We now wish to report that by the use of an as yet unidentified fungus of the *Rhizopus* family (our strain SY 152) isolated[5] from a Mexican soil sample (Molino de Bezares, D.F., Mexico) it has been possible to achieve the oxidation of progesterone to its 11α-hydroxy analog I in 45% yield. In striking contrast to the catalytic hydrogenation of 11-keto[6] and 11β-hydroxy[7] steroids which yields predominantly the 5α(*allo*) dihydro derivative, it was observed that catalytic hydrogenation of I with palladized charcoal catalyst in ethanol solution preferably in the presence of potassium hydroxide for 30 minutes yields only small amounts of the *allo* isomer,[3] the main product being the *normal* derivative, pregnane-3,20-dione-11α-ol (II) [m.p. 116–118°, $[\alpha]^{20}D$ +91° (all rotations in chloroform), $\lambda_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and free –OH; found: C, 75.97; H, 9.92; *acetate*, m.p. 148–149°. $[\alpha]^{20}D$ +65°, $\lambda_{max}^{CHCl_3}$ 1736, 1720 and 1700 cm.$^{-1}$]. This reversal of the stereochemical course of the catalytic hydrogenation of 11-oxygenated Δ^4-3-ketosteroids in the case of the 11α-epimer thus permits the conversion of ring C unsubstituted precursors (progesterone, diosgenin, stigmasterol) to cortisone by way of the desirable 5β(normal) series.

Chromium trioxide oxidation of II furnished pregnane-3,11,20-trione (m.p. 158–160°, [$]^{20}D$ +128°, $\lambda_{max}^{CHCl_3}$ 1702 cm.$^{-1}$, identified by comparison with an authentic specimen,[8] m.p. 160–162°, $[\alpha]^{20}D$ +126°) and reduction of the latter with sodium borohydride in *pyridine solution*[9] smoothly yielded the known[8] pregnane-11,20-dione-3α-ol (m.p. 169–171°, $[\alpha]^{20}D$ + 105°, $\lambda_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and free –OH, identified by comparison with an authentic sample, m.p. 168–171°, $[\alpha]^{20}D$ +103°) and upon acetylation pregnane-11,20-dione-3α-ol acetate m.p. 134–135°, $[\alpha]^{20}D$ +135°). Experimental details of the further transformations of this substance to cortisone have already been recorded.[10] The consistently high yields, the ready availability of the starting materials and the paucity of steps (ten from progesterone or fourteen from diosgenin) appear to make this combined microbiological-chemical route the best yet described synthesis of cortisone.

RESEARCH LABORATORIES
SYNTEX, S. A.
LAGUNA MAYRAN 413
MEXICO CITY 17, D.F.

O. MANCERA
ALEJANDRO ZAFFARONI
B. A. RUBIN
FRANZ SONDHEIMER
G. ROSENKRANZ
CARL DJERASSI[11]

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(5) This work was aided by the use of a screening technique based on a color reaction specific for 11α-hydroxyprogesterone (A. Zaffaroni, *et al.*, to be published).

(6) C. Djerassi, G. Rosenkranz, J. Pataki, and St. Kaufmann, *J. Biol. Chem.*, **194**, 115 (1952).

(7) J. Pataki, G. Rosenkranz and C. Djerassi, *ibid.*, **195**, 751 (1952), and references cited therein.

(8) J. von Euw, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **27**, 821 (1944).

(9) In ethanol solution, the product was chiefly the known pregnane-$3\alpha,20\beta$-diol-11-one (*cf.* L. H. Sarett, THIS JOURNAL, **70**, 1690 (1948).

(10) T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, *ibid.*, **74**, 483 (1952).

(11) Department of Chemistry, Wayne University, Detroit, Michigan.

ON A PHOSPHO-TRI-ANHYDRIDE FORMULA FOR THE NUCLEIC ACIDS

Sir:

Last year Dr. Edward Ronwin[1] suggested a phospho-tri-anhydride formula for the nucleic acids, with as its core a polymer chain of phosphorus atoms held together by oxygen atoms, each phosphorus atom having five oxygen atoms attached to it, of which three bind it to adjacent phosphorus atoms, one is in a hydroxyl group, and one is in a sugar ester group. We then stated[2] that in formulating a hypothetical structure for a substance one must take care that the structural elements of which use is made are reasonable ones or one must show that there is an overwhelming necessity for a radical proposal, that there is no precedent for a structure in which phosphorus is bonded to five oxygen atoms, that in every one of the scores of quinquepositive phosphorus compounds that have been subjected to complete structural investigation the phosphorus atom is surrounded by four oxygen atoms, and that the ligation of five oxygen atoms about each phosphorus atom is such an unlikely structural feature that the proposed phospho-tri-anhydride formula for the nucleic acids deserves no serious consideration.

Dr. Ronwin has now kindly informed us that he has become aware of earlier references in the literature to compounds to which structures have been attributed involving quinquepositive phosphorus bonded to five oxygen atoms or to a total of five oxygen atoms and similar atoms. Anschütz[3] prepared four compounds to which he assigned structures involving ligation of five oxygen atoms to a phosphorus atom. The synthesis of several compounds described as having one oxygen atom and four NHR groups bonded to a phosphorus atom has been reported by Lemoult,[4] and Autenrieth and Meyer[5] have reported similar compounds with two oxygen atoms, two NHR groups, and one SH group presumed to be bonded to a phosphorus atom.

Although there may be some question about the correctness of the structures attributed to some of these compounds, and although no complete structure determination has been made for any of them, the compounds reported by Anschütz may indeed have the structures suggested by him, involving five oxygen atoms ligated to a quinquepositive phosphorus atom. His suggested formulas for the four substances are $P(OC_6H_5)_5$, $PO_2C_6H_4(OC_6H_5)_3$, $P(OC_6H_5)(O_2C_6H_4)_2$, and $P_2(O_2C_6H_4)_5$, in which $O_2C_6H_4$ is the *o*-phenylene group. Our statement that there is no precedent for a structure in which a phosphorus atom is bonded to five oxygen atoms must accordingly be withdrawn.

It is pertinent to the proposed phospho-tri-anhydride formula for the nucleic acids that the four compounds reported by Anschütz are described by him as being extremely sensitive to moisture, so sensitive as to make it impossible to

(1) E. Ronwin, THIS JOURNAL, **73**, 5141 (1951).

(2) L. Pauling and V. Schomaker, *ibid.*, **74**, 1111 (1952).

(3) L. Anschütz, *Ann.*, **454**, 71 (1927).

(4) P. Lemoult, *Compt. rend.*, **141**, 1241 (1905).

(5) W. Autenrieth and W. Meyer, *Ber.*, **58**, 840 (1925).

determine, except roughly, their melting points and other physical properties.

GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA 4, CALIFORNIA
LINUS PAULING
VERNER SCHOMAKER

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PARTIAL PURIFICATION AND AMINO ACID CONTENT OF VASOPRESSIN FROM HOG POSTERIOR PITUITARY GLANDS

Sir:

A highly purified vasopressin preparation (400–500 pressor units per mg.) from beef posterior pituitary glands has recently been prepared[1] by countercurrent distribution of concentrates between *n*-butyl alcohol and 0.09 *M* *p*-toluenesulfonic acid. Analysis of hydrolysates by chromatography on starch columns[2] showed phenylalanine, tyrosine, proline, glutamic acid, aspartic acid, glycine, arginine and cystine in approximately equimolar amounts, plus three moles of ammonia per mole of any one amino acid. The preparation and the amino acid analysis have been verified on several batches of posterior pituitary material of bovine origin.

We wish to report here an unexpected result encountered when pressor concentrates from hog posterior pituitary lobes were used as starting material for vasopressin preparation. A mixture of pressor fractions obtained by a solvent fractionation procedure (fractions "e" and "f" of Kamm, *et al.*[3]) were subjected to a twenty-transfer countercurrent distribution at room temperature in an all-glass machine[4] in the system *s*-butyl alcohol and 0.1% acetic acid. The material from tubes 1–4 inclusive was then submitted to a fifty-transfer countercurrent distribution at 5–10° in the system *n*-butyl alcohol and 0.09 *M* *p*-toluenesulfonic acid. The peak of pressor activity seemed to be in the vicinity of tube 20, which indicated a distribution constant of 0.66. The vasopressin of bovine origin had a distribution constant of 1.25 in this solvent system.[1]

Material from tubes 10–24 inclusive was subjected to a 150-transfer distribution in the same solvent system. Analysis of the distribution pattern by quantitative ninhydrin reaction[5] on aliquots of the lower phase showed a peak at tube 59 (distribution constant 0.65) which corresponded to the peak of pressor activity. The combined material from tubes 54–65 inclusive had a potency of approximately 175 pressor units per mg. This potency probably does not represent the highest obtainable for this principle. We have reason to believe that some inactivation has occurred in the process of working up the material.

Analysis of a hydrolysate of this material by starch column chromatography showed a pattern similar to that of vasopressin of bovine origin except that arginine was absent and a peak occupying the position of lysine was present.[6] If calculated as lysine this peak represented approximately one mole per mole of each of the other amino acids. One-half mg. of this preparation gave a negative Sakaguchi test both before and after acid hydrolysis. The same amount of a purified beef vasopressin preparation or an equimolar amount of arginine gave a strong positive test. Two-dimensional paper chromatograms of a hydrolysate with arginine or lysine added showed clearly that the basic amino acid present was not arginine, and was inseparable from lysine under these conditions. Microbiological assay of a hydrolysate for L-lysine[7] gave a value in reasonable agreement with the value from the starch column analysis.

Efforts are being continued toward further purification of lysine-vasopressin. Additional efforts are being made to ascertain whether lysine-vasopressin can be found in beef glands and arginine-vasopressin in hog glands, or whether this interesting and unexpected result represents a qualitative species difference.

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DEPARTMENT OF BIOCHEMISTRY
CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK, N. Y.
EDWIN A. POPENOE[8]
H. CLAIRE LAWLER
VINCENT DU VIGNEAUD[9]

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(1) R. A. Turner, J. G. Pierce and V. du Vigneaud, *J. Biol. Chem.*, **191**, 21 (1951).

(2) S. Moore and W. H. Stein, *ibid.*, **178**, 53 (1949).

(3) O. Kamm, T. B. Aldrich, I. W. Grote, L. W. Rowe and E. P. Bugbee, THIS JOURNAL, **50**, 573 (1928).

(4) L. C. Craig, *Anal. Chem.*, **22**, 1346 (1950).

(5) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

(6) It is of interest that *oxytocin* preparations from beef and hog sources had shown no difference in amino acid composition (Pierce, Gordon and du Vigneaud, manuscript in preparation).

(7) L. M. Henderson and E. E. Snell, *J. Biol. Chem.*, **172**, 15 (1948).

(8) Public Health Service Postdoctorate Research Fellow of the National Institutes of Health.

(9) Appreciation is expressed to Lederle Laboratories, American Cyanamid Company for a grant-in-aid and to Parke, Davis and Company and Armour and Company for gifts of material.

STREPTOLIN. THE STRUCTURE AND SYNTHESIS OF ISOLYSINE

Sir:

Hydrochloric–formic acid hydrolysis of the antibiotic streptolin[1] followed by chromatographic separation on Dowex-50 has given five major fractions; the last to be eluted possesses the empirical formula $C_6H_{14}O_2N_2$ for the free base and is designated as "*iso*-lysine." This substance, which is also a hydrolysis product of viomycin[2] and streptothricin,[3] we have characterized as the di-(*p*-hydroxyazobenzene-*p'*-sulfonate)[4] (I), dec. 243.5–244°, $[\alpha]^{25}D$ +6.5 ± 1 (alc.) (found: C, 50.91; H, 5.08; N, 12.00) and the dipicrate,[4] m.p. 200–201° (found: C, 35.38; H, 3.63; N, 18.06).

Isolysine gave a positive hydroxamic acid test[5]

(1) R. W. Rivett and W. H. Peterson, THIS JOURNAL, **69**, 3006 (1947).

(2) T. H. Haskell, S. A. Fusari, R. P. Frohardt and Q. R. Bartz, *ibid.*, **74**, 599 (1952).

(3) H. E. Carter, W. R. Hearn and W. R. Taylor, "Abstracts of Papers" 119th Meeting, American Chemical Society, Cleveland, Ohio, April 1951, p. 25A.

(4) These derivatives have been previously reported by Haskell *et al.* (ref. 2) and Carter, *et al.* (ref. 3).

(5) F. Feigl, "Qualitative Analysis by Spot Tests," Elsevier Publishing Co., Inc., New York, N. Y., 1946, p. 369.