the bovine enzyme.

Stopped-flow kinetic experiments were performed at 410 mμ using the reporter group labeled sulfonamide as an inhibitor and employing 2-hydroxy-5-nitro-α-toluenesulfonic acid sultone as the substrate.[3] Both of these compounds were present in excess over the enzyme. The rate data were analyzed by eq 4 where

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