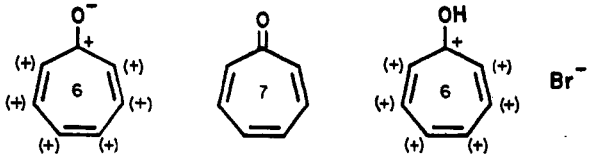
has prominent maxima at 3425, 2970, 1638, 1582, 1524, 1475, 1225, 1217, 1009, 936, 898, 834 and 785 cm.$^{-1}$. The ultraviolet spectrum in isoöctane shows maxima at 225 mu (log E 4.33), 297 (3.74) and 310 (3.67). Both III and IV react with three



IIIa IIIb IV

molecules of hydrogen with Adams platinum catalyst in ethanol to give *cycloheptanone*, identified as the semicarbazone, m.p. and m.p. mixed with authentic material 162.5–163°. The possible presence of an O–H bond inferred from the 3425 cm.$^{-1}$ band is excluded by the failure of III to exchange more than negligibly with deuterium oxide (analysis by Mr. Arthur K. Hoffmann, Columbia University). The arrangement of atoms in III is unquestionable (IIIa or IIIb).

As an extreme electronic structure, IIIa and not IIIb satisfactorily expresses (a) the basicity which reflects a high electron density on oxygen and a stabilization of positive charge (IV), (b) the large dipole moment implied by the high boiling point (*cf.* benzaldehyde, b.p. 68° at 15 mm.), miscibility with water and the large exaltation, and (c) the shift of the carbonyl frequency. The stability of IIIa relative to IIIb and the very existence of III in contrast to the non-existence of cyclopentadienones (having fewer than two phenyl substituents),[2] find insufficient theoretical explanation in terms of resonance structures (IIIa) alone, but are explained by the molecular orbital theory which predicts peculiar stability from *six* electrons in a cyclic resonating system.

This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

(2) C. F. H. Allen and J. A. VanAllan, THIS JOURNAL, **72**, 5165 (1950).

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RECEIVED JANUARY 16, 1951

COUNTERCURRENT DISTRIBUTION OF INSULIN

Sir:

Our success in applying the technique of countercurrent distribution to the study of the purity of polypeptides in the molecular weight range of a few thousand has encouraged us to attempt a similar study with proteins. Insulin as a type substance has given promise from the first attempts and has now been studied in several systems.

Active material has been found to give an interchange between phases sufficiently rapid to permit a true partition ratio to hold. This in itself would appear to be a finding of considerable interest. Although runs involving approximately 100 transfers did not clearly reveal more than a single major component, higher numbers of transfers were more revealing. The major band did not continue to behave as a single component.

For example, an experiment made with 500 mg. of a sample of beef insulin (activity 27 μ/mg. Sample No. T 2344), supplied by the Eli Lilly Company, gave the result shown in Fig. 1 at 424 transfers (System 2-butanol/1% aqueous dichloroacetic acid; temp. 24°; pH of system 2.7; single withdrawal procedure[1] used). The distribution apparatus contained 220 equilibration cells in the train and was operated automatically.[2]

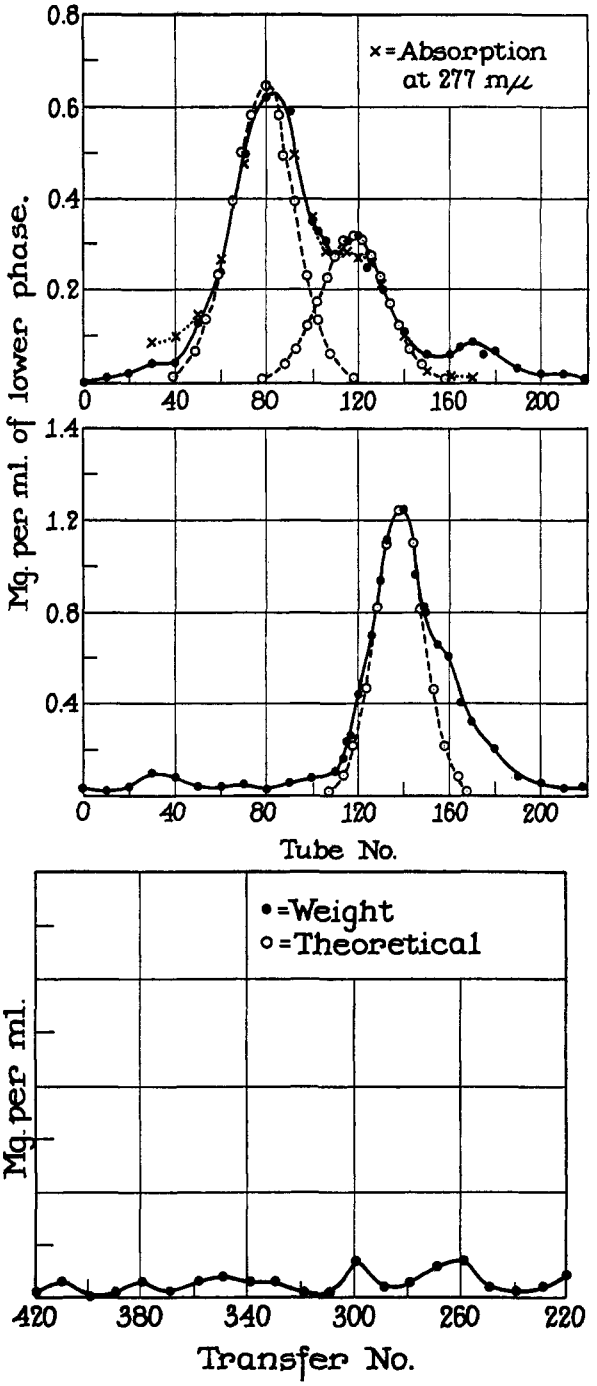

Fig. 1.—Distribution patterns of insulin: ●, weight; ○, theoretical: lower 424 transfers; upper 909 transfers.

(1) L. C. Craig and O. Post, *Anal. Chem.*, **21**, 500 (1949).
(2) L. C. Craig, *ibid.*, **22**, 1345 (1950).

Aside from small percentages of impurities or transformation products appearing in most of the fractions, the main solute band showed an interesting shoulder. In order to learn whether or not the shoulder really indicated a major second component, the apparatus was adjusted for the "recycling procedure"[2] and permitted to operate until 909 transfers had been accomplished. The upper pattern shown in Fig. 1 was thus obtained.

Although such a result strongly indicates two major components with slightly different partition ratios, further study is required, particularly since Fredericq and Neurath[3] have studied this same sample and, among other criteria, found it to give a solubility curve indicative of a single component.

It proved relatively easy to crystallize material from each peak of Fig. 1. Though identical in crystalline form, the largest component, A, showed a lower partition ratio in the system than did the faster moving B component. The partition ratios calculated from the pattern are 0.49 and 0.59. The activity[4] of the recovered material, if at all different, appeared to be slightly lower, *e. g.*, 22 and 26 μ/mg., respectively, for A and B, rather than higher than that of the starting material. No difference was found in the C, H and N analyses of A and B.

An attempt to redistribute material from each peak and to determine the quantitative amino acid composition will be made at the earliest opportunity. It is possible these results may have a bearing on the inconsistency of the proposed minimum molecular weight[3] of 6000 for the dissociated form and the published quantitative amino acid analyses[4] for insulin.

We are indebted to Dr. E. D. Campbell of the Eli Lilly Company for the insulin and for the bioassays.

(3) E. Fredericq and H. Neurath, THIS JOURNAL, **72**, 2684 (1950).

(4) F. Sanger, *Ann. Repts. on Progress Chem. (Chem. Soc. London)*, **XLV**, 287 (1948).

THE ROCKEFELLER INSTITUTE
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RECEIVED JANUARY 17, 1951

THE PARTIAL SYNTHESIS OF ESTRONE-16[1] AND OF ISOANDROSTERONE-16 (HEARD'S OXYKETONE)

Sir:

In our studies[2] of the various reductive methods as applied to 16-keto-17-hydroxysteroids we have found that the Clemmensen reduction of such a steroid unexpectedly gives rise to the 16-keto-17-desoxy compound. Thus, from 16-keto-estradiol[3] is obtained 3-hydroxy-16-keto-$\Delta^{1,3,5}$-estratriene (estrone-16) melting[4] at 243.5–245.5° dec. and having an optical rotation of $[\alpha]^{25}D$ −87° (in 95% ethanol). *Anal.*[5] Calcd. for $C_{18}H_{22}O_2$: C, 79.96, H, 8.20. Found: C, 80.04, 79.93; H, 8.22, 8.15. That this compound possesses the unaltered naturally-occurring $\Delta^{1,3,5}$-estratriene nucleus was established by hydrogenolysis of the 3-benzoxy-16-diethyl thioketal[2] to desoxoestrone benzoate (followed by saponification to desoxoestrone) as shown by mixed melting point comparison using authentic desoxoestrone benzoate[6] (and using authentic desoxoestrone[6]).

Estrone-16 was further characterized by preparation of the analytically pure semicarbazone (m.p. 246.5–248° dec.), acetate (m.p. 132–133°), benzoate (m.p. 223.5–224.5°, slight dec.), palmitate (m.p. 110.5–111.5°), methyl ether (m.p. 124–124.5°), and benzyl ether (m.p. 156–156.5°).

In 1939 Heard and McKay[7] isolated from mares' pregnancy urine a 3β-hydroxy-keto-androstane in which the position of the ketonic oxygen was not determined. Oppenauer[8] later confirmed this isolation. Much speculation has ensued concerning the exact location of the carbonyl in this androstane derivative.

The Clemmensen reduction of 3β,17-dihydroxy-16-keto-androstane (m.p. 217–218° dec.), prepared by the sequence of nitrosation[9] and Stodola reduction[9] of isoandrosterone, furnished 3β-hydroxy-16-keto-androstane (isoandrosterone-16) melting at 186–186.5° and possessing an optical rotation of $[\alpha]^{25}D$ −180° (in dioxane). *Anal.* Calcd. for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 78.55, 78.62; H, 10.36, 10.37. Isoandrosterone-16 gave a benzoate melting at 208.5–209° and an oxime melting at 199°.

Heard characterized his oxyketone ($C_{19}H_{30}O_2$) in part as follows: melting point, 187–187.5°; optical rotation, $[\alpha]^{24}D$ −160° (in dioxane); benzoate, m.p. 206–208°; oxime, m.p. 194–195°. Although a direct comparison between our isoandrosterone-16 and Heard's oxyketone has not yet been possible, it is highly probable that they are identical.

[Since this manuscript was submitted for publication a direct comparison between synthetic isoandrosterone-16 and the urinary androstanolone of Heard and McKay (supplied by Professor R. D. H. Heard) has been possible. A mixed melting point test showed no depression.]

We wish to thank G. D. Searle and Company and the Graduate Research Institute of Baylor University at Dallas for financial support of this research.

(1) The research concerning estrone-16 was completed in the Department of Biochemistry, Southwestern Medical School, Dallas, Texas.

(2) M. N. Huffman and M. H. Lott, THIS JOURNAL, **71**, 719 (1949).

(3) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **172**, 325 (1948).

(4) All melting points are uncorrected.

(5) Analyses were performed and optical rotations determined by Dr. E. W. D. Huffman, Denver.

(6) Kindly supplied by Dr. O. Wintersteiner of the Squibb Institute for Medical Research.

(7) R. D. H. Heard and A. F. McKay, *J. Biol. Chem.*, **131**, 371 (1939).

(8) R. Oppenauer, *Z. physiol. Chem.*, **270**, 97 (1941).

(9) F. H. Stodola, E. C. Kendall and B. F. McKenzie, *J. Org. Chem.*, **6**, 841 (1941).

THE OKLAHOMA MEDICAL RESEARCH INSTITUTE
MAX N. HUFFMAN
OKLAHOMA CITY, OKLAHOMA MARY HARRIET LOTT

RECEIVED DECEMBER 14, 1950

TOMATIDINE, A STEROID SECONDARY AMINE[1]

Sir:

Crystalline tomatine, a new glycosidal alkaloid having antifungal activity, was first isolated in our laboratory from the tomato plant and found to consist of an aglycone portion, tomatidine,[2] and a tet-

(1) Report of a study in which certain phases were carried on under the Research and Marketing Act of 1946.

(2) T. D. Fontaine, G. W. Irving, Jr., R. M. Ma, J. B. Poole, and S. P. Doolittle, *Arch. Biochem.*, **18**, 467 (1948).