proline do not appear to have been satisfactorily described. It is well established that it exists in a strongly levorotatory form, designated as II, in water and organic acids; Blout, *et al.*,[4] report a strong negative Cotton band centered at 203 mμ ($[m']_{216}$ *ca.* −32,000°) for this form in water. It is also known that, as normally prepared, poly-L-proline exists in a different form, designated as I, which is weakly dextrorotatory at 589 mμ and is stable in aliphatic alcohols[3,6] but unstable in water and organic acids. Upon dissolving in these latter solvents, it mutarotates over a period of several hours to give form II.[1,2,3] The structures of solid state forms corresponding to I and II have been established; I[7] is a right-handed helix with a residue translation of 1.85 A and with the peptide bonds in the *cis* configuration, whereas II[8,9] is left-handed with a residue translation of 3.12 A and with the peptide bonds *trans*.[10] The mutarotation in solution very probably arises principally from *cis–trans* isomerization about the peptide bonds.

Form I has been stated by Blout, *et al.*,[4] to exhibit a negative Cotton band in the far ultraviolet even stronger than that of II. We have investigated this question further by observing the mutarotation of poly-L-proline at ambient temperature (*ca.* 30°) in trifluoroethanol solution, observing both ORD (at 1–2 × 10^{-2} *M* in residues) and absorption over the range 280 to 185 mμ.[11] Some results of a typical experiment are shown in Figure 1. It is observed that initially the poly-L-proline (degree of polymerization 140) exhibits a strong *positive* Cotton band, apparently centered at about 217 mμ.[12] This gives place (half-life about 1.5 hr at *ca.* 30°) to a weaker negative Cotton band, centered at 202 mμ, corresponding to that reported by Blout, *et al.*[4]

We find the absorption spectrum (in trifluoroethanol) of the trifluoroethanol-stable form (presumably form II) to have a maximum at 202 mμ (ε 7100) while that of the initial form (assumed to be I) is at 210 mμ (ε 8900), a wavelength difference even greater than that reported by Gratzer, *et al.*,[5] for aqueous solutions. The absorption maximum for II corresponds to the inflection point of the ORD curve, but that for I is markedly shifted to shorter wavelength. The positive lobe of the form I ORD curve is considerably weaker ($[m]_{223}$ = +47,500 ± 500°) than the negative lobe ($[m]_{208}$ = −90,000 ± 500°),[13] both values extrapolated to zero time; they are more nearly equal (Figure 1 and ref 4) for form II.

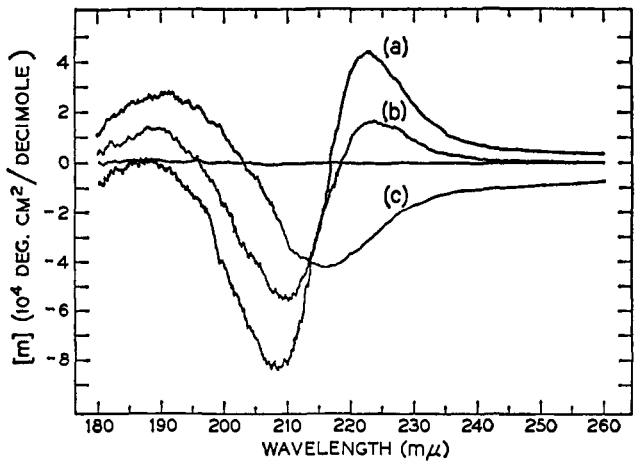

Figure 1. ORD spectra of poly-L-proline, 2.05 × 10^{-2} *M* in trifluoroethanol, *ca.* 30°. Curves a, b, and c are run at successive times. Because the rates of scanning and of mutarotation are comparable, curves a and b show some kinetic distortion. For these, the three intervals given below represent the times (minutes after initial dissolution of polymer) at which the first maximum, first minimum, and second maximum were observed: (a) 8, 11, 22 min; (b) 64, 68, 80 min; (c) 20 hr.

The assignment of the form I Cotton band is not yet certain. It is probable that it is a π–π* band, but in view of the marked n-π* Cotton band observed in the closely related model compound cyclic di-L-proline,[14] an n–π* contribution cannot be excluded.

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(2) W. F. Harrington and M. Sela, *Biochim. Biophys. Acta*, **27**, 24 (1958).
(3) I. Z. Steinberg, A. Berger, and E. Katchalski, *ibid.*, **28**, 647 (1958).
(4) E. R. Blout, J. P. Carver, and J. Gross, *J. Am. Chem. Soc.*, **85**, 644 (1963).
(5) W. B. Gratzer, W. Rhodes, and G. D. Fasman, *Biopolymers*, **1**, 319 (1963).
(6) F. Gornick, L. Mandelkern, A. F. Dorio, and D. E. Roberts, *J. Am. Chem. Soc.*, **86**, 2549 (1964).
(7) W. Traub and U. Shmueli, *Nature*, **198**, 1165 (1963).
(8) P. M. Cowan and S. McGavin, *ibid.*, **176**, 501 (1955).
(9) V. Sasisekharan, *Acta Cryst.*, **12**, 897 (1959).
(10) For a review and illustrations of these structures see W. F. Harrington and P. H. von Hippel, *Advan. Protein Chem.*, **16**, 1 (1961).
(11) Absorption and ORD spectra were obtained using a Durrum-Jasco Model ORD/UV-5 spectrophotometer and ORD recorder. Quartz cells (Opticell Cell Co., Inc., Brentwood, Md.) of 0.1- and 1-mm path length were employed. Poly-L-proline was obtained from Yeda (Rohovoth, Israel) in two lots having degrees of polymerization of 15 and 140.
(12) Since the completion of this work, Carver and Blout (private communication) have reported a similar band in polyproline I. E. R. Blout and E. Schechter (*Biopolymers*, **1**, 565 (1963)) have reported similar bands for poly-L-proline films grown from a catalytic surface.
(13) Very similar positive Cotton bands are observed in both 90:10 (v/v) 1-butanol–trifluoroethanol and 90:10 acetonitrile–trifluoroethanol, and these show no change after 30 days at room temperature. We interpret this to mean that the high molecular weight polymer is substantially entirely in form I in these solvents (and at zero time in trifluoroethanol) as it would not be expected that one would coincidentally choose three solvent systems in all of which the same equilibrium mixture of I and II happened to prevail. On the other hand, the low molecular weight polymer, while showing generally similar behavior, exhibits at the start an ORD curve indicating the presence of form II; it also shows somewhat weaker rotations for both forms.
(14) F. A. Bovey, F. P. Hood, and J. Longworth, unpublished observations.

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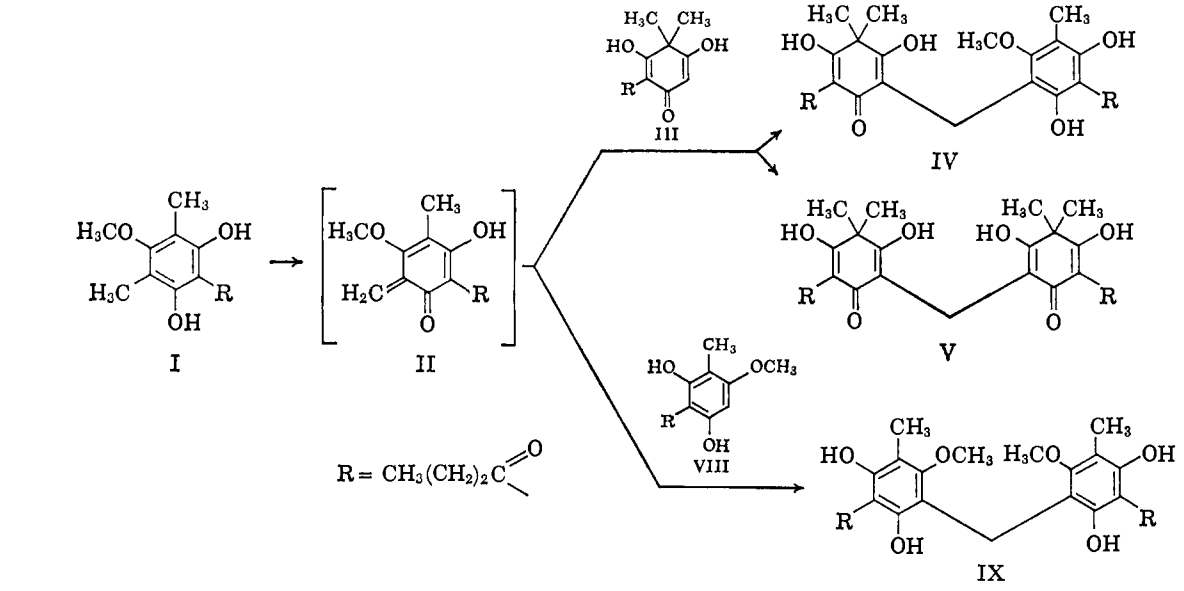
The Biosynthesis *in Vitro* of Methylenebisphloroglucinol Derivatives

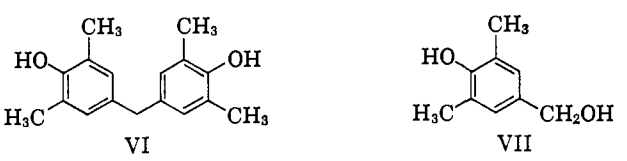
Sir:

We have recently shown that the methyl groups as well as the central methylene unit of *Dryopteris* fern constituents such as *p*-aspidin (IV) are derived from methionine *in vivo*.[1] In addition, methylaspidinol (I) has been shown[1] to be incorporated in IV, presumably *via* oxidation to the quinone methide species (II), followed by Michael addition of the anion of butyryl-

(1) A. Penttila, G. J. Kapadia, and H. M. Fales, *J. Am. Chem. Soc.*, **87**, 4402 (1965).

filicinic acid (III). Because of the similarity of this process to the chemical coupling of mesitol to VII,[2] as well as the horseradish peroxidase induced oxidation of mesitol to VII,[3] we were led to study the action of this enzyme upon an equimolar mixture of I and III in phosphate buffer at pH 7.8.[4] A fair yield (~30%) of a mixture of *p*-aspidin (IV) and albaspidin (V) was obtained and the products were identified by paper chromatography[5] and mass spectra.[6] The albaspidin (V) resulted from the further reaction of *p*-aspidin with III, since a mixture of III and IV at pH 7.8 without enzyme was shown to form albaspidin (V) with the liberation





of a corresponding amount of aspidinol (VIII); *cf.* "rottleron change."[7]

In an analogous example, methylaspidinol (I) was combined with an equimolar quantity of aspidinol (VIII) and, after a few minutes incubation with peroxidase, methylenebisaspidinol (IX) was obtained in crystalline form and identified by comparison of melting point (190–191°) and infrared spectrum with authentic material.[8] Both I and VIII were required; incubation of either I or VIII alone gave rise to no discernible dimeric species.[9]

(2) S. L. Cosgrove and W. A. Waters, *J. Chem. Soc.*, 1726 (195).
(3) H. Booth and B. C. Saunders, *ibid.*, 940 (1956).
(4) Compounds I and III (3 μmoles each) were stirred with 0.6 ml of phosphate buffer (pH 7.8), 0.75 ml of hydrogen peroxide (0.015%), and 0.06 mg of crystalline horseradish peroxidase (Boehringer) for 15 min.
(5) A. Penttila and J. Sundman, *J. Pharm. Pharmacol.*, **13**, 531 (1961).
(6) Mass spectra were obtained by slowly volatilizing the crude reaction mixture from the end of a probe inserted in the ion source of an Associated Electrical Industries MS-9 double-focussing mass spectrometer. The monomers I and III (or I and VIII) volatilized at <100° showing the parent ions at *m/e* 238 (I) and 224 (III and VIII). Complete evaporation left the dimers IV and V, or IX, which volatilized at ~170°, showing a parent ion at *m/e* 460 in each case as well as characteristic fragment ions.
(7) T. Backhouse, A. McGookin, J. Matchett, A. Robertson, and E. Tittensor, *J. Chem. Soc.*, 113 (1948).
(8) R. Boehm, *Ann.*, **329**, 269 (1903).
(9) Unless demethylation of I occurs, only VIII could oxidize to a dimer; however the former process has been observed; *cf.* ref 2.

The facility with which these reactions proceed led us to look for peroxidase activity within the plant. Highest activity is usually found in the roots;[10] in contrast no activity was found in *Dryopteris* rhizomes while the leaves gave a highly positive test. This suggests that the dimerization, and presumably the main biosynthetic activity, is taking place in the leaves while the rhizomes are a storage location for the dimeric compounds.

Although a wide variety of materials (anilines, phenols, dyes, etc.) have been used as hydrogen donors with peroxidase, this appears to be one of the few examples[11] of the formation of a natural product with the aid of this versatile enzyme.

(10) K. Wachholder, *Biochem. Z.*, **213**, 394 (1942).
(11) For example, pyrogallol → purpurogallin: R. Willstätter and J. Weiss, *Ann.*, **433**, 17 (1923).
(12) Visiting Scientist at the National Heart Institute, on leave from Medica, Ltd., Helsinki, Finland (1966).

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A New Hydrogen-Abstracting Reaction with Diethyl Azodicarboxylate

Sir:

Azo-disubstituted compounds would be expected to be strong electron acceptors when their substituents are electron-attracting groups such as alkoxycarbonyl, acyl, nitrile, and so on. The treatment of these compounds with Hückel MO theory indicates that they show the special case of possessing a vacant bonding orbital.[1] For example, the energies of the vacant bonding orbitals of dimethyl azodicarboxylate, diethyl azodicarboxylate, azodiacetyl, and Δ^1-1,2,4-triazoline-3,5-dione[2] showed +0.369, +0.369, +0.357, and

(1) Recently, F. D. Marsh and M. E. Hermes, *J. Am. Chem. Soc.*, **87**, 1819 (1965), reported that molecular orbital calculation on azodicarbonitrile indicated an unfilled bonding orbital was present at $+0.42\beta$.
(2) Although this compound is unknown, the 4-phenyl derivative was synthesized already (R. C. Cookson, S. S. H. Gilani, and I. D. R. Stevens, *Tetrahedron Letters*, 615 (1962)).