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
FOR MEDICAL RESEARCH ELIZABETH J. HARFENIST
NEW YORK 21, N. Y. LYMAN C. CRAIG

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).

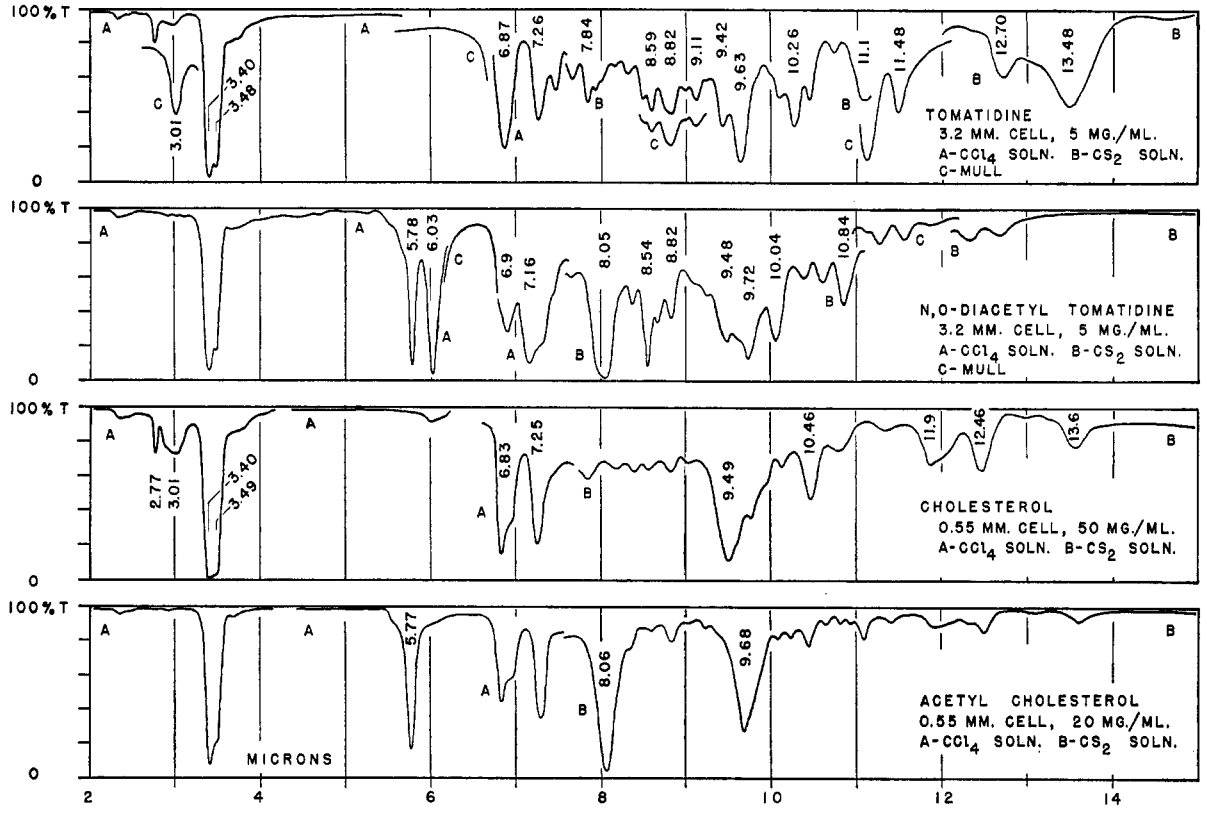

Fig. 1.—Infrared spectra of tomatidine, N,O-diacetyltomatidine, cholesterol, and acetylcholesterol.

rasaccharide moiety, composed of xylose, galactose, and two glucose units.[3] We now wish to report some of the chemical and physical data which have suggested a steroid structure for tomatidine. These products and some derivatives are here assigned the following empirical formulas:

Tomatine.—*Anal.* Calcd. for $C_{50}H_{83}O_{21}N$: C, 58.07; H, 8.09; N, 1.35. Found: C, 57.62; H, 8.15; N, 1.37.

Tomatidine, m.p. 210–211°[4]. *Anal.* Calcd. for $C_{27}H_{45}O_2N$: C, 78.02; H, 10.91; N, 3.37. Found: C, 78.02; H, 10.97; N, 3.37; CH_3O, absent.

N,O-Diacetyltomatidine.—By the treatment of tomatidine with acetic anhydride in pyridine at room temperature, m.p. 193–194°. *Anal.* Calcd. for $C_{31}H_{49}O_4N$: C, 74.51; H, 9.88; N, 2.80. Found: C, 74.36; H, 9.78; N, 2.84.

Dihydrotomatidine.—By the reduction of tomatidine with $LiAlH_4$ in diethyl ether solution, m.p. 194–195°. *Anal.* Calcd. for $C_{27}H_{47}O_2N$: C, 77.64; H, 11.34. Found: C, 76.92; H, 11.14.

N,O,O'-Triacetyldihydrotomatidine.—By the treatment of dihydrotomatidine with acetic anhydride in pyridine at room temperature. *Anal.* Calcd. for $C_{33}H_{53}O_5N$: C, 72.89; H, 9.83. Found: C, 72.87; H, 9.78.

Kuhn, *et al.*,[5] have confirmed our results on the sugars in tomatine but have differed by assigning an ethylenic linkage and, therefore, two less hydrogen atoms to these compounds.[5,6] We have found no evidence of an ethylenic group by either ultraviolet or infrared analysis (Fig. 1). The absorption of one mole of hydrogen by tomatidine[5] may have resulted in the opening of an oxidic linkage, as reported by Marker, *et al.*,[7] for sapogenins. Further evidence of the opening of an oxidic ring is that after $LiAlH_4$ reduction, as reported here, an additional hydroxyl group appeared, after which acetylation added three instead of two acetyl groups.

Tomatidine forms an alcohol insoluble digitonide, which is evidence of a 3(β)-ol sterol configuration. The steroid structure of tomatidine has been confirmed by Sato, *et al.*,[8] who have degraded it to Δ^{16}-allo-pregnen-3(β)-ol-20-one.

The infrared spectra of tomatidine and diacetyltomatine are compared with those of cholesterol and acetylcholesterol in Fig. 1. In the spectrum of diacetyltomatidine, a strong band appears at 6.03 microns from acetylation of the nitrogen, which together with the absence of bands at 3.0 and 6.5 microns, is characteristic of a secondary amine structure of the original base.[9,10]

(3) R. M. Ma and T. D. Fontaine, *ibid.*, **27**, 461 (1950).

(4) All melting points were taken on samples sealed in evacuated tubes and are corrected.

(5) R. Kuhn, I. Löw and A. Gauhe, *Ber.*, **83**, 448 (1950).

(6) R. Kuhn and I. Löw, *ibid.*, **81**, 552 (1948).

(7) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, THIS JOURNAL, **69**, 2167 (1947).

(8) Y. Sato, A. Katz, E. Mosettig, *ibid.*, **73**, 880 (1951).

(9) H. M. Randall, R. G. Fowler, N. Fuson, and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949.

(10) A preliminary report of some of this work was presented at the American Society of Biological Chemists Meeting, Atlantic City, N. J., April 17–21, 1950; T. D. Fontaine, J. S. Ard, R. M. Ma, C. L. Ogg and C. O. Willits, *Federation Proc.*, **9**, 171 (1950).

BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY
THOMAS D. FONTAINE
AGRICULTURAL RESEARCH CENTER
J. S. ARD
BELTSVILLE, MARYLAND
ROBERTA M. MA

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