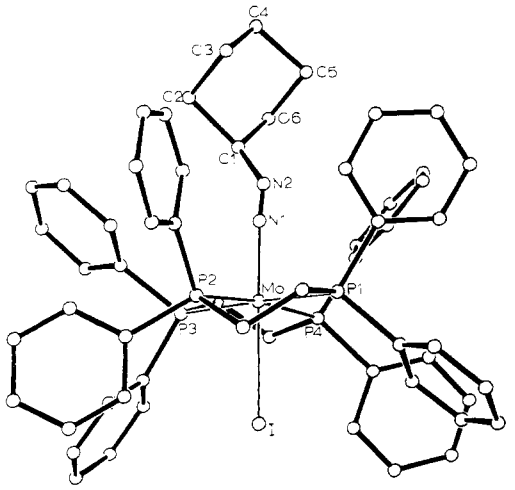


Figure 1. Perspective view (derived from an ORTEP drawing) of the molecular structure of $MoI(N_2C_6H_{11})(dppe)_2$ (**3**) as seen in crystals of its benzene solvate.

amagnetism) at 21° of 1.83 B_μ. This value is comparable to μ_{eff} for other molybdenum(I)–dppe containing compounds.[14]

Under similar conditions chloromethane and **1** reacted to produce methane, dinitrogen, dihydrogen, and $MoCl(N_2)(dppe)_2$ (**6**). **6** was crystallized from benzene–methanol solution with one methanol of solvation. **6** was first reported by Smith and coworkers.[15] In the infrared spectrum (CsI pellet) noteworthy absorptions were observed at 1966 and 311 cm^{-1} due to $\nu_{N\equiv N}$ and ν_{Mo-Cl}, respectively.[16]

Two points critical to discussions of mechanisms for these reactions can be noted at this time. Firstly, **1** has an absorption maximum at 376 nm (ϵ 14,500) that may be tentatively assigned[17] to a charge transfer transition between metal orbitals and dinitrogen antibonding orbitals. Secondly, in all the reactions reported herein one dinitrogen is retained by the metal, either as an unreacted dinitrogen ligand or incorporated in an alkyldiazenido ligand.

Investigation of the chemical and physical properties of these interesting compounds and the mechanisms of their formation are in progress, as are attempts to grow high-quality single crystals of **3** and the other compounds reported herein for X-ray diffraction studies.

References and Notes

(1) Presented in part at the International Symposium on Nitrogen Fixation, Pullman, Wash., June 3–7, 1974.
(2) T. A. George and C. D. Selbold, *Inorg. Chem.*, **12**, 2548 (1973).
(3) Iodomethane reacts with **1** in benzene, at room temperature, and in the dark to produce $MoI(N_2CH_3)(dppe)_2$ (>60 hr). However, no reaction between **1** and chloro- and bromomethane occurs in the dark.
(4) Irradiation at 366 nm with a 100-W B-100A Blak-Ray Lamp, Ultra-Violet Products, Inc., San Gabriel, Calif.
(5) Calculated for **2**·CH_2Cl_2; $C_{54}H_{53}Cl_2I_2MoN_2P_4$: C, 56.51; H, 4.66; N, 2.44; halogen, 17.24. Found: C, 54.70; H, 4.51; N, 2.33; halogen, 16.67. An ion cyclotron resonance spectrum of gases evolved when a sample of **2**·CH_2Cl_2 was decomposed at 220° showed dichloromethane to be present.
(6) A. P. Gaughan, Jr., B. L. Haymore, J. A. Ibers, W. H. Myers, T. E. Nappier, and D. W. Meek, *J. Am. Chem. Soc.*, **95**, 6859 (1973).
(7) The first number in parentheses is the root mean square estimated standard deviation of an individual datum. The second and third numbers, when given, are the average and maximum deviations from the average value, respectively.
(8) A. P. Gaughan, Jr., and J. A. Ibers, *Inorg. Chem.*, **14**, 352 (1975).
(9) J. V. McArdle, A. J. Schultz, B. J. Corden, and R. Eisenberg, *Inorg. Chem.*, **12**, 1676 (1973); R. Mason, K. M. Thomas, J. A. Zubieta, P. G. Douglas, A. R. Galbraith, and B. L. Shaw, *J. Am. Chem. Soc.*, **96**, 260 (1974); G. Avitabile, P. Ganis, and M. Nemiroff, *Acta Crystallogr., Sect. B*, **27**, 725 (1971).
(10) We thank Dr. E. R. Domb of our physics department for determining the magnetic moments of compounds **2**, **3** and **4**.
(11) We thank one of the referees for suggesting this alternative bonding scheme.
(12) We thank Dr. M. L. Gross for ion cyclotron resonance spectra of gases evolved in these reactions.
(13) Similar products from the photochemical reactions of bromoalkanes have been observed: A. A. Diamantis, J. Chatt, G. J. Leigh, and G. A. Heath, *J. Organomet. Chem.*, **84**, C11 (1975).
(14) For examples see ref 1.
(15) J. K. Alkinson, A. H. Mawby, and D. C. Smith, *Chem. Commun.*, 157 (1971).
(16) In $Mo(N_2)_2(dppe)_2$ and $MoCl_2(dppe)_2$, $\nu_{N\equiv N}$ and ν_{Mo-Cl} occur at 1979 and 305 cm^{-1}, respectively.
(17) I. M. Trietel, M. T. Flood, R. E. Marsh, and H. B. Gray, *J. Am. Chem. Soc.*, **91**, 6512 (1969).
(18) One of us (S.D.A.I.) thanks Conoco for a Summer Fellowship, 1974.

Victor W. Day,* T. Adrian George,* S. D. Allen Iske[18]

Department of Chemistry, University of Nebraska–Lincoln
Lincoln, Nebraska 68508

Received February 18, 1975

7-*cis*,9-*cis*- and 7-*cis*,9-*cis*,13-*cis*-Retinal. A Stereoselective Synthesis of 7-*cis*,9-*cis*-β-Ionylideneacetaldehyde[1]

Sir:

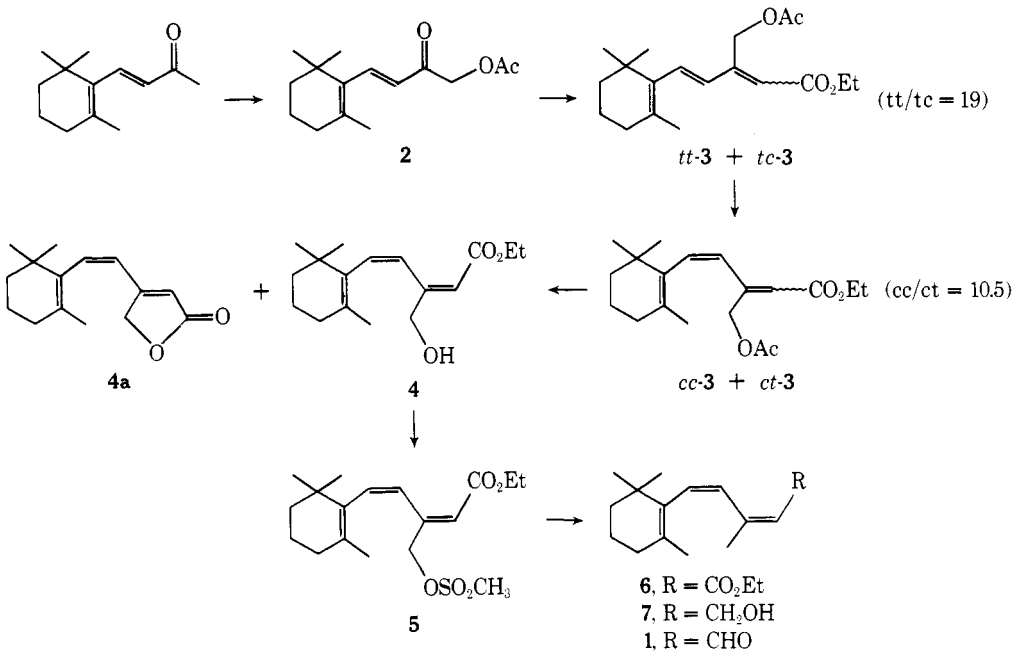
Earlier we reported the preparation of four 7-cis isomers of retinal by a six-step nonselective reaction sequence.[2] By this scheme the separation of isomers, particularly pairs around the 9,10-double bond, presented difficulty. Since then, we have been able to effect HPLC separation of 7-*cis*- and 7-*cis*,9-*cis*-retinal but are still unable to resolve the remaining isomeric pair (7-cis,13-cis and 7-cis,9-cis,13-cis) by this technique.[3] Similar difficulties exist in separating retinal precursors, such as the C_{15}-trienals and C_{18}-tetraenones. We therefore sought to undertake the completely stereoselective synthesis of the trienals[1] in order to prepare specific retinal isomers wherein the configuration of the trisubstituted 9,10-double bond would be totally unambiguous.

In designing schemes to a single isomer of β-ionylideneacetaldehyde (**1**) we found little guidance from available literature. There were no reports of stereoselective syntheses of the two 7-trans trienals despite the fact that these compounds are important precursors to vitamin A.[4] Pure *tt*-**1** and *tc*-**1** were only obtained by fractional crystallization of an aldehyde derivative followed by regeneration of the free aldehyde. This procedure was clearly inapplicable to the preparation of 7-cis isomers because of the sensitivity of the 7-cis geometry to regeneration reactions involving carbonium ion intermediates and failure on our part to obtain satisfactory crystalline derivatives.

Of the several approaches we have attempted, Scheme I was found to be highly stereoselective and led to isomerically pure 7-*cis*,9-*cis*-β-ionylideneacetaldehyde.

Acetoxylation of β-ionone with $Pb(OAc)_4$ in refluxing benzene (3 hr) gave *trans*-10-acetoxy-β-ionone (**2**) bp 120–124° (0.5 Torr), in 35–45% yield.[5,6] The Horner reaction of **2** with the sodium salt of triethylphosphonoacetate in benzene (room temperature, 3 hr) was highly stereoselective (*tt*-**3**/*tc*-**3** = 19),[7] giving *tt*-**3** in 78% yield as a nearly colorless crystalline solid, mp 75.5–76.0° (from aqueous ethanol). Irradiation of *tt*-**3** (0.0425 *M* in benzene) for 8 hr at 10° (uranium glass filter, 200-W medium pressure Hg lamp) in the presence of benzanthrone (0.0045 *M*, E_T = 47 kcal/mol) as sensitizer gave 7-*cis*- and 7-*cis*,9-*cis*-**3** in a ratio of 1:10.5.[8] After crystallization from aqueous methanol 7-*cis*,9-*cis*-**3** was obtained as a low melting solid, mp 50–52°. Upon acid-catalyzed methanolysis, 7-*cis*,9-*cis*-**3** gave the corresponding 7-*cis*,9-*cis*-9-hydroxymethyltriene ester, **4**. In this reaction the corresponding hydroxy ester derivative of 7-*cis*-**3** was converted to butenolide, **4a**.[8] This minor product was also prepared by acid-catalyzed methanolysis of *tt*-**3** followed by photosensitized isomerization of

Scheme I



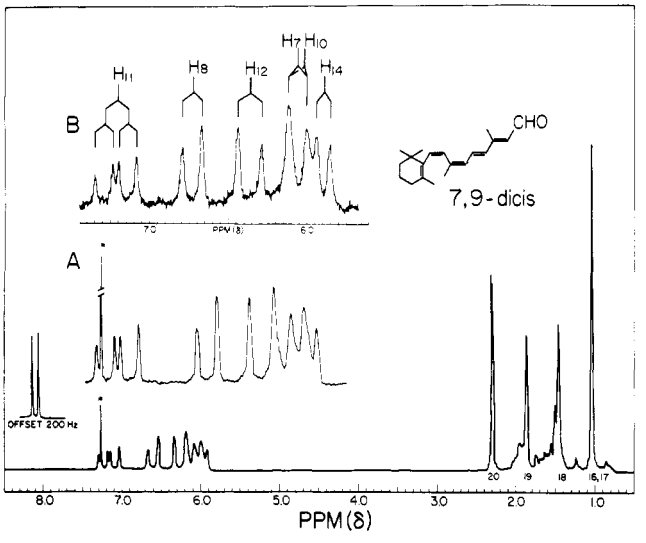


Figure 1. ^{1}H NMR spectrum (FT, 100 MHz) of 7-*cis*,9-*cis*-retinal in $CDCl_3$: (A) vinyl region expanded ($CDCl_3$); (B) vinyl region expanded, in DMSO-d_6.

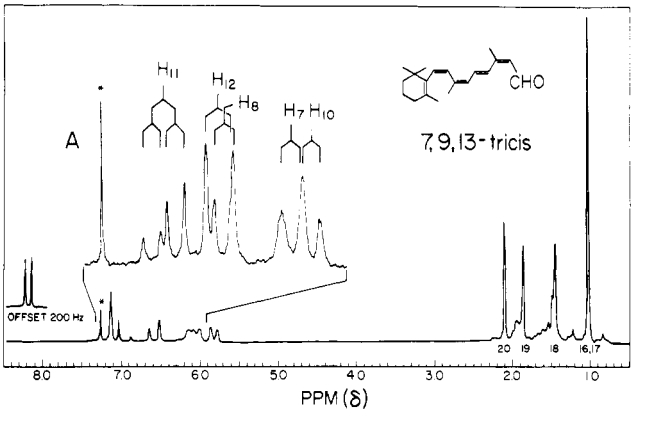


Figure 2. ^{1}H NMR spectrum (FT, 100 MHz) of 7-*cis*,9-*cis*,13-*cis*-retinal in $CDCl_3$: (A) vinyl region after addition of Pr(fod)$_3$ shift reagent.

the 7-*trans*-butenolide. Mesylation of alcohol **4** (mesyl chloride, triethylamine and CH_2Cl_2)[9] gave mesylate **5** in essentially quantitative yield. Crude mesylate **5** was immediately reduced with $NaBH_4$ in HMPA to give the desired isomerically pure triene ester **6**.[10,11] The NMR spectrum of **6** was identical with that previously reported from the spectrum of a mixture of isomers.[12] The overall conversion of *tt*-**3** to *cc*-**6** was effected in 87% yield without intervening purification steps. Reduction of dicis triene ester **6** with $LiAlH_4$ in ether afforded the alcohol **7** in high yield. Lastly, oxidation of alcohol **7** with freshly prepared active MnO_2 (room temperature, 1 hr, CH_2Cl_2) gave pure 7-*cis*,9-*cis*-β-ionylideneacetaldehyde (*cc*-**1**) in 74% yield. Its NMR spectrum was identical with that previously reported.[12]

The condensation of methyl *trans*-4-diethylphosphono-3-methyl-2-butenoate (**8**) with α,β-unsaturated aldehydes (e.g., cis or trans citral) has been reported to proceed stereoselectively to give only ditrans diene esters. Similarly, the reaction of pure *cis*-**8** with aldehydes resulted in extensive isomerization around the 2 double bond.[13] In our hands the Horner reaction of 7,9-*cc*-**1** with excess **8** (NaH, THF, 5 hr, room temperature) gave a ~1:1 mixture of methyl 7-*cis*,9-*cis*- and 7-*cis*,9-*cis*,13-*cis*-retinoates (**9**) in 71% yield. The isomers were separated by column chromatography (Biosil A, 25% $CHCl_3$–hexanes) with the tricis isomer eluting in the earlier fractions:[14] nmr δ ($CDCl_3$), 7,9-*cc*-**9**, 1.07 (6 H), 1.49 (3 H, 18-CH_3), 1.86 (3 H, 19-CH_3), 2.34 (3 H, 20-CH_3), 3.69 (3 H, -OCH_3), 5.77 (bs, H_{14}), 5.9–6.3 (m, H_7, H_{10}, and H_{12}), 6.59 (d, J = 13 Hz, H_8), and 7.02 (d of d, J = 11.5 and 15.0 Hz, H_{11}); 7,9,13-*ccc*-**9**, 1.07 (6 H), 1.49 (3 H, 18-CH_3), 1.86 (3 H, 19-CH_3), 2.05 (3 H, 20-CH_3), 3.68 (3 H, -OCH_3), 5.62 (bs, H_{14}), 5.95–6.25 (m, H_7 and H_{10}), 6.59 (d, J = 12.5 Hz, H_8), 6.99 (d of d, J = 11 and 15 Hz, H_{11}), and 7.70 (d, J = 15.5 Hz, H_{12}).

The formation of the 7-cis,9-cis,13-cis isomer suggests either a loss of stereochemistry in the betaine intermediate from **8** and 7,9-*cc*-**1** or isomerization of the anion of **8** prior to its reaction with the aldehyde. We favor the latter possibility since, in a separate experiment, treatment of **8** with NaH followed immediately by quenching of the salt mixture with water gave a ca. 1:1 mixture of both *cis*- and *trans*-**8**. These observations are clearly not in accord with earlier reports concerning the selectivity of **8** in Horner reactions. At this stage we are unsure of the cause of this discrepancy.

Subsequent reduction of the mixture of methyl 7-*cis*,9-*cis*- *and* 7-*cis*,9-*cis*,13-*cis*-retinoates with $LiAlH_4$ (room temperature, 2 hr) followed by MnO_2 oxidation (CH_2Cl_2, room temperature, 0.75 hr) gave the corresponding mixture of retinal isomers. Separation of 7-*cis*,9-*cis*- and 7-*cis*,9-*cis*,13-*cis*-retinal was readily effected by column chromatography (Biosil A, 25% $CHCl_3$–hexane) with the latter eluting first.[14] The NMR spectra of the pure isomers are presented in Figures 1 and 2. The spectrum of 7-*cis*,9-*cis*-retinal is identical with that of HPLC purified sample from the mixture prepared previously. The olefinic region is well resolved and all vinylic hydrogens can be unambiguously assigned. The spectrum of 7-*cis*,9-*cis*,13-*cis*-retinal is complicated by the accidental equivalence of H-11 and H-12; however, with $Pr(fod)_3$ shift reagent, the familiar first order d and d of d signals for H-12 and H-11, respectively, are again present (see A in Figure 2).

We are currently examining alternate approaches to stereoselective 15 + 5 condensations of 7,9-*cc*-**1** as well as new routes to pure 7-*c*-**1**.

Acknowledgment. This work was supported by grants from the Public Health Services (EY00918, AM17806).

References and Notes

(1) New Geometric Isomers of Vitamin A and Other Carotenoids. II. For paper I, see ref 2.
(2) V. Ramamurthy and R. S. H. Liu, *Tetrahedron*, **31**, 201 (1975).
(3) In 7 ft × 0.25 in.-Corasil-II or 1 ft × 0.25 in. μ-Porasil columns.
(4) For a summary of synthesis of 7-trans of retinal and carotenoids, see O. Isler, Ed., "Carotenoids", Birkhauser, Verlag, Basel and Stuttgart, 1971.
(5) When β-ionone was treated with 1 equiv of $Pb(OAc)_4$, considerable amounts of 10,10-diacetate were formed (10%). With 2 equiv of $Pb(OAc)_4$, the diacetate became the major product (60%) with **2** being formed in 30–35% yield. Diacetoxylation was effectively suppressed by using a 1.5-fold excess of β-ionone. See J. W. Ellis, *J. Org. Chem.*, **34**, 1154 (1969), for analogous preparations.
(6) Compounds **2** to **7** show expected spectroscopic properties which will be disclosed in a full paper in the future.
(7) With more polar solvents the Horner reaction exhibited decreased selectivity. Thus, in THF or 25% HMPA–THF, the ratio of *tt*-**3** to *tc*-**3** was 5:1 and 7:3, respectively. Similar stereoselectivity has been reported for other systems: G. R. Pettit, C. L. Herland, and J. P. Yardley, *J. Org. Chem.*, **35**, 1389 (1970), and ref 8 therein.
(8) Prolonged irradiation of *tt*-**3** greatly decreased the overall yield of both *cc*-**3** and *ct*-**3**. Careful monitoring of the photoreaction by NMR spectroscopy circumvented this problem.
(9) R. K. Crossland and K. L. Servis, *J. Org. Chem.*, **35**, 3195 (1970).
(10) A lower yield (66–70%) of **6** was obtained when DMSO was used as solvent. This was presumably due to a slow interaction of $NaBH_4$ with this solvent. On the other hand, see H. M. Bell, C. W. Vanderslice, and A. Spehar, *J. Org. Chem.*, **34**, 3923 (1969).
(11) The use of more reactive reducing reagents (e.g., LAH) in an attempt to convert **5** directly to **7** only resulted in the formation of allylic rearranged isomer.
(12) V. Ramamurthy, G. Tustin, C. C. Yau, and R. S. H. Liu, *Tetrahedron*, **31**, 193 (1975).
(13) G. Pattenden and B. C. L. Weedon, *J. Chem. Soc. C*, 1984, 1997 (1968).
(14) This elution pattern is consistent with 7-cis isomers of retinal (ref 2).
(15) Fellow of the John Simon Guggenheim Foundation, 1974–1975.

A. E. Asato, R. S. H. Liu*[15]
Department of Chemistry, University of Hawaii
Honolulu, Hawaii 96822
Received April 19, 1975

Inhibition of Jack Bean Urease (EC 3.5.1.5) by Acetohydroxamic Acid and by Phosphoramidate. An Equivalent Weight for Urease

Sir:

On the basis of earlier reports of new substrates for urease,[1,2] and our finding that semicarbazide was a substrate,[3] we quite wrongly predicted that phosphoramidate would also be a substrate. Instead we found that it produced reversible inhibition with kinetic characteristics surprisingly similar to those of acetohydroxamic acid. This finding has enabled us to obtain two totally independent assessments of the operational equivalent weight[4] of urease, measured by correlation of specific enzymatic activity with the incorporation of the reversibly bound inhibitors, [^{14}C]acetohydroxamic acid[5,6] and [^{32}P]phosphoramidate. Further, it led us to reexamine the metal ion content of urease which is reported in the following communication.[7]

[U-^{14}C]Acetohydroxamic acid was prepared from [U-^{14}C]acetamide[8] by treatment with hydroxylammonium chloride for 1 hr at 86–96° with the exclusion of moisture. The recrystallized product had constant specific radioactivity (17.70 μCi/mmol) and mp 88.7–89.1°, lit.[9] mp 89°. [^{14}C]Acetohydroxamic acid was assayed spectrophotometrically,[10] using acetohydroxamic acid as standard.

Ammonium [^{32}P]phosphoramidate was prepared essentially as described by Sheridan et al.[11] The recrystallized product had constant specific radioactivity (177.3 μCi/mmol), and was free of contaminating radioactivity,[14] and of material which did not hydrolyze in acid to give inorganic phosphate.[15]

Scintillation counting of aqueous samples was carried out in Instagel (Packard Instrument Co., Inc.) or in a medium prepared from toluene (Mallinckrodt, scintillation grade; 48 vol), Triton X-100 (35 vol), and Liquifluor (New England Nuclear; 2 vol) using a Nuclear Chicago Mark I or a Beckman LS 250 liquid scintillation system. Counting efficiencies were measured using an internal standard of [^{14}C]toluene (New England Nuclear) or ammonium [^{32}P]phosphoramidate (25 μl of a 3.442 m*M* aqueous solution) dispensed with a Grunbaum pipet (Labindustries). All dilutions and measurements were made in duplicate.

Urease was prepared as previously described,[16] except that the storage buffer was 5 m*M* in β-mercaptoethanol. Two totally independently prepared samples (I and II) of the enzyme were used. Ureases I and II had specific activities[17] of 84,620 and 84,610 $(\mu kat/l.)/A_{280}$, respectively. Assays of inhibited enzyme samples and of control samples of native urease were performed at 10°, at which temperature the reactivation of inhibited enzyme during assay is negligible.

Urease I (5.10 ml, 5.62 mg/ml, in oxygen-free 0.02 *M* phosphate buffer, 1 m*M* each in EDTA and β-mercaptoethanol, pH 7.0) was equilibrated at 38° with 4.9 m*M* [U-^{14}C]acetohydroxamic acid for 10 min. Urease II (5.30 ml, 2.46 mg/ml, in 0.05 *M* *N*-ethylmorpholine buffer, 1 m*M* in EDTA and 5 m*M* in β-mercaptoethanol, pH 7.12) was equilibrated at 38° with 23.2 m*M* 2-(*N*-morpholino)ethanesulfonic acid (to produce pH ~6.0) and 11.9 m*M* ammonium [^{32}P]phosphoramidate for 10 min.

In each experiment, the sample of inhibited enzyme was cooled rapidly to 0°, and passed at 4° through a column (3.0 × 35 cm) of Sephadex G-50 preequilibrated with the appropriate oxygen-free buffer. As expected from the slow reactivation at 4° of the enzyme-inhibitor complexes,[6] appropriate assays showed that the protein-inhibitor peaks were completely separated from the unbound radioactive inhibitors. The peak protein fraction ("maximally inhibited enzyme") was assayed immediately for enzymatic activity, protein concentration, and radioactivity. Aliquots (3.0 ml) of the maximally inhibited enzyme were equilibrated at 38° for varying lengths of time, cooled to 0°, passed through columns (2.2 × 14.5 cm) of Sephadex G-25 at 4°, and similarly assayed. The results of these assays are given in Table I.

A plot of the residual specific enzymatic activity of the effluent protein (expressed as a percentage of its specific activity before treatment with radioactive inhibitor) vs. the ratio [protein-bound inhibitor]/[protein] is strictly linear (Figure 1). The least-squares line so obtained extrapolates