cording to scheme B, instead of A, will alter the arrangement of acetate carbons in cholesterol only at the four following positions: C_7, C_8, C_{12} and C_{13}. Evidence for the origin of one of these crucial carbon atoms is presented in this communication. Epiandrosterone, obtained by degradation of labeled dihydrocholesterol,[4,6] was oxidized under the conditions used for C-methyl determination[7] to yield 1.7 moles of acetic acid (theory 2 moles). The acetic acid, which is derived from the angular methyl groups and the adjoining carbon atoms, was analyzed for C^{14} and degraded for separate assay of $C_{10} + C_{18}$ and $C_{18} + C_{19}$. The table shows that in cholesterol which had been synthesized biologically from methyl-labeled acetate, either C_{10} or C_{13}, in addition to the angular methyl groups, contains C^{14}. Since it is known from the work of Cornforth, *et al.*,[8] that C_{10} is derived from an acetate carboxyl, it can be concluded that the labeled carbon in the present case is C_{13}.[9] Now if C_{13} has its origin in a methyl group of acetic acid cyclization scheme A is untenable. Scheme B, which is consistent with earlier as well as with the present new isotopic data, provides also a particularly reasonable basis for the biosynthesis of the triterpenoid alcohol, lanosterol (II), whose remarkable resemblance to the sterols has recently been demonstrated.[10] Formation from squalene, accompanied by a methyl shift, would rationalize the fact that lanosterol is not constituted in accordance with the isoprene rule.

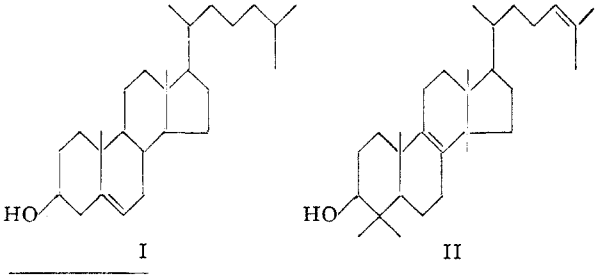
TABLE I

CHEMICAL DEGRADATION OF STEROID SYNTHESIZED FROM METHYL-LABELED ACETATE C^{14}, C.P.M.[a]

All values calculated for a ratio of 10 methyl to 9 carboxyl carbon atoms of acetate in epiandrosterone (*cf.* H. N. Little and K. Bloch, *J. Biol. Chem.*, **183**, 33 (1950)).

Products analyzed	Scheme A[b]	Scheme B[c]	Found
(1) Epiandrosterone	...	...	100
(2) Acetic acid from (1)	95	143	135
(3) Carboxyl-C from (2) $C_{10} + C_{13}$	0	95	105
(4) Methyl-C from (2) $C_{18} + C_{19}$	190	190	165

[a] As infinitely thick samples of $BaCO_3$. [b] Calcd. A: C_{18}, C_{19} derived biologically from acetate-methyl; C_{10}, C_{13} derived biologically from acetate-carboxyl. [c] Calcd. B: C_{18}, C_{19}, C_{13} derived biologically from acetate-methyl; C_{10} derived biologically from acetate-carboxyl.



I II

(6) Isolated by Dr. Josef Würsch.

(7) R. Kuhn and F. L'Orsa, *Z. angew. Chem.*, **44**, 847 (1931).

(8) J. W. Cornforth, G. D. Hunter and G. Popjak, private communication.

(9) These arguments will not be valid in the very unlikely event that the angular methyl carbon C_{18} shifts quantitatively to another position during the course of the oxidation.

(10) W. Voser, M. V. Mijovic, H. Heusser, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **35**, 2414 (1952).

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RECEIVED MARCH 11, 1953

ENZYMATIC REDUCTION OF COMPOUND E-21-ALDEHYDE TO COMPOUND E

Sir:

Δ^4-Pregnene-17α-ol-21-al-3,11,20-trione (Compound E-21-aldehyde) has been prepared from Δ^4-pregnene-17α,21-diol-3,11,20-trione (Compound E) by Rogers, *et al.*,[1] who noted that the aldehyde has approximately the same biological activity as the parent hormone. We believe that the observed activity of the aldehyde is due to its rapid reduction to Compound E *in vivo*, and wish to report the isolation of Compound E following the incubation of the aldehyde in a simple cell-free *in vitro* system.

Six grams of rat liver acetone powder was extracted with 60 ml. of Kreb's phosphosaline buffer *p*H 7.4 for 30 min. at room temp. The suspension was centrifuged, and the supernatant (48 ml.) added to 57.8 mg. of the aldehyde hydrate[2] in 68 ml. of the above buffer containing 100 mg. of dihydrodiphosphopyridine nucleotide. After 30 min. at 38° (gas phase air) the incubate was diluted with acetone, filtered, and the acetone removed *in vacuo*. The aqueous residue was diluted with water and extracted with ethyl acetate. After removal of the solvent, the extract was chromatographed on magnesium silicate Celite. Elution with ethyl acetate gave a single crystalline fraction which after recrystallization from methanol weighed 22 mg. It was identified as Compound E by its m.p. (218–220°, not depressed on admixture with authentic Compound E), the observed absorption maxima in sulfuric acid solution (280–285 mμ, 340 mμ and 410–415 mμ, corresponding to the published maxima for authentic Compound E,[3] the m.p. of the acetate (242–244°[4]), and the analysis of the acetate (Calcd. for $C_{23}H_{30}O_6$: C, 68.65; H, 7.46. Found: C, 68.44; H, 7.20).

A detailed study of this enzymatic reduction including efforts to effect its reversal will be published at a later date

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(1) E. F. Rogers, W. J. Leanza, J. P. Conbere and K. Pfister 3rd, THIS JOURNAL, **74**, 2947 (1952).

(2) The author wishes to thank Dr. Karl Pfister and Dr. E. F. Rogers, Merck and Company, Inc., for the Compound E-21-aldehyde hydrate used in this experiment.

(3) A. Zaffaroni, *ibid.*, **72**, 3828 (1950).

(4) L. H. Sarett, *ibid.*, **70**, 1454 (1948).