COMMUNICATIONS TO THE EDITOR

THE NITROGEN INVERSION FREQUENCY IN CYCLIC IMINES

Sir:

Considerable effort has been expended in attempts to prepare substances with optical forms due to asymmetry of trivalent nitrogen.[1] Suitably substituted ethylenimines are thought to be particularly favorably constituted for existence of stable, optically active antipodes.[1,2] Successful resolution of such imines has not yet been achieved.

The nuclear magnetic resonance spectra of N-ethylethylenimine (I) and N-ethylallenimine[3] (II) has now been studied as a function of temperature (Fig. 1).

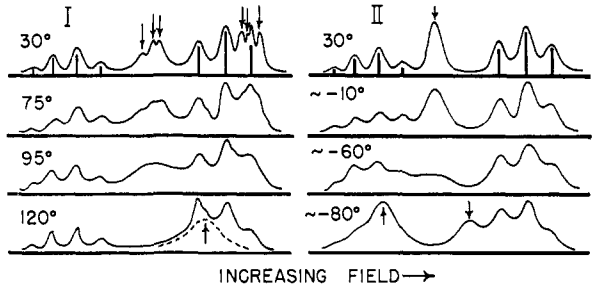
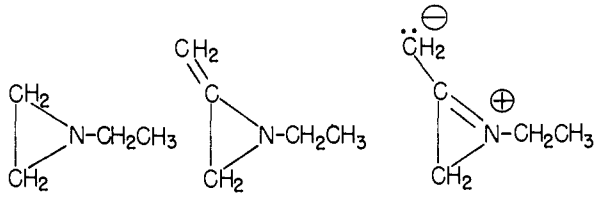


Fig. 1.—Nuclear magnetic resonance spectra of protons of N-ethylethylenimine (I) and N-ethylallenimine (II) as a function of temperature. Samples in 0.5 mm. tubes and Varian Associates High Resolution Spectrometer (V-4300) at 40 mc. with vacuum-jacketed probe insert and 12-in. magnet equipped with Super Stabilizer. Heavy vertical lines mark characteristic bands of ethyl group while vertical arrows indicate absorption of ring hydrogens. The temperature-invariant absorption of the double-bond methylene protons of II is off scale on the left.

At room temperature, I shows the characteristic bands of the ethyl group and two slightly split triplet band systems separated by 27 c.p.s. at 40 mc. These latter band systems are best interpreted as being due to the two groups of ring hydrogens which are either *cis* or *trans* to the N-ethyl group. In such event, the mean lifetime of a given imine molecule before the nitrogen inverts must be much greater than 0.04 sec.[4] On heating to 120–130°, the ring hydrogens appear to completely lose their identity with respect to the position of the ethyl group and the mean lifetime before nitrogen inversion must be substantially less than 0.04 sec. The intermediate temperature with a mean lifetime of 0.04 sec. is estimated to be about 110°. The rate of nitrogen inversion of I would thus seem to preclude successful resolution of substituted ethylenimines except at rather low temperatures.



I II III

N-Ethylallenimine (II) shows only one band for the ring methylene group at room temperature. However, at and below −80°, this band is sharply split into two components separated by about 30 c.p.s. The intermediate temperature is estimated to be between −60 and −70°, at which point the mean lifetime before inversion of the nitrogen is about 0.03 sec.

The tremendously faster inversion rate of II compared to I is expected on the basis of contribution of electron delocalization involving the nitrogen and double bond as indicated by structure III. Such delocalization would markedly aid the attainment of a planar inversion transition state.

So far, no similar temperature behavior has been noted in the n–m–r spectra N-methylpyrrolidine or N-methylpiperidine and related compounds. These studies are being continued.

(1) *Cf.*, R. L. Shriner, R. Adams and C. S. Marvel in H. Gilman, "Organic Chemistry, An Advanced Treatise," John Wiley and Sons, Inc., New York, N. Y., Second Edition, 1943, Vol. I, pp. 402–413; V. Prelog and P. Wieland, *Helv. Chim. Acta*, **27**, 1127 (1944).

(2) R. Adams and T. L. Cairns, THIS JOURNAL, **61**, 2464 (1939); J. F. Kincaid and F. C. Henriques, Jr., *ibid.*, **62**, 1474 (1940); T. L. Cairns, *ibid.*, **63**, 871 (1941); H. M. Kissman, D. S. Tarbell and J. Williams, *ibid.*, **75**, 2959 (1933), and earlier references there cited to papers by Tarbell and co-workers and others.

(3) (a) M. G. Ettlinger and F. Kennedy, *Chem. and Ind.*, 166 (1956); (b) A. T. Bottini and J. D. Roberts, THIS JOURNAL, **79**, 0000 (1957).

(4) H. S. Gutowsky and A. Saika, *J. Chem. Phys.*, **21**, 1688 (1953).

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ENZYMATIC FORMATION OF OXALATE AND ACETATE FROM OXALOACETATE

Sir:

Oxalic acid is one of the major products of carbohydrate metabolism in molds and higher plants but the precise metabolic pathway by which it is produced has not been elucidated to date. In this communication we are reporting the formation of oxalate and acetate from oxaloacetate by a soluble enzyme preparation obtained from *Aspergillus niger* (ATCC 10582).

The organism was grown at 25° with shaking on a medium containing 10% dextrose, 0.3% $(NH_4)_2SO_4$, 0.1% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 1% Difco malt extract at *p*H 6.0. After 72 hours the *p*H of the medium was adjusted between 7.5 and 8.5 with sterile 2 *M* Na_2CO_3 solution and the mycelia were harvested 12 hours later. The enzyme was prepared by grinding the well-washed and frozen mycelia and extracting with three volumes of tris-hydroxymethylaminomethane buffer

(0.02 *M*, *p*H 7.0). The clear supernatant obtained by high speed centrifugation (25,000 *g*, 30 minutes) was treated with aluminum hydroxide Cγ gel. The enzyme was eluted with 0.2 *M* potassium phosphate buffer (*p*H 7.5) and the eluate was dialyzed against tris-hydroxymethylaminomethane buffer (0.01 *M*, *p*H 7.0) for 5 hours at 0°.

The stoichiometry of the reaction is shown in Table I. When corrected for the spontaneous decarboxylation of oxaloacetate, the disappearance of oxaloacetate[1] was matched by the appearance of almost equal amounts of oxalate and acetate. Under these conditions, none of the following compounds yielded oxalate: malate, fumarate, succinate, tartrate, oxalosuccinate,[2] citrate, *cis*-aconitate, isocitrate, pyruvate, glycolate, glyoxylate and acetate.

TABLE I

STOICHIOMETRY OF OXALOACETATE CLEAVAGE

Expt. I: a reaction mixture (2.0 ml.) containing 20 μmoles of oxaloacetate, 10 μmoles of $MnCl_2$, 200 μmoles of tris-hydroxymethylaminomethane buffer *p*H 8.1 and the enzyme (3.7 mg. protein) was incubated at 37° for 30 minutes. Expt. II: without $MnCl_2$. Expt. III: boiled enzyme control. Zero time samples did not contain any oxalate. Numbers are expressed in μmoles.

	Δ Oxaloacetate[a]	Δ Pyruvate[b]	Δ Acetate[c]	Δ Oxalate[d]
Expt. I	−17.3	5.6	11.6	11.1
Expt. II	−12.2	5.2	6.8	6.0
Expt. III	−6.7	7.0	0	0

[a] Determined by the method of A. H. Mehler, A. Kornberg, S. Grisolia and S. Ochoa, *J. Biol. Chem.*, **174**, 961 (1948). [b] Determined by the method of A. Kornberg and W. E. Pricer, Jr., *ibid.*, **184**, 769 (1950). [c] Acetate was determined with acetokinase from *E. coli:* I. A. Rose, M. Grunberg-Manago, S. R. Korey and S. Ochoa, *ibid.*, **211**, 737 (1954). We are indebted to Drs. E. Heath and J. Hurwitz for their help and a generous gift of a purified acetokinase preparation. The acetohydroxamate formed in this assay was further identified by paper chromatography according to E. R. Stadtman and H. A. Barker, *ibid.*, **184**, 769 (1950). [d] Oxalate was determined manometrically with a highly purified preparation of oxalic decarboxylase from *Collyvia veltipes* (H. Shimazono and O. Hayaishi, unpublished procedure).

In a large scale experiment oxalate was precipitated as the calcium salt, dissolved in 0.1 *N* HCl, passed through a Dowex-50 (H^+-form) column and crystallized as the free acid. Its identity was established by melting point, analysis and a comparison of the infrared spectrum with that of an authentic sample.

The enzyme required Mn^{++} for maximum activity but no other cofactors appeared to be involved. The hydrolytic cleavage of oxaloacetate to yield acetate and oxalate has been postulated by previous investigators.[3,4] Although further purification of the enzyme appears to be necessary to clarify the precise mechanism of the reaction, the results presented in this paper represent the first experimental evidence for this hypothetical reaction. This enzyme is therefore tentatively designated as oxaloacetic hydrolase.

(1) Samples of oxaloacetic acid (m.p. 150 ~ 152°) were purchased from California Foundation for Biochemical Research and Sigma Chemical Co. Oxaloacetic acid (m.p. 182 ~ 5°), presumably hydroxyfumaric acid, was obtained from Sigma Chemical Co. The three preparations gave essentially identical results.

(2) A commercial preparation of oxalosuccinic acid contained approximately 16% oxalic acid as an impurity.

(3) H. Raistrick and A. B. Clark, *Biochem. J.*, **13**, 329 (1919).

(4) F. Lynen and F. Lynen, *Ann.*, **560**, 149 (1948).

(5) Fellow of The Jane Coffin Childs Memorial Fund for Medical Research.

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THE VINYL SIDE-CHAIN IN GELSEMINE[1]

Sir:

Gelsemine contains a double bond that readily can be hydrogenated[2] and has been considered heretofore as being located in an exocyclic methylene group.[3,4] This assignment was based on the interpretation of two reactions.[3,4] Dr. E. Wenkert at a recent meeting of the American Chemical Society questioned the validity of the interpretation, thus prompting a further study of the nature of the double bond.

Cleavage of the olefinic bond in N(a)-methylgelsemine ($C_{21}H_{24}O_2N_2$) by sodium metaperiodate in the presence of a catalytic amount of osmium tetroxide[5] gave rise to a substance, m.p. 192–194°, (Calcd. for $C_{20}H_{22}O_3N_2$: C, 70.98; H, 6.55; N, 8.28; 2N-CH_3, 8.88; C-CH_3, nil. Found: C, 71.10; H, 6.62; N, 8.53; N-CH_3, 8.61; C-CH_3, nil.) The infrared absorption spectrum of this substance contained, besides a band at 1702 cm.$^{-1}$ due to the oxindole carbonyl, a second band in the same region (1719 cm.$^{-1}$) and one at 2735 cm.$^{-1}$ characteristic of the carbonyl and of the CH stretching respectively of an aldehyde. Reaction of the oxidation product with hydroxylamine gave an oxime, m.p. 267–268°, (calcd. for $C_{20}H_{23}O_3N_3$: C, 67.97; H, 6.56; N, 11.89. Found: C, 68.21; H, 6.75; N, 11.81) which, on dehydration with acetic anhydride and pyridine, produced a nitrile, m.p. 240–241°, (calcd. for $C_{20}H_{21}O_2N_3$: C, 71.62; H, 6.31; N, 12.53. Found: C, 71.57; H, 6.42; N, 12.49). The infrared absorption spectrum of this nitrile showed a band at 1711 cm.$^{-1}$ attributable to the oxindole carbonyl and a sharp band at 2255 cm.$^{-1}$ characteristic of the C≡N vibration. The formation of a nitrile under these conditions confirms that the oxidation product was indeed an aldehyde, and not a ketone. This was further confirmed by the Wolff–Kishner reduction of the oxidation product which gave rise to a compound, m.p. 172–174°, (calcd. for $C_{20}H_{24}O_2N_2$: C, 74.04; H, 7.46; N, 8.64; 1C-CH_3: 4.63. Found: C, 74.19; H, 7.56; N, 8.79; C-CH_3, 4.11). The infrared absorption spectrum of this compound contained the oxindole carbonyl band at 1707 cm.$^{-1}$, but no

(1) Issued as N.R.C. Bull. No. 4105.

(2) T. T. Chu and T. Q. Chou, THIS JOURNAL, **62**, 1935 (1940).

(3) M. S. Gibson and R. Robinson, *Chem. and Ind.*, 93 (1951).

(4) R. Goutarel, M. M. Janot, V. Prelog, R. P. A. Sneeden and W. I. Taylor, *Helv. Chim. Acta*, **34**, 1139 (1951).

(5) R. Pappo. D. S. Allen, Jr., R. U. Lemieux and W. S. Johnson, *J. Org. Chem.*, **21**, 478 (1956).