loss of water already mentioned; (2) the ultraviolet spectrum shows $\lambda_{max}^{CHCl_3}$ 269 mμ (ε, 12000); (3) the infrared spectrum shows absorption at 5.52 μ (strained β-lactam), 5.9 μ (strained and conjugated γ-thiolactone), 6.0 μ (amide) and 6.1 μ (double bond); (4) the n.m.r. spectrum[11] shows absence of the tertiary hydrogen at carbon-3 and a doublet at $\tau = 7.83$ and 7.92 for the isopropylidene group; (5) ozonolysis affords acetone in excellent yield; no acetone is obtained upon ozonolysis of the parent penicillin.

The *anhydro*penicillins possess extraordinary chemical stability as compared with the parent penicillins. This is surprising in view of the highly strained bicyclic system of these compounds (see infrared data above). Thus they are recovered unchanged after prolonged refluxing in ethyl alcohol, aqueous dioxane or xylene. The *anhydro*penicillins display only weak antibacterial activity.[12] However, their increased chemical stability and the activation of the methyl groups by the adjacent double bond makes the introduction of substituents on the methyl groups possible by allylic attack. Some of the products thus obtained show antibacterial activity *and* stability to penicillinase. Details of these and related experiments will be reported in a subsequent paper.

Acknowledgments.—We thank Professor B. Belleau of the University of Ottawa for stimulating discussions which led to the correct structure, D. L. Evans for valuable assistance with the infrared spectra and Dr. L. C. Cheney for his advice and encouragement.

(11) Obtained in methylene chloride on the Varian A60 N.M.R. spectrometer. We thank D. L. Whitehead for this spectrum.

(12) Whereas penicillin G. shows a minimum inhibitory concentration *versus Staph. aureus Smith* of 0.02–0.05 γ/ml., *anhydro*benzylpenicillin inhibits growth at 70 γ/ml.

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RECEIVED JANUARY 4, 1963

SYNTHETIC PORPHYRINS RELATED TO CHLOROBIUM CHLOROPHYLLS

Sir:

Analytical evidence indicated that a tricarboxylic acid derived from fraction 5 of chlorobium phaeophorbide (660) degraded to a homolog of δ-phylloporphyrin wherein the distribution of methyl and ethyl groups on the 5- and δ-positions was uncertain.[1] This phylloporphyrin has since been isolated and characterized[2] as has a pyrroporphyrin[2] obtained in the same way from fraction 4 of chlorobium phaeophorbide (650).

We have synthesized the methyl ester of 1,3,8-trimethyl-2,4,5-triethylporphin-7-propionic acid (*Anal.* Calcd. for $C_{33}H_{38}O_2N_4$: C, 75.83; H, 7.33; N, 10.72. Found: C, 75.91; H, 7.48; N, 10.61) and of its δ-methyl derivative (*Anal.* Calcd. for $C_{34}H_{40}O_2N_4$: C, 76.09; H, 7.51; N, 10.44. Found: C, 76.27; H, 7.21; N, 10.61). These syntheses were analogous to a synthesis of pyrroporphyrin XV[3] and utilized the pyrromethenes from 2-formyl-3-bromo-4-ethyl-pyrrole-5-carboxylic acid with 2-methyl- or 2-ethyl-3-methyl-pyrrole-4-propionic acid.

The identity of the methyl ester of the above chlorobium pyrroporphyrin and the first synthetic porphyrin seemed assured but not clear-cut because of their polymorphism. Their copper complexes, however, showed identical m.p. (220–226°), mixed m.p. and X-ray powder photographs. This proved the 5-ethyl group in fraction 4 of chlorobium phaeophorbide (650), also proved by degradation to ethylmaleimide.[2] It also proved that the pairs of substituents have the hitherto assumed arrangements on the pyrrole rings as in phaeophorbide-a; this was also proved in the case of chlorobium phaeophorbide (650) fraction 6 by conversion to pyrophaeophorbide-a.[2]

The methyl ester of the above phylloporphyrin homolog from chlorobium and the second synthetic porphyrin were identical in m.p. (214–215.5°), mixed m.p., and in their exceptionally well defined X-ray powder photographs. A difficulty in the analytical evidence was resolved when it was found that our synthetic porphyrin, like the analytical one[1] but not as reported for δ-phylloporphyrin IV,[4] had bands II and III of its visible spectrum equal in intensity. The 5-ethyl group and the δ-methyl group, the preferred alternative on general grounds,[2] as well as the hitherto assumed arrangement of the substituents are thus proved in chlorobium phaeophorbide (660) fraction 5; the proton magnetic resonance data is consistent with a δ-alkyl group.[1]

We are also synthesizing porphyrins with the 4-*n*-propyl- and 4-isobutyl-substituents proved by the analytical work,[2] including 4-*n*-propyl-4-des-ethyl-des-oxo-phylloerytherin methyl ester, m.p. 236–238° (*Anal.* Calcd. for $C_{35}H_{40}O_2N_4$: C, 76.61; H, 7.35; N, 10.21. Found: C, 77.02; H, 7.15; N, 10.55), copper complex, m.p. 244–246° (*Anal.* Calcd. for $C_{35}H_{38}O_2N_4$: C, 68.90; H, 6.28; CuO, 12.39. Found: C, 68.56; H, 6.34; residue, 12.39). This awaits analytical material for comparison.

The authors wish to thank Dr. A. S. Holt of the Division of Applied Biology for his generous coöperation.

(1) A. S. Holt, D. W. Hughes, H. J. Kende and J. W. Purdie, *J. Am. Chem. Soc.*, **84**, 2835 (1962).

(2) A. S. Holt and J. W. Purdie, in preparation.

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(4) H. Fischer and H. Orth, "Chemie des Pyrrols," II/1, Leipzig, 1937, p. 360.

(5) N. R. C. Postdoctoral Fellow 1960–1962.

(6) N. R. C. Postdoctoral Fellow 1959–1961.

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RECEIVED NOVEMBER 24, 1962

INTRINSIC COTTON EFFECTS IN COLLAGEN AND POLY-L-PROLINE[1,2]

Sir:

Previous investigations of collagen solutions have shown that the optical rotation in the visible and near ultraviolet regions undergoes large changes from highly negative to less negative values upon denaturation.[3] Rotatory dispersion measurements, in spectral regions removed from absorption bands, on both native and denatured collagen solutions fit the one-term Drude equation as do dispersion data from random coil polypeptides and proteins with low helix contents.[4] Recently, acceptable models for the molecular structure of collagen have been proposed[5,6] which involve three left-handed polyglycine-poly-L-proline II type helices wound in a right-handed super helix. In this com-

(1) This is Polypeptides XLII. For the preceding paper in this series see reference 16.

(2) This work was supported in part by U. S. Public Health Service Grant A2558 and in part by the Office of the Surgeon General, Department of the Army.

(3) See for example: C. Cohen, *J. Biophys. and Biochem. Cytology*, **1**, 203 (1955).

(4) For reviews on rotatory dispersion measurements see: (a) E. R. Blout, Chapter 17 in "Optical Rotatory Dispersion" by C. Dierassi, McGraw-Hill Book Company, New York, N. Y., 1960; (b) P. Urnes and P. Doty in "Advances in Protein Chemistry," Vol. 16, C. B. Anfinsen, N. L. Anson, K. Bailey and J. T. Edsall, editors, Academic Press, Inc., New York, N. Y., 1961, p. 401.

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(6) (a) A. Rich and F. H. C. Crick, *Nature*, **176**, 915 (1955); (b) A. Rich and F. H. C. Crick, *J. Mol. Biol.*, **3**, 483 (1961).

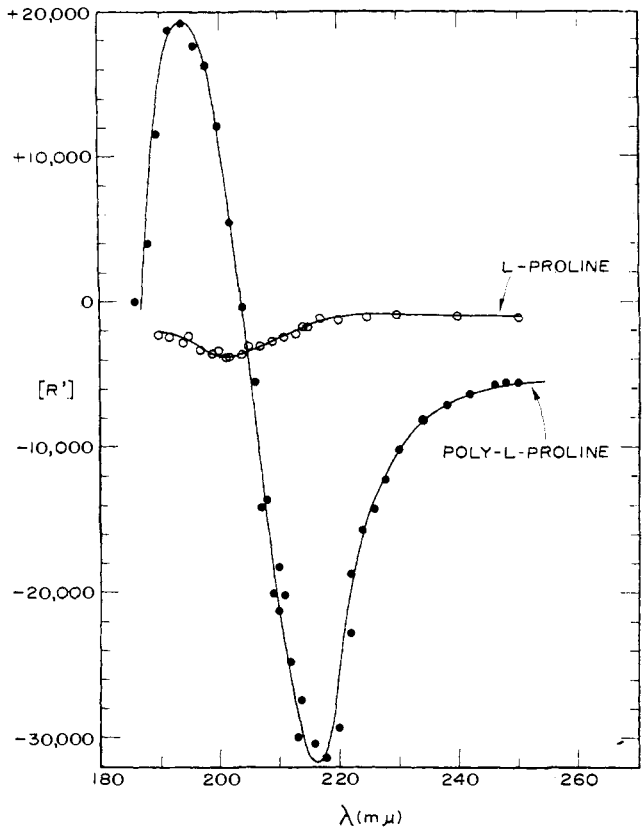

Fig. 1.—The far ultraviolet rotatory dispersions of poly-L-proline II ●—●—● and L-proline ○—○—○ in water solution. Concentrations were between 0.015 and 0.147% in a 1-mm. cell. The same spectropolarimeter was used as noted in reference 11.

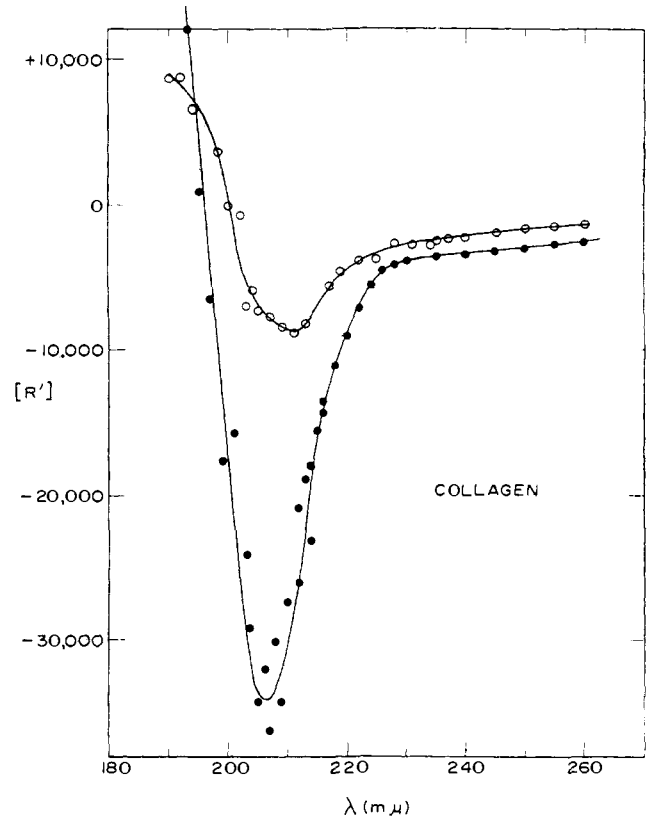

Fig. 2.—The far ultraviolet rotatory dispersion of native calf skin collagen ●—●—● in 0.01 molar acetic acid solution. The ultraviolet rotatory dispersion of the same preparation of calf skin collagen heated at 50° for 30 min., cooled to 25°, and measured immediately, ○—○—○. Concentrations were between 0.0076 and 0.076%. The same spectropolarimeter was used as noted in reference 11.

munication we report the finding of an intrinsic Cotton effect in the far ultraviolet optical rotatory dispersion of native collagen and poly-L-proline II solutions which appears to be characteristic of their molecular structure.

Many synthetic L-polypeptides have been shown to possess the α-helical conformation in the solid state.[7,8] In solution the optical rotatory dispersion of such materials obeys the Moffitt equation[9] in the region 240 to 600 mμ when the α-helical conformation is maintained. At shorter wave lengths two Cotton effects are observed, one at 225 mμ (negative),[10] and a second at 190 mμ (positive)[11] which appears to arise from the fundamental $\pi \rightarrow \pi^*$ transition of the peptide group. The sign of these Cotton effects is directly related to the right-handed helical sense of most L-synthetic polypeptides.

Poly-L-proline has been investigated as a possible model for some aspects of collagen structure,[12,13] and two forms, designated I and II, differing markedly in solubility and rotatory properties have been described.[14,15] We have shown recently that these two forms possess ultraviolet absorption bands at 206 and 203 mμ, respectively.[16] These bands probably are related to the $\pi \rightarrow \pi^*$ transitions of the polypeptide (amide) grouping. The far ultraviolet rotatory dispersion of poly-L-proline II reveals a large negative Cotton effect (Fig. 1) with an inflection point at 203 mμ and minima and maxima at 216 mμ and 194 mμ, respectively. This large Cotton effect is not observed in the far ultraviolet optical rotatory dispersion of L-proline (Fig. 1). The sign of the poly-L-proline II Cotton effect is opposite to that observed from the same transition in most L-α-helical polypeptides. If the correlation between the sign of the 190 mμ Cotton effect and the sense of the helix found for α helices can be applied to polyproline-type helices, then one would expect the sense of helix of poly-L-proline II in solution to be left-handed. This is consistent with the structure proposed by Cowan and McGavin[17] for poly-L-proline II in the solid state on the basis of X-ray diffraction measurements.

Investigations of native calf skin collagen solutions have shown the presence of an absorption maximum at 190 mμ and a large Cotton effect (also negative) at about 195 mμ (Fig. 2). The similarity of the Cotton effects described here (sign and magnitude) suggests a close relation between the left-handed poly-L-proline II helix and that of the structural components of collagen. Furthermore, the position of the inflection points in the Cotton effects of poly-L-proline II and collagen and the absence of a 225 mμ Cotton effect distinguishes these structures from α-helical conformations.

Treatment of a solution of native collagen at 50°

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(8) C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides," Academic Press, Inc., New York, N. Y., 1956.

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(11) E. R. Blout, I. Schmier and N. S. Simmons, *ibid.*, **84**, 3193 (1962).

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for one-half hour at least partially denatures the protein, and markedly reduces the magnitude of the Cotton effect (Fig. 2); thus it appears that the collagen Cotton effect is conformation-dependent.

Finally, we note some preliminary observations on the "reverse mutarotation" of poly-L-proline II to poly-L-proline I in 1-propanol–water solutions.[12b] Upon standing, the absorption spectrum shifts from 204 to 206 mμ and the near ultraviolet and visible optical rotation becomes much less negative. The Cotton effect in the far ultraviolet, however, remains negative in sign and, in fact, becomes larger in magnitude. Recent investigations[18] indicate that poly-L-proline I, in the solid state, is right-handed as earlier proposed. If one assumes this sense is maintained in solution, then one must conclude that profound changes in peptide group environment in going from II → I are responsible for the sign of the Cotton effect remaining the same despite the altered sense of helix.

(18) W. Traub and U. Shmueli, Paper A7, International Symposium on Protein Structure and Crystallography, Madras, India, January 14–18, 1963.

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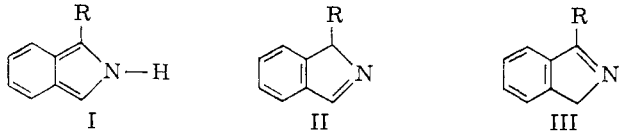
RECEIVED JANUARY 9, 1963

1-ARYL ISOINDOLES

Sir:

Isoindole and its N-unsubstituted derivatives (I) might be expected to be in tautomeric equilibrium with the corresponding isoindolenines (II) and (III)

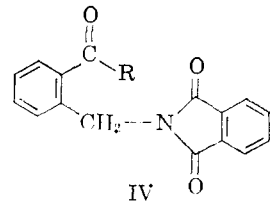


I II III

Investigation of this equilibrium would yield valuable data on the stability of the electronic system in (I) compared to that in the common systems (II) and (III). Zero order molecular orbital calculations give resonance energies of 50.0 kcal./mole for isoindole, 42.1 kcal./mole for isoindolenine and 79.0 kcal./mole for the 1-phenylisoindolenine (III).[1]

No experimental data have been available, since all reported isoindoles[2,3,4] are substituted on the nitrogen and are thus not capable of tautomerism. They are very reactive and unstable. Only one compound of type (III) has been described:[5] 1-ethoxyisoindolenine (III, R = OC_2H_5). Its stability and its infrared and n.m.r. spectra indicate that it exists entirely in the isoindolenine form. Attempts to prepare the parent isoindole (I) (or its tautomer) have failed.[6,7]

We have now prepared 1-aryl-isoindoles and found them to be in equilibrium with, and thus of stabilization comparable to, the tautomeric isoindolenines (III). The isoindoles are made by treatment of 2-(phthalimidomethyl)-benzophenone derivatives, such as (IVa) and (IVb) with hydrazine in refluxing ethanol. (IVa) and (IVb) are prepared from α-phthalimido-*o*-toluyl



IV

IVa, R = C_6H_5; m.p. 107–108°
b, R = *p*-H_3CO-C_6H_4; m.p. 102.5–104°
c, R = Cl

chloride (IVc)[7] and benzene or anisole, using 3 to 4 equivalents of aluminum chloride.

Both isoindoles (I, R = C_6H_5– or *p*-H_3CO-C_6H_4–) are solids, give blue Ehrlich tests and resinify when exposed to acid or air.

1-Phenylisoindole (I, R = C_6H_5) dec. 90–100°: $C_{14}H_{11}N$. *Anal.* Calcd.: C, 87.01; H, 5.74; N, 7.25. Found: C, 87.20; H, 5.80; N, 7.26. Infrared: NH at 3460 cm.$^{-1}$, no strong absorption above 1600 cm.$^{-1}$ in $CHCl_3$.

1-*p*-Methoxyphenylisoindole (I, R = *p*-H_3CO-C_6H_4): $C_{15}H_{13}NO$ (dec. 60–65°). *Anal.* Calcd.: C, 80.69; H, 5.87; N, 6.27. Found: C, 80.82; H, 5.92; N, 6.00. Infrared NH at 3460 cm.$^{-1}$, in $CHCl_3$.

The isoindole structure is confirmed by both the ultraviolet and the n.m.r. spectra. These spectra, taken in different solvents, also reveal the presence of isoindole–isoindolenine equilibria. The spectra show the tautomer to be the cross conjugated form (III), form (II) could not be detected. The ultraviolet spectra (Table I) of 1-phenyl- and 1-*p*-methoxyphenylisoindole in ethanol are very similar to that of Theilacker's 1,3-diphenyl-N-methylisoindole.[8] As expected, their extinction coefficients are somewhat lower, the difference being comparable to the difference in extinction coefficients of 2-phenylpyrrole and 2,5-diphenyl-N-methylpyrrole.[8]

TABLE I

Isoindole	λ_{max} (log ε)			
1-Phenyl-	357 (3.10)	325 (2.99)	282 (2.92)	272 (2.86)
1-*p*-Methoxyphenyl-	358 (3.00)	309 (2.93)	282 (3.25)	272 (3.10)
1,3-Diphenyl-N-methyl-	376 (4.30)	334 (4.03)	277 (4.13)	270 (4.10)
Maleic anhydride adduct of 1-phenyl-	357 (3.06)	324 (2.95)	282 (2.88)	272 (2.84)

The ratio of absorbance of the 358 mμ peak to that of the 272 mμ peak of 1-*p*-methoxyphenylisoindole changes with solvent. If it is assumed that (1) all the absorption at 358 mμ comes from the isoindole tautomer and (2) the extinction coefficient at 272 mμ of the isoindolenine is twice that of the isoindole (as is reasonable from comparison of benzophenone imines with isoindoles), the fraction of isoindole in the equilibrium can be determined: 1-*p*-methoxyphenylisoindole in various solvents

Solvent	A_{358}/A_{272}	% isoindole
Ethyl ether	1.138	100
Ethanol	0.925	90
Acetonitrile	0.874	87
Chloroform	0.614	70

The value of 100% isoindole in ether is based on the n.m.r. spectrum in ether-d_{10} (see below). The solvent dependency parallels that of keto–enol equilibria.[9] In the n.m.r. spectra the same solvent dependency is observed. The n.m.r. spectrum of 1-phenylisoindole in $CDCl_3$ shows the N–H signal at −0.0 to +0.9 τ (broad); aromatic protons 1.9 to 3.2 τ (composite);

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