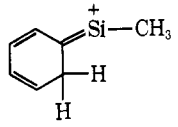

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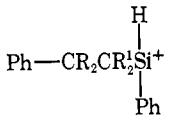

9, R = H; R′ = D
10. R = D; R′ = H

yet deuterium atoms are scrambled prior to the loss of ethylene from **10**. Our work on this rearrangement and other interesting electron-impact-induced rearrangements of organosilanes is continuing.

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(17) NDEA Fellow, 1967–1968.

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Crystal and Molecular Structure of Narcissidine[1]

Sir:

Despite extensive studies, the chemistry of the Amaryllidaceae alkaloid narcissidine has remained anomalous. The initial formulation, **1a**, which resulted from degradation studies,[2] was revised to **1b** after mass spectral studies on other members of this class of alkaloids showed a fragmentation pattern for C_1–C_2 inconsistent with **1a**.[3] No definitive reaction sequence or spectroscopic study was able to elucidate the configuration of the hydroxyl at C_4. Although most alkaloids containing this ring system but lacking the C_4 substituent have been shown to undergo relatively simple degradations, comparable degradations of narcissidine have failed. Two additional alkaloids, parkacine[4] (**1c**) and ungiminorine[5] (**1d**), have been reported to possess structures related to that of narcissidine. To determine the structure unambiguously, we carried out a single-crystal X-ray structure determination of narcissidine hydrobromide and found the structure to be significantly different from either **1a** or **1b**.

Narcissidine hydrobromide crystallizes in the orthorhombic system with $a = 16.10 \pm 0.01$ Å, $b = 15.53 \pm 0.01$ Å, and $c = 7.34 \pm 0.01$ Å. Density measurements indicate four molecules per unit cell and the systematic extinctions $h00$ ($h = 2n + 1$), $0k0$ ($k = 2n + 1$), and $00L$ ($L = 2n + 1$) uniquely determine the space group $P2_12_12_1$ (D_2^4). The unique reflections ($2\theta \leq 110°$) were collected on an automated Hilger–Watts four-circle diffractometer using filtered Cu (1.5418 Å) radiation. Of the 1376 reflections measured, 1259 were judged observed after background and LP corrections.

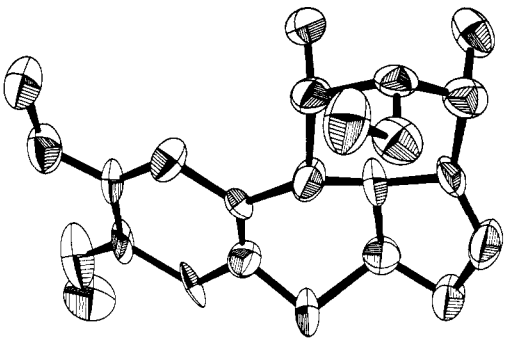
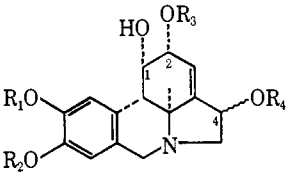
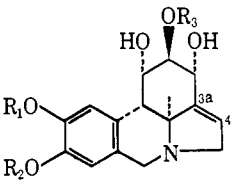


Figure 1.



1a, $R_1 = R_2 = R_4 = CH_3$; $R_3 = H$
1b, $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$
1c, $R_1 = R_2 = CH_3$; $R_3 = R_4 = H$
1d, $R_1 - R_2 = CH_2$; $R_3 = CH_3$; $R_4 = H$

The initial position of the bromine atom was determined from the three-dimensional Patterson synthesis, and the 24 nonhydrogen atoms were located in subsequent electron density syntheses. Full matrix least-squares refinements in which all atomic positions and anisotropic temperature factors were varied and the atomic scattering factor of bromine was corrected for anomalous dispersion gave a final unweighted R of 0.124 for the 1259 observed reflections. A final difference map showed no peaks larger than 0.4 e/Å^3. The estimated standard deviations are ±0.02 Å for bond lengths and ±1.0° for bond angles. All distances and angles compare well with generally accepted values.[6] Figure 1 is a drawing of the final X-ray model, less the Br. The absolute configuration shown for narcissidine is that found for dihydrolycorine.[7] No abnormally short intermolecular contacts were found.



2a, $R_1 = R_2 = R_3 = CH_3$
2b, $R_1 = R_2 = CH_3$; $R_3 = H$
2c, $R_1 - R_2 = -CH_2-$; $R_3 = H$

Ironically, this structure (**2a**) was once considered for narcissidine, but discarded because a satisfactory vicinal

(1) Work was performed in the Ames Laboratory of the U. S. Atomic Energy Commission. Additional support was derived from a grant from the National Institutes of Health (HE-07503).
(2) H. M. Fales and W. C. Wildman, *J. Amer. Chem. Soc.*, **80**, 4395 (1958).
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(5) M. Normatov, K. A. Ahduazimov, and S. Y. Yunusov, *Dokl. Akad. Nauk Uzb. SSR*, **19**, 27 (1962); *Chem. Abstr.*, **59**, 6456a (1963); M. R. Oagudaev, K. A. Ahduazimov, and S. Y. Yunusov, *Khim. Prir. Soedin.*, 99–101 (1969).
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(7) M. Shiro, T. Sato, and H. Koyama, *Chem. Ind.* (London), 1229 (1966); for the intricate series of chemical transformations interrelating narcissidine and dihydrolycorine hydrobromide see: W. C. Wildman, "The Alkaloids," Vol. 11, Academic Press, Inc., New York, N. Y., 1968, pp. 322–334.

glycol analysis was obtained for dihydronarcissidine.[2,8] Chemical and spectral data for narcissidine are consistent with structure **2a** and will be reported in detail later. The double bond is now placed between C_{3a} and C_4 (1.32 ± 0.02 Å). The hydroxyl groups are placed in a *cis*-diaxial orientation on C_1 and C_3. The short O–O distance of these hydroxyls (2.83 ± 0.02 Å) is in good agreement with the infrared hydroxyl stretching frequencies[9] observed at 3544 and 3612 cm^{-1} in dilute solution.[10] The methoxyl group at C_2 is axial and *trans* to the adjacent hydroxyl groups at C_1 and C_3.

In view of this new structure, **2a**, for narcissidine, it should be necessary to revise the structures proposed for parkacine[4] from **1c** to **2b** and ungiminorine[5] from **1d** to **2c**.

Acknowledgment. Particular thanks are due James Benson and James R. Clark for technical assistance.

(8) This glycol analysis appears to have been fortuitous. A reexamination of several alkaloids containing the ring system of 2 has shown that even monohydroxy derivatives are cleaved by periodate.

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(10) W. C. Wildman, C. L. Brown, and N. E. Heimer, unpublished observations (1967).

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Design and Synthesis of Inhibitors for Crystallographic Studies on the Active Site of Chymotrypsin

Sir:

With the X-ray analysis of the structures of bovine α-chymotrypsin[1] and γ-chymotrypsin[2] now at high resolution, that is, at a Bragg spacing of 2.0–3.0 Å, it is possible to carry out detailed studies of the active site regions in these two forms of this serine protease. Difference Fourier maps of complexes between the protein and inhibitors will provide information about binding modes, recognition sites, and disposition of catalytic groups toward the peptide bond that is cleaved in a natural substrate.

We have designed and synthesized several peptide chloromethyl ketones which are effective inhibitors of γ-chymotrypsin crystals and have demonstrated that one of the crystalline derivatives is isomorphous with native protein crystals. Peptide chloromethyl ketones were chosen as the inhibitors to be investigated initially since tosyl-L-phenylalanyl chloromethyl ketone (TPCK) has been demonstrated to be an active site-specific reagent for α-chymotrypsin in solution, reacting only with His-57.[3]

The key intermediate for the synthesis of all the inhibitors was benzyloxycarbonyl-L-phenylalanyl chloromethyl ketone.[3] Deblocking with a saturated solution of HBr in acetic acid followed by the addition of ether gave the crystalline phenylalanyl chloromethyl ketone hydrobromide.[4] This could be acylated with simple anhydrides to yield, for example, Ac-PheCH_2Cl or could be coupled with blocked peptide acids using a mixed anhydride procedure.[5] Crystalline products were obtained in all cases in yields generally greater than 50%.

Chymotrypsin was prepared from bovine chymotrypsinogen by a 90-min rapid-activation procedure using acetylated trypsin. A chromatographically pure fraction (CM-cellulose, 2.0 × 60 cm column, linear gradient 0.075–0.225 *M* K^+ phosphate, pH 6.2) was concentrated and allowed to crystallize (after seeding with tetragonal chymotrypsin crystals) from a 2.0 *M* $(NH_4)_2SO_4$ solution at pH 5.6–5.9 and 20°. Well-formed tetragonal bipyramids formed within 2–4 days and grew to 0.5 mm in size during 1–3 weeks. The crystalline habit is characteristic for bovine chymotrypsin of the π, δ, and γ family.[6] End-group analysis has indicated that such a preparation is predominantly γ-chymotrypsin. α-Chymotrypsin crystals were prepared using the procedure of Sigler, *et al.*[7] The inhibition experiments with γ-chymotrypsin were performed by soaking the crystals in 2.4 *M* phosphate, pH 5.6, for 1 day to remove $(NH_4)_2SO_4$ and then placing them in a saturated solution of inhibitor in 2.4 *M* phosphate. The α-chymotrypsin crystals were treated in a similar fashion at pH 4.5. At the conclusion of the experiment, the crystals were washed with fresh salt solution and dissolved in 0.001 *M* HCl. The activity of the protein solution was then measured using a spectrophotometric assay with benzyloxycarbonyl-L-tyrosine *p*-nitrophenyl ester as substrate.[8]

The results in Table I demonstrate that a variety of peptide chloromethyl ketones are able to inhibit γ-chymotrypsin crystals within a reasonable length of time. Our best inhibitor, BOC-Gly-PheCH_2Cl, is as effective as phenylmethanesulfonyl chloride (PMSF), a known crystal inhibitor.[9] The results obtained with Ac-Leu-PheCH_2Cl were surprising since Yamashita[10]

Table I. Per Cent Inhibition of γ-Chymotrypsin Crystals[a]

Inhibitor[b]	Series I, 2 weeks	Series II, 1 week
PMSF	100	
Z-PheCH_2Cl	30	
BOC-Gly-PheCH_2Cl	100	99
Ac-PheCH_2Cl		65
Ac-Gly-PheCH_2Cl		75
Ac-Ala-PheCH_2Cl		71
Ac-Leu-PheCH_2Cl		17

[a] Inhibitions were carried out with a saturated solution of inhibitor in 2.4 *M* phosphate containing 2% CH_2CN at pH 5.6. The amino acid residues Phe, Ala, and Leu are optically active and have the L configuration.

(1) P. B. Sigler, D. M. Blow, B. W. Matthews, and R. Henderson, *J. Mol. Biol.*, **35**, 143 (1968).

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(3) E. Shaw in "Methods of Enzymology," Vol. XI, C. H. W. Hirs, Ed., Academic Press, New York, N. Y., 1967, p 677 ff.

(4) Satisfactory analyses were obtained for all new compounds.

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