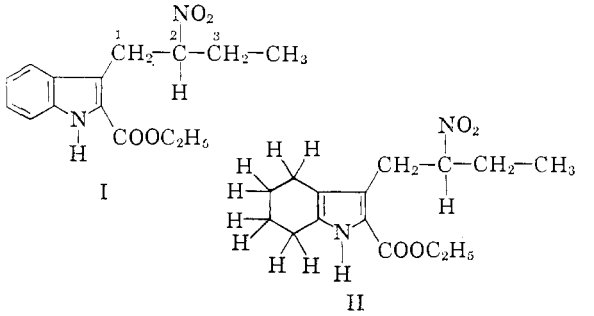
Prolonged (72 hr.) catalytic hydrogenation of 1-(α-carbethoxy-β-indolyl)-2-nitrobutane (I) in glacial acetic acid over 30% Pd/C at about 3 atm. and at room temperature led to II (48%), m.p. 122.5–124.5°, $\lambda_{max.}^{95\% \text{ ethanol}}$ 288, 227 (ϵ, 16,240, 6,380), n.m.r., no aromatic protons. *Anal.* Calcd. for $C_{15}H_{22}N_2O_4$: C, 61.20; H, 7.53; N, 9.52; mol. wt., 294.3. Found: C, 61.30; H, 7.40; N, 9.48; mol. wt. (Rast, camphor), 291. When the reaction was repeated in a different hydrogenator with catalyst from a different lot, II was isolated in 50% yield after 50 hr. Some of the Bz-tetrahydroamine (III, about 22%) was isolated in each experiment as the perchlorate, m.p. 232–234° dec., $\lambda_{max.}^{95\% \text{ ethanol}}$ 287, 245 (ϵ, 18,420, 4,240). *Anal.* Calcd. for $C_{15}H_{25}N_2O_6Cl$: C, 49.39; H, 6.91; N, 7.68. Found: C, 49.52; H, 7.17; N, 7.69. Compound I could be regenerated from II by chloranil dehydrogenation. Hydrogenation of 1-(α-carbethoxy-β-indolyl)-2-nitropropane (IV) under essentially the same conditions (except for a shorter time employed because of the more rapid consumption of hydrogen) occurred in normal fashion, producing a mixture of 1-(α-carbethoxy-β-indolyl)-2-aminopropane (V, 46%) and the corresponding lactam, 1-oxo-3-methyl-1,2,3,4-tetrahydropyrido[3,4-b]indole (VI, 13%). The amine (V) was isolated as the perchlorate, m.p. 240.5–241.5° dec.; $\lambda_{max.}^{95\% \text{ ethanol}}$ 296, 228 (ϵ, 21,240, 24,900). *Anal.*, Calcd. for $C_{14}H_{19}N_2O_6Cl$: C, 48.47; H, 5.52; N, 8.08. Found: C, 48.57; H, 5.60; N, 7.77.

Compounds I and IV also were reduced by a chemical method to form the respective lactams (VII) and (VI). Treatment of I with zinc in aqueous acetic acid for 5 hr. at 40° gave VII (41%), m.p. 190–191.5° subl., infrared, lactam carbonyl at 1660 cm.$^{-1}$. *Anal.* Calcd. for $C_{13}H_{14}N_2O$: C, 72.88; H, 6.59; N, 13.08. Found: C, 73.12; H, 6.31; N, 12.92. Similar treatment of IV gave VI (59%), m.p. 226–227° subl., infrared, lactam carbonyl 1660 cm.$^{-1}$. *Anal.* Calcd. for $C_{12}H_{12}N_2O$: C, 71.97; H, 6.04; N, 13.99. Found: C, 71.92; H, 5.94; N, 13.82.



The slow hydrogenation of the nitro function in I, in contrast to the relatively rapid hydrogenation of that in IV, suggests that the methyl group attached to the 3-carbon atom of I, in conjunction with the steric requirements of the remainder of the molecule I, hinders contact between the nitro function and the catalyst surface. Indeed, an examination of models (Stuart and Briegleb) reveals that the nitro function in both I and IV is in a sterically crowded environment. The fundamental structure of IV, having the carbethoxy group coplanar with the aromatic nucleus (carbonyl infrared absorption of the ester function appears near 1695 cm.$^{-1}$), is such that the nitro group can occupy only a restricted number of positions. Addition of the methyl group to the 3-carbon atom (to give I) blocks the only remaining approach to the close proximity of the nitro group. The observation that hydrogenation of the benzene ring in I occurs when the nitro function is isolated from the catalyst surface further suggests that electron-attraction by the nitro group facilitates hydrogenation of the benzene ring. Several known examples of hydrogenation of the benzene ring of an indole nucleus,[2,3] especially in the field of β-carboline alkaloids,[4,5] support the hypothesis that a more or less remote electron-attracting group may produce such an effect.

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GLUTAMIC ACID DEHYDROGENASE—A PROTEIN OF UNUSUAL CONFORMATION[1]

Sir:

A systematic study of the optical rotatory dispersion of globular proteins showed that about 40% of them have relatively high rotatory dispersion constants (λ_c) of 250–290 mμ, and that all proteins are levorotatory.[2,3,4] Among the enzymes, the slightly levorotatory dehydrogenases exhibit high λ_c values indicating a high α-helix content in these macromolecules.[5,6,7,8] It was, however, most surprising that the glutamic acid dehydrogenase (GAD)[9] was *dextrorotatory*. Upon a mild denaturation with alkali of *p*H 9.5 a negative shift of the rotation was observed, and a λ_c of 316 ($\pm$15) mμ was obtained at the beginning of the alkali treatment. The negative shift could be followed about 4–5 hours after the addition of alkali. At the end of this period the λ_c dropped to 240 mμ, and further observation was impossible because of increasing turbidity of the solution. The data, which were obtained with a Rudolph model 80 photoelectric spectropolarimeter, are shown in Table I.

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(9) The GAD preparations were obtained from California Corporation for Biochemical Research, Los Angeles; the crystallized enzyme was isolated from bovine liver by the C. F. Boehringer & Soehne G.m.b.H., Mannheim, Germany. The enzyme was obtained as a suspension of the crystals in either sodium sulfate or ammonium sulfate solutions. The crystals were dissolved by dialysis against dilute solutions of sodium phosphate or sulfate.

TABLE I

SPECIFIC ROTATION OF 0.80% GAD IN 0.02–0.10 *M* SODIUM SULFATE OR PHOSPHATE (+ NaOH), *T* 25°

λ, mμ	Native GAD, *p*H 6.9–8.6	*p*H 9.5, 30 minutes after adding NaOH[a]	*p*H 9.5, 4 hours later
546.1	+13° ± 5	−22° ±3	−49° ± 4
492		−28	−72
435.8	+21	−50	−97
404.7	+20	−63	−122
366	+9	−121	−164
b_0	−170 ± 40	−357	−70

[a] These values are corrected for the change occurring during observation.

Although the accuracy, because of the weak rotatory power and turbidity, was low, the dextrorotation was ascertained using three different preparations, *e.g.*, the $[\alpha]_{435.8}$ of one of them (in 0.02*M* sodium sulfate, *p*H 7.1) was +24°, while another preparation (in 0.1 *M* phosphate, *p*H 6.9) yielded at the same wave length +18°, and a third (in 0.01 *M* sulfate, *p*H 7.4) +19°. Treatment of the native enzyme with sodium decyl sulfate (0.05 *M*, *p*H 7.8) at 25° resulted in a negative shift of the specific rotation ($[\alpha]_{435.}$ −87°), and upon this treatment the turbidity disappeared. The λ_c of the detergent treated GAD was 247 mμ.

The rotatory data of the native GAD did not fit the one-term Drude rule. The data then were evaluated according to the Moffitt–Yang method,[10] *i.e.*, the $[\alpha]_\lambda \times (\lambda^2 - \lambda_0^2)$ values were plotted against $\lambda^4/(\lambda^2 - \lambda_0^2)$, where λ_0 is 212 mμ, and the b_0 values of the Moffitt–Yang equation[10] were calculated (see Table I). The b_0 for the detergent treated GAD was found to be −98. (The refractive index and residual molecular weight factors were disregarded, since they do not affect b_0 greatly.[11]) Recently the b_0 of another preparation of native GAD was rechecked with an improved Rudolph model 80-AQ6 instrument, and the b_0 was found to be −208 (±20). The $[\alpha]_\lambda = f(\lambda)$ curve had a flat minimum at 290–300 mμ. The Moffitt–Yang equation was applicable only within the near ultraviolet and visible spectral range.

The enzymic activity was tested according to Strecker,[12] and the neutral solutions were found as active as the most active preparations described in the literature.[12,13,14] At *p*H 8.6, however, the activity was considerably diminished, whereas no significant change could be seen in the rotatory power. Denaturation with alkali at *p*H 9.5 (4.5 hours at 25°) or with sodium decyl sulfate resulted in complete inactivation.

Since the molecular weight of native GAD is known to be about 10^6, and since the enzyme dissociates and aggregates readily,[13] it seems likely that the $M = 10^6$ particle may be composed of several polypeptide chains or subunits.[15] Terminal amino acid analysis of three specimens after Sanger[16] showed that 17–23 moles of N-terminal alanine and 1–2 moles of N-terminal aspartic and/or glutamic acids are present per mole of enzyme. Thus the $M = 10^6$ particle is composed at least of 17–23 peptide chains. After adding the decyl sulfate, a complete dissociation occurred, since a low s_{20}^0 of 3.7 *S* and a low intrinsic viscosity of 0.025 dl./g. was observed. Application of the Scheraga–Mandelkern treatment[17] by taking a β factor of 2.16, yielded for the subunits an *M* of 43,000. This is in reasonable agreement with the N-terminal group analysis, *i.e.*, each subunit possibly represents one polypeptide chain.

The results indicate that GAD is a protein of unusual conformation. The negative b_0 and the dextrorotation of the native enzyme suggest the presence of α-helical conformation.

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THE COPPER SALT CATALYZED PEROXIDE REACTIONS

Sir:

The versatility of the copper salt catalyzed reaction of peroxides with various substrates has been well described.[1,2,3,4,5] In particular, the reaction between peroxides and olefins has been postulated to proceed via a complex containing copper, peroxide and olefin.[1,4,5] This termolecular mechanism is based in part on the *non-rearrangement* of allylic systems.[1,4]

We have examined this reaction between *tert*-butyl peracetate and perbenzoate with the three isomeric butenes in the presence of cuprous bromide at 75–85°. The stoichiometry is described by the equation

$$RCO_3Bu\text{-}t + C_4H_8 \rightarrow RCO_2C_4H_7 + t\text{-}BuOH$$

The over-all yields of butenyl acetates and benzoates are in the range of 70–85%. The compositions of the butenyl ester mixtures were examined by gas–liquid chromatography and found to be *invariant* (within 5%) with the reactant butene. Thus, butene-1, *cis*-butene-2 and *trans*-butene-2 all yield a mixture of butenyl esters consisting of 89–94% 3-acyloxybutene-1 and 11–6% crotyl esters. Under the conditions of these experiments there is no allylic isomerization of either the reactant butenes[6] or product esters.

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