

## COMMUNICATIONS TO THE EDITOR

## ON THE CHEMICAL BEHAVIOR OF CAFESTEROL

Sir:

In view of the recent publication of Wettstein, *et al.* [*Helv. Chim. Acta*, **24**, 332E (1941)], which we have just received, it appears desirable to record some of our observations in this field. The existence of a benzenoid ring in cafesterol, as suggested by Slotta and Neisser [*Ber.*, **71**, 2342 (1938)], is highly improbable, since nitric acid oxidation of this compound gives neither benzene tetra- or dicarboxylic acid; from the reaction mixture was obtained only a non-acidic substance, apparently a nitro derivative, m. p. 220–230°. Cafesterol possesses a highly reactive conjugated double bond system in one ring, a fact shown by the formation of the maleic anhydride adduct (m. p. 185–192°), in benzene solution at room temperature or on gentle warming. Boiling such a solution promptly causes decomposition. In absolute alcoholic solution cafesterol takes up two moles of hydrogen in presence of palladized charcoal (20% Pd) giving a tetrahydro derivative, m. p. 153–155°, acetate 150–152°. Neither this tetrahydrocafeesterol nor its acetate gives any coloration with concentrated hydrochloric acid, while cafesterol in alcoholic solution gives an intense blue to blue-green color reaction with this reagent (Slotta and Neisser). Contrary to the observations of Wettstein, *et al.*, and Slotta and Neisser, on treatment with sodium and alcohol (or amyl alcohol), cafesterol gives a new product, m. p. 153–156°, acetate 162–165°. Despite similar melting points, the difference of this product from cafesterol is shown by the fact that with concentrated hydrochloric acid, its alcoholic solution gives a purple coloration which *does not turn blue even on boiling*. Its acetate on the other hand gives a yellow to orange coloration with the same reagent. Sodium-alcohol treatment does not appear to affect the conjugated double bond system, because the acetate of the product gives a maleic anhydride adduct, m. p. 185°. The adduct gives no coloration with hydrochloric acid at room temperature.

A detailed report will be published at a later date.

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## DERIVATIVES OF ESTRONE CONTAINING OXYGEN AT POSITION 16

Sir:

The interesting hypothesis, recently advanced by Marrian,<sup>1</sup> that 16-ketoestrone is an estrogen metabolite in the human, leads to the speculation that estriol (theelol) may be formed from estrone by the reduction of 16-ketoestrone. If such a reduction occurs, it is logical to assume that 16-hydroxyestrone may also lie on this metabolic pathway as an intermediate between the dione and glycol forms. In the reduction of 16-ketoestrone, two stereoisomeric 16-hydroxyestrones and four stereoisomeric estriols are theoretically possible. The question also arises whether or not a compound in this series may be regarded as an abnormal estrogen metabolite, which might conceivably play a role in the etiology of carcinoma of the uterus and of the mammary gland.

We have succeeded in preparing (i) the methyl ether of 16-ketoestrone, (ii) a compound believed to be one of the two epimeric 16-hydroxyestrones, and (iii) an estriol which is isomeric with the naturally-occurring theelol.

Estrone was converted to the 16-isonitroso derivative by the method of Litvan and Robinson,<sup>2</sup> and this derivative on reduction with zinc and acetic acid<sup>3</sup> yielded a mixture of  $\alpha$ -ketols, from which there was isolated in pure form a compound which is probably a 16-hydroxyestrone (m. p. 234–237°;  $[\alpha]^{20}_D - 102^\circ$  in ethanol). This compound was characterized by the following derivatives: oxime (m. p. 222.5–223°), monobenzoate (m. p. 241.5–243.5°), methyl ether (m. p. 174–177°), and oxime of the methyl ether (m. p. 175–177°).

Reduction of a similar  $\alpha$ -ketol mixture with hydrogen and Adams catalyst yielded a mixture of estriols, one of the components of which proved to be an isomer of theelol. The isomer of estriol obtained has m. p. of 267–269° and  $[\alpha]^{20}_D + 88^\circ$  (in ethanol). A mixed melting point with theelol shows a depression of 10°. It gives a methyl ether melting at 141–142° and a triacetate melting at 152°.

(1) Marrian, *Bul. New York Acad. Med.*, **15**, 27 (1939).

(2) Litvan and Robinson, *J. Chem. Soc.*, 1997 (1938).

(3) Stodola, Kendall and McKenzie, *J. Org. Chem.*, **6**, 841 (1941).

Oxidation with copper acetate of the mixture of  $\alpha$ -ketols obtained from estrone methyl ether gave 16-ketoestrone methyl ether, obtained as flat needles, orange in color (m. p. 176–178°). It gives a dioxime (m. p. 230°) identical with that prepared by oximating 16-isonitrosoestrone methyl ether. The dione gives an intense violet color with concentrated sulfuric acid.

The dioxime of 16-ketoestrone (free phenol) was also prepared in the hope that this derivative might be useful in detecting the dione in tissue or fluid, should it be present as predicted by Marrian. 16-Ketoestrone dioxime (m. p. 230–231°) gives no colored complex with nickelous or cobaltous ions, but produces a yellowish-green solution with alcoholic copper acetate. The copper complex may be extracted with chloroform, and the color intensity is such that the eye can detect it at the level of 10  $\gamma$  per cc.

The melting points listed above are uncorrected.

We are now attempting to prepare the other isomeric 16-hydroxyestrone and the remaining two isomeric estriols.

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# THE CRYSTAL STRUCTURE OF $\beta$ -GLYCYLGLYCINE

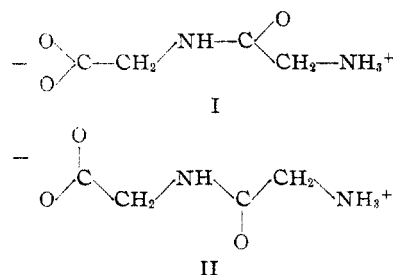
Sir:

Some time ago the determination of the crystal structure of glycylglycine was undertaken as a continuation of the X-ray diffraction studies<sup>1</sup> which are a part of a program of research upon the constitution and configuration of proteins. This determination, the first concerned with a linear peptide, was stopped last year because of the war, and in view of the uncertainty of completing the work the results at hand are briefly described in this letter.

The crystals were grown from aqueous *n*-propyl alcohol and all three modifications described by Bernal<sup>2</sup> were eventually obtained, although not simultaneously as in his crystallization. Because of the shortness of the *b* axis the needle-like beta form was selected for investigation. Using Cu K $\alpha$  rays complete sets of oscillation pictures were

prepared about *a* and *b* and some oscillations were made about *c* to confirm the length of that axis. Weissenberg pictures of the [010] zone were made for intensity comparisons. The cell has *a* = 17.89 Å., *b* = 4.62 Å. and *c* = 17.06 Å., with  $\beta$  = 125°10', and contains eight molecules. This *c* is twice that given by Bernal and the space-group instead of P2<sub>1</sub>/*a* as given by him, is either A*a* or A2/*a*. The latter was tentatively assumed and present results indicate it is probably correct.

A Patterson projection parallel to *b* yielded preliminary *x* and *z* parameters. The configurations I, suggested by Bernal, and II were tried, and it was found that only with II could the projection be interpreted. The parameters were



improved by Fourier projections and least squares,<sup>3</sup> the latter to resolve CO groups not resolved in the projections. The present agreement between calculated and observed intensities in this zone is good, but minor discrepancies indicate that there may be a few errors in signs or that the contributions from hydrogen atoms should be considered. A tentative set of *y* parameters has also been selected so as to yield reasonable interatomic distances both within and between molecules and these give qualitative agreement between calculated and observed intensities in the [100] zone.

Because of the approximate nature of the parameters, particularly the *y*'s, there is no point in listing interatomic distances. The chief results are first, that the configuration is that of II with the molecule coplanar within present errors except for the terminal nitrogen atom, which lies out of the plane by several tenths of an ångström, and, second, that the zwitterion form is correct. The terminal nitrogen is surrounded at the usual N–H  $\cdots$  O distance by three oxygen atoms of other molecules, two carboxyl and one carbonyl. All the indicated hydrogen bond angles about the nitrogen are tetrahedral to within 10°. The imino nitrogen is also forming a hydrogen bond to a carboxyl oxygen of a neighboring molecule.

(1) Diketopiperazine, R. B. Corey, *THIS JOURNAL*, **60**, 1598 (1938); glycine, G. Albrecht and R. B. Corey, *ibid.*, **61**, 1087 (1939); *dl*-alanine, H. A. Levy and R. B. Corey, *ibid.*, **63**, 2095 (1941).

(2) J. D. Bernal, *Z. Krist.*, **78**, 363 (1931).

(3) E. W. Hughes, *THIS JOURNAL*, **63**, 1737 (1941).