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.

CHILDREN'S CANCER RESEARCH FOUNDATION
MASSACHUSETTS GENERAL HOSPITAL   E. R. BLOUT
AND DEPARTMENTS OF BIOLOGICAL   J. P. CARVER
CHEMISTRY AND MEDICINE   J. GROSS
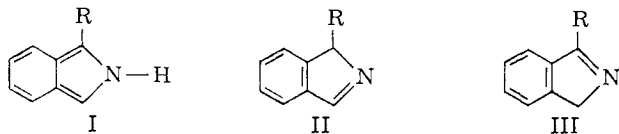HARVARD MEDICAL SCHOOL
BOSTON 15, MASSACHUSETTS

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-phenyl-isoindolenine (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$_2$H$_5$). 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-(phthal-imidomethyl)-benzophenone derivatives, such as (IVa) and (IVb) with hydrazine in refluxing ethanol. (IVa) and (IVb) are prepared from α-phthalimido-*o*-toluyl

(1) Using 16 kcal./mole for β.
(2) G. Wittig, H. Tenhaeff, W. Schoh and G. Koenig, *Ann.*, **572**, 1, 8 (1951).
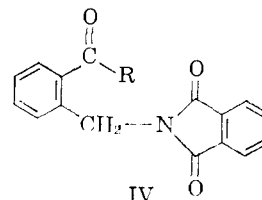(3) W. Theilacker and W. Schmidt, *ibid.*, **605**, 43 (1957), and earlier papers.
(4) G. Wittig, E. Knauss and K. Niethammer, *ibid.*, **630**, 10 (1960), and earlier papers.
(5) S. Petersen and E. Tietze, *ibid.*, **623**, 166 (1959).
(6) G. W. Fenton and C. K. Ingold, *J. Chem. Soc.*, 3925 (1928).
(7) J. Bornstein, S. F. Bedell, P. E. Drummond and C. L. Kosloski, *J. Am. Chem. Soc.*, **78**, 83 (1956).

IVa, R = C$_6$H$_5$; m.p. 107–108°
   b, R = *p*-H$_3$CO-C$_6$H$_4$; 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$_6$H$_5$– or *p*-H$_3$CO-C$_6$H$_4$–) are solids, give blue Ehrlich tests and resinify when exposed to acid or air.

1-Phenylisoindole (I, R = C$_6$H$_5$) 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$_3$CO-C$_6$H$_4$): 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*-methoxyphenyl-isoindole 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);

(8) B. Elpern and F. C. Nachod, *ibid.*, **72**, 3379 (1950); S. M. King, C. R. Bauer and R. E. Lutz, *ibid.*, **73**, 2253 (1951).
(9) A. S. N. Murthy, A. Balasubranian, C. N. R. Rao and T. R. Kastur, *Can. J. Chem.*, **40**, 2267 (1962).

and a peak at 5.13 τ attributed to the CH_2 group in the tautomeric isoindolenine (III, R = C_6H_5). The ratio of the areas of the aromatic proton signal plus $1/2$ of the CH_2 signal to the area of the N–H signal plus $1/2$ of the CH_2 signal was found to be 10.2:1, within experimental error of 10:1.[10] The area of the CH_2 peak corresponds to a fraction of 9% isoindolenine. In CCl_4 the relative area of the CH_2 peak corresponds to 4% isoindolenine. The changes in tautomeric equilibrium are more drastic for 1-p-methoxyphenylisoindole. The n.m.r. spectra of one and the same sample were measured in ethyl ether-d_{10} and in $CDCl_3$. In ether-d_{10}, the N–H signal was too broad to be integrated at -1.3 to 0.0 τ, aromatic protons at 1.9 to 3.1 τ, CH_2 at 5.2 τ (very small), and OCH_3 at 6.32 τ (single sharp peak). The integrals of aromatic to CH_3 signals had the ratio 2.9:1. The isoindolenine content was very small, judged by the CH_2 area and the single OCH_3 peak. In $CDCl_3$:N–H at -0.3 to $+0.3$ τ, aromatic protons at 1.9 to 3.2 τ, CH_2 at 5.19 τ, and two OCH_3 peaks at 6.26 and 6.29 τ, with relative peak heights about 1:2. The ratio NH $+ 1/2$ CH_2:$1/2$ CH_2 $+$ aromatic CH: OCH_3 was measured as 1.01:9.04:3.00. From the relative area of the CH_2 peak an isoindolenine content of 30.8% is calculated. This is consistent with the relative heights of the OCH_3 peaks and with the above determination from the ultraviolet spectrum (30%).

1-Phenylisoindole readily forms a maleic anhydride adduct. Its ultraviolet spectrum is that of an isoindole (Table I). The infrared spectrum shows N–H (3280 cm.$^{-1}$) and carboxanhydride (1861, 1831, 1770 cm.$^{-1}$) (in KBr). It therefore seems to be a condensation product (in the 3 position?), rather than a Diels–Alder adduct.

We are further investigating the equilibria and chemical reactions of isoindoles.

Acknowledgment.—We are greatly indebted to Drs. Agahigian, Rittner and Siggia of the Olin–Mathieson Chemical Corp., at New Haven, for their invaluable help with the n.m.r. spectra and elemental analyses.

(10) Since the two CH_2 protons in the isoindolenine correspond to one aromatic and one NH proton in the isoindole, adding half of the CH_2 signal to the aromatic and half to the NH signals corrects to 100% isoindole.

(11) NSF Summer Research Fellow, 1962; NIH Predoctoral Fellow, Sept. 1962–present.

Sterling Chemistry Laboratory
Yale University   Daniel F. Veber[11]
New Haven, Connecticut   Walter Lwowski

A SYNTHESIS OF HOLOMYCIN

Sir:

Various Streptomyces species elaborate yellow, sulfur containing metabolites which exhibit high activity against fungi, Gram-positive and Gram-negative bacteria. Four representatives of this group of antibiotics are presently known in pure form. Degradative studies on thiolutin,[1] aureothricin,[1] holomycin[2] and isobutyropyrrothine[3] revealed structures which differ only in the nature of the N-acyl side chain and the substituent attached to the lactam nitrogen atom of the pyrrothine[1] nucleus. The nine steps outlined below led to synthetic holomycin.[4]

S-Benzylcysteine ethyl ester[5] was acylated with di-

(1) W. D. Celmer and I. A. Solomons, J. Am. Chem. Soc., 77, 2861 (1955), and earlier papers cited.

(2) L. Ettlinger, E. Gäumann, R. Hütter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog and H. Zähner, Helv. Chim. Acta, 42, 563 (1959).

(3) D. S. Bhate, R. K. Hulyalkar and S. K. Menon, Experientia, 16, 504 (1960).

(4) A synthesis of holomycin following a different sequence has been announced in a brief note by U. Schmidt and F. Geiger, Ang. Chem., 74, 328 (1962).

(5) C. R. Harington and R. V. Pitt Rivers, Biochem. J., 38, 417 (1944).

ketene in ethanol solution.[6] Dieckmann condensation[6] of the crude acetoacetamide with sodium ethoxide in ethanol–benzene (80°, 3 hr.) yielded α-acetyl-γ-benzylthiomethyltetramic acid (I) (42% for both steps), m.p. 114°; γ_{max}^{KBr} 3400–2500, 1710, 1665, 1610 cm.$^{-1}$; λ_{max}^{EtOH} 244, 277 mμ (ϵ 5100, 12700). Dehydrogenation with thionyl chloride in benzene solution (25°, 24 hr.) provided the yellow benzylthiomethylene derivative (II or its stereoisomer) (84%), m.p. 170°, ν_{max}^{KBr} 3500–2500, 1710, 1690, 1660, 1600 cm.$^{-1}$; λ_{max}^{EtOH} 288, 350 mμ (ϵ 18500, 11600); n.m.r. (d_7-DMF) 0.85 (1H), 2.9 (5H), 3.7 (1H); 6.05 (2H), 7.85 (3H) τ. Treatment with hydroxylamine in aqueous tetrahydrofuran (25°, 24 hr.) led to a single oxime (III) (71%), m.p. 160°, ν_{max}^{KBr} 3400–2600, 1680–1620, 1600 cm.$^{-1}$; λ_{max}^{EtOH} 297, 334 mμ (ϵ 15600, 18700) which on heating with p-toluenesulfonyl chloride and sodium hydroxide in aqueous tetrahydrofuran (65°, 30 min.) was transformed to α-acetylamino-γ-benzylthiomethylenetetramic acid (IV) (28%), m.p. 200°, $\nu_{max}^{CHCl_3}$ 3440, 3390, 3250, 1690, 1650, 1600, 1525 cm.$^{-1}$; λ_{max}^{EtOH} 235, 330 mμ (ϵ 10000, 23700); n.m.r. ($CDCl_3$) -1.33 (1H), 2.7 (5H), 3.88 (1H), 6.0 (2H), 7.85 (3H) τ.
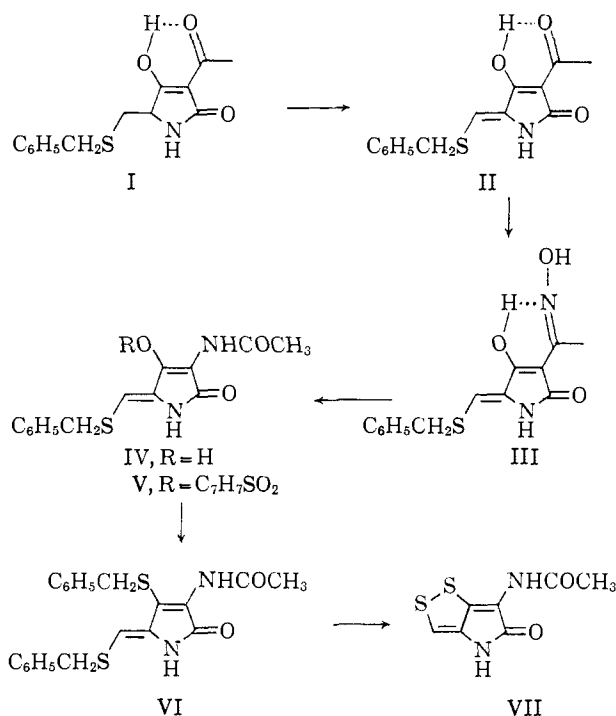
Direct conversion[7] of tetramic acid (IV) to its benzylthio derivative (VI) was not possible but this intermediate became available by a two-stage sequence[7,8] when it was found that IV was converted readily to its O-toluenesulfonyl derivative (V) (80%) m.p. 200–204° (dec.) with tosyl chloride in a mixture of tetrahydrofuran and triethylamine (25°, 16 hr.). Displacement of the toluenesulfonate group with sodium benzylmercaptide in ethanol–tetrahydrofuran (70°, 8 hr.) produced the desired sulfide (VI) (40%); m.p. 180°; ν_{max}^{KBr} 3400,3250,1690,1660,1625,1600,1525 cm.$^{-1}$; λ_{max}^{EtOH} 360 mμ (ϵ 23400); n.m.r. ($CDCl_3$) 2.6 (10 H), 3.8 (1H), 6.05 (2H), 6.15 (2H), 7.9 (3H) τ. Debenzylation with lithium in liquid ammonia[9] and air oxidation of the crude dithiol in methanol at pH 2 (25°) completed the synthesis. The product obtained (15%) had m.p.

(6) Method of R. N. Lacey, J. Chem. Soc., 850 (1954).

(7) Method of R. E. Ireland and J. A. Marshall, J. Am. Chem. Soc., 81, 6336 (1959).

(8) R. B. Woodward, M. P. Cava, W. D. Ollis, W. D. Hunger, H. U. Daeniker and K. Schenker, ibid., 76, 4749 (1954).

(9) K.-D. Gundermann and G. Pape, Chem. Ber., 95, 2076 (1962).