nally present was further demonstrated by a Van Slyke amino nitrogen assay.

Acid hydrolysis yielded two aglycons which were separated by chromatography; I, m.p. 111–115°, $C_{21}H_{33}N$, ultraviolet absorption at 229–230 mμ (ε 15,260); 234 mμ (ε 16,700), 243 mμ shld. (ε 12,190); II, m.p. 168–173°, $C_{21}H_{35}NO$. Compound II has now been identified as 20α-amino-3β-hydroxy-5-pregnene[2] and, therefore, I corresponds to 20α-amino-3,5-pregnadiene. The sugar fraction of the hydrolysis was shown to be D-glucose by formation of its osazone, by paper chromatography and by its oxidation to potassium gluconate and subsequent formation of the characteristic aldobenzimidazole.[3] From the above evidence and from other considerations it seemed quite probable that the new steroid alkaloid was 20α-amino-3β-hydroxy-5-pregnene β-D-glucoside. This has been confirmed by synthesis.

20α-Amino-3β-hydroxy-5-pregnene was converted to 3β-hydroxy-20α-trifluoroacetamido-5-pregnene, m.p. 199–201°. Reaction with acetobromoglucose gave the 3β-D-glucoside acetate, m.p. 200–205°. Alkaline hydrolysis removed the blocking groups and the resulting base, m.p. 285–287°, yielded a hydrochloride, m.p. 257–259°, which was identical in every respect with isolated hypotensively active material. Several derivatives and compounds related to this substance have been investigated and these together with more complete details of the above experiments will be reported later.

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DISSOCIATION OF γ-GLOBULIN

Sir:

Reaction of human γ-globulin with sulfhydryl compounds, sulfite, or performic acid resulted in marked diminution in the sedimentation coefficient and molecular weight. Efficient reduction required the presence of denaturing agents. Because the products were insoluble after removal of the denaturing agent, molecular weights were determined in urea solutions[1] by the Archibald principle[2]

$$q_a \equiv \frac{RT(\partial c/\partial r)_{r_a}}{\omega^2 r_a} = M_{app}(1 - \bar{v}\rho)[(c_a - c_0) + c_0]$$

R is the gas constant, T the absolute temperature, $(\partial c/\partial r)_{r_a}$ the concentration gradient at the meniscus r_a, ω the constant angular velocity, M_{app} the apparent molecular weight of solute calculated as if its activity coefficient were unity, $\bar{v}$ the partial specific volume, ρ the density of the solvent, c_a the concentration at r_a, and c_0 the initial concentration. Plotting q_a against $(c_a - c_0)$ yields $M_{app}(1 - \bar{v}\rho)$ as the slope of a least squares line. This allows calculation of a molecular weight average which initially emphasizes the heavier components of a polydisperse solution.[3]

Fifteen milligrams of human γ-globulin was reduced with 5 ml. of 0.1 M β-mercaptoethylamine·HCl in 6 M urea at room temperature for four hours. Some samples then were dialyzed against a large volume of 6 M urea that was 0.02 M in iodoacetamide. The products appeared as a polydisperse peak in the ultracentrifuge. Corrected for the density and viscosity of urea, the s^0_{20w} of reduced iodoacetamide-treated γ-globulin was 2.3S. In Table I are the measured values of $M_{app}(1 - \bar{v}\rho)$ and M_{app} values calculated assuming $\bar{v} = 0.74$.[4] Simultaneous determinations of $M_{app}(1 - \bar{v}\rho)$ with solutions in D_2O and H_2O that were 6 M in urea and 0.1 M in β-mercaptoethylamine·HCl, allowed estimation of $\bar{v} = 0.71$. Using this value, and $\rho = 1.097$ for the H_2O solution yields $M_{app} = 42{,}000$.

TABLE I

EFFECT OF VARIOUS REAGENTS ON $M_{app}(1 - \bar{v}\rho)$ VALUES OF HUMAN γ-GLOBULIN

(Fraction II of Cohn, Lederle lot C 543[a])

Solvent	$M_{app}(1 - \bar{v}\rho)$ ± standard deviation	M_{app}
0.2 M KCl	$(4.8 \pm 0.1) \times 10^4$	192,000
6 M urea + 0.2 M KCl	$(3.0 \pm 0.3) \times 10^4$	158,000
0.1 M MEA[b] + 0.2 M KCl	$(3.5 \pm 0.1) \times 10^4$	140,000
0.1 M MEA + 6 M urea + 0.2 M KCl	$(0.93 \pm 0.07) \times 10^4$	48,000
Reduced in 8 M urea + 0.1 M MEA, next dialyzed against 6 M urea + 0.02 M iodoacetamide then 6 M urea + 0.2 M KCl	$(0.92 \pm 0.05) \times 10^4$	48,000

[a] This sample contained a small amount of heavy material sedimenting faster than the main 7S component. [b] MEA = β-mercaptoethylamine·HCl.

A pathological macroglobulin reduced in 6 M urea had a value of 41,000 for M_{app}. Macroglobulins have been found[5] to dissociate in mercaptoethanol solutions without urea to sub-units of about the same molecular weight as normal γ-globulin.

Reaction of human γ-globulin with sulfite[6] in urea[7] yielded a water soluble aggregated S-sulfoprotein. In tris-(hydroxymethyl)-aminomethane buffer, pH 8, made 6 M in urea, the smallest non-dialyzable component had a value of 0.81×10^4 for $M_{app}(1 - \bar{v}\rho)$. Performic acid oxidation[8] of human γ-globulin gave a product with $M_{app} = 32{,}000$ that was slightly soluble in citrate buffer, pH 10.9, in the absence of urea.

These findings suggest that human γ-globulin contains subunits linked at least in part by disulfide

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bonds. The possibility that linkages other than disulfide bonds are involved has not been excluded.

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RECEIVED APRIL 24, 1959

THE HOMOPOLYMERIZATION OF MONOISOCYANATES

Sir:

Beyond the formation of cyclic dimers[1] and trimers[2] from monoisocyanates (Reactions I and II in Fig. 1) and the suggestion that cyamelide[3] is a linear polyisocyanic acid, there has been no evidence to indicate the possibility of obtaining addition polymers from monofunctional compounds containing >C=N– groups. I have recently found that monoisocyanates can be polymerized to linear high molecular weight polymers in a manner similar to vinyl compounds in accordance with the Reaction III in Fig. 1. These polymers may be regarded as N-substituted "1-nylons."

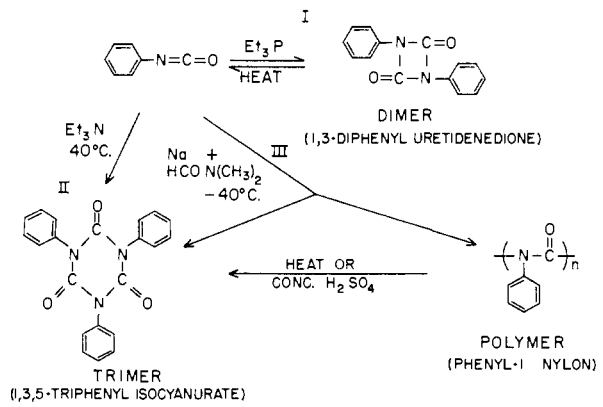

Fig. 1.—Reactions of phenyl isocyanate.

The polymerization takes place at low temperatures (−20° to −100°) in polar solvents, such as N,N-dimethylformamide, N,N-dimethylacetamide and triethylamine, through the use of anionic initiators. Ethyl isocyanate and phenyl isocyanate, examples of an aliphatic and aromatic isocyanate, were polymerized in the manner to be described.

Ethyl isocyanate, 25 ml., and dry triethylamine, 25 ml., both previously cooled to −40°, were stirred together rapidly under dry nitrogen, and further cooled to −100° with liquid nitrogen. Next 10 ml. of a sodium catalyst solution in N,N-dimethylformamide[4] was added to the rapidly stirring solution during about 30 seconds. A white, fibrous solid precipitated immediately upon contact of the catalyst with the monomer solution. The solid was filtered off, washed with methanol and dried to give 8.5 g. (39%) of ethyl 1-Nylon polymer (m.p. 250° with decomposition; found: C, 50.76; H, 6.88; N, 19.34). From the filtrate the cyclic trimer, 1,3,5-triethyl isocyanurate, was isolated and identified by its solubility and melting (m.p. 94°) characteristics.

Ethyl 1-Nylon was soluble in trifluoroacetic acid (TFA) and concentrated sulfuric acid. Clear films were obtained when TFA solutions were air dried and extracted with methanol at room temperature. Prolonged standing of the polymer in TFA resulted in degradation: a change in the inherent viscosity at 0.5% concentration from 0.3 to 0.04 occurred in two days. Light scattering studies on the aged solution gave a molecular weight value of 1600–3000 (*i.e.*, a D.P. of 23 to 42).[5] The polymer was further characterized by its distinct infrared spectrum where the only significant bands were those corresponding to carbonyl at 5.85μ, N,N-disubstituted amide at 7.4μ and a conspicuous absence of isocyanate and NH bands.[5]

With phenyl isocyanate, a polymer (m.p. 197° with decomposition) was obtained in 86% yield in N,N-dimethylformamide using the sodium catalyst[4a] at −40°. The polymer was soluble in concentrated sulfuric acid, but insoluble in TFA and chlorinated hydrocarbons. Its infrared spectrum was consistent with a phenyl 1-Nylon structure and quite distinct from either the dimer or trimer of phenyl isocyanate.

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(4) (a) The catalyst solution was prepared by adding 10 drops of a 50% sodium dispersion in xylene (du Pont Electrochemicals Department) to 50 ml. of dry N,N-dimethylformamide. The mixture was stirred for 1 hour before use. (b) The use of this combination as a catalyst for the anionic polymerization for vinyl compounds was demonstrated by L. Grandine, E. I. du Pont de Nemours & Company (private communication).

(5) The author wishes to thank Drs. D. Akeley and R. Zbinden for the light scattering data and infrared analysis, respectively.

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STEROIDS. CXXII.[1] 6α-FLUORO-16α-METHYLCORTICOIDS—SYNTHESIS AND BIOLOGICAL ACTIVITY

Sir:

Among the most recent chemical modifications of the hydrocortisone and prednisolone molecule—resulting in increased anti-inflammatory activity and diminution of salt retention—have been introduction of a 6α-fluorine atom[1,2,3] *or* addition of a methyl group in the 16α-position.[4,5] We now wish to report the synthesis and preliminary biological evaluation of analogs of the most important cortical hormones combining *both* of these structural features.

The preparation in this Laboratory of 6α-fluoro-16α-methylhydrocortisone acetate (I) *via* 6α-fluoro-16α-methyl "Substance S" already has been recorded.[6] Dehydration of I with mesyl chloride in

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