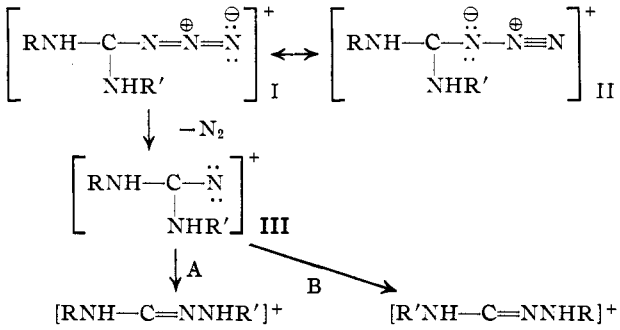
MIGRATION OF NITROGEN IN THE SCHMIDT REACTION

Sir:

From a consideration of the mechanism proposed for the isomerization of substituted 5-aminotetrazoles[1,2] together with a study of the products formed by the acid induced degradation of 5-substituted tetrazoles,[3] we have concluded that guanyl azides can undergo a Curtius or Schmidt type reaction.



Under the conditions used the carbodiimides hydrolyze to carbon dioxide, an amine and a hydrazine. The rearrangement of III involves an interchange in which the electrons are transported to the electronically deficient nitrogen by a nitrogen atom rather than a carbon atom. This has not been previously observed and affords another opportunity for studying the competitive migration aptitudes of groups. The degradation of 5-hydrazinotetrazole[4] in concentrated hydrochloric acid at 170° is an example. Opening of the tetrazole ring[2] yields a guanyl azide where R = NH_2 and R′ = H. Since ammonia is absent in the products, the rearrangement must occur almost exclusively by route A; *i.e.*, the amino group migrates in preference to the hydrazino group. We have found that 5-phenylaminotetrazole (IV) degrades largely to aniline and hydrazine (route A, where R = C_6H_5 and R′ = H): A solution of 0.5 g. of IV in 10 ml. of 85% phosphoric acid was heated at 190–200° for 2.5 hours, cooled, diluted with 10 ml. of water, refluxed for 1.5 hours, re-cooled, partially neutralized, and treated with benzaldehyde. Benzalazine (0.51 g. 79%; m.p. 92–93°) was removed and the solution was made alkaline. Benzalaniline (0.38 g., 68%; m.p. 46–49°) was recovered.

The Schmidt reaction should be capable of extension to the ammonocarbonic acids[5] provided that one of the contributing forms is a carbonium ion. With guanidine the product should be aminoguanidine and experimentally the latter has been recovered in about 1% yield. Ten grams of sodium azide was added portionwise during 2 hours to an agitated slurry of 10.8 g. of guanidine sulfate, 35 ml. of 96% sulfuric acid and 150 ml. of benzene at 25–28°. The temperature was next raised to and held at 40–50° for 4 hours. After the benzene layer was decanted, the acid layer was diluted with water, partially neutralized, shaken with benzaldehyde, neutralized to *p*H 9, and cooled to 5°. The hydrazone was removed, washed with water, extracted with petroleum ether until free of benzalazine, and converted to a picrate (0.3 g., 1%) which alone and in admixture with an authentic sample of benzalaminoguanidine picrate melted at 252–254°. X-Ray powder patterns were identical. Under similar conditions hydrazine and guanidine sulfates do not react to give aminoguanidine.

This extension of the Schmidt reaction will be fully explored.

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(1) W. G. Finnegan, R. A. Henry and E. Lieber, submitted to *J. Org. Chem.*

(2) R. A. Henry, W. G. Finnegan and E. Lieber, accepted for presentation at the 123 National Meeting of the American Chemical Society, Los Angeles, California.

(3) F. R. Benson, *Chem. Revs.*, **41**, 55 (1947).

(4) J. Thiele and H. Ingle, *Ann.*, **287**, 233 (1895).

(5) E. C. Franklin, "Nitrogen System of Compounds," Reinhold Publ. Corp., New York, N. Y., 1935, p. 86.

THE CYCLIZATION OF SQUALENE IN CHOLESTEROL SYNTHESIS

Sir:

The hypothesis that the triterpenoid hydrocarbon squalene is an intermediate in the biological synthesis of cholesterol (I) has recently received direct experimental support.[1,2] It has further been shown[3] that acetic acid, the principal carbon source in cholesterol synthesis, is a precursor also for squalene. The squalene hypothesis can be harmonized with the hitherto known distribution of acetate carbon in cholesterol[4] if one assumes that (a) each isoprene unit of the hydrocarbon contains 3 methyl and 2 carboxyl carbons of acetate, arranged as shown: 

(o = acetate methyl, x = acetate carboxyl); and (b) that in the transformation to sterol, squalene cyclizes as suggested by Robinson[5] (*cf.* Fig. 1, A).

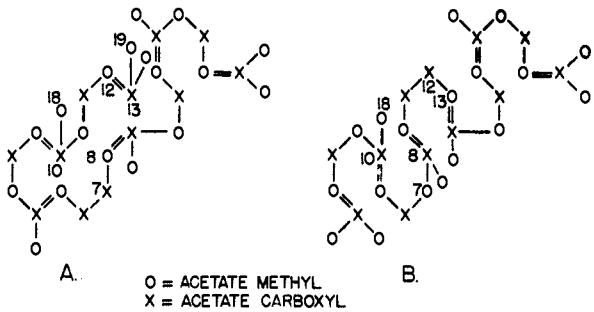


Fig. 1.

We now wish to propose the alternative mechanism shown in Fig. 1 B, as a more likely one to be involved in this transformation. Robinson's scheme provides for a conversion without rearrangement of the carbon skeleton of squalene. In mechanism B, on the other hand, one or more methyl migrations is necessary at some stage for the construction of the quaternary center at C_{13}. As is clear from Fig. 1, cyclization of squalene ac-

(1) R. G. Langdon and K. Bloch, THIS JOURNAL, **74**, 1869 (1952).

(2) R. G. Langdon and K. Bloch, *J. Biol. Chem.*, **200**, 135 (1953).

(3) R. G. Langdon and K. Bloch, *ibid.*, **200**, 129 (1953).

(4) J. Würsch, R. L. Huang and K. Bloch, *ibid.*, **195**, 439 (1952).

(5) R. Robinson, *J. Soc. Chem. Ind.*, **53**, 1062 (1934).

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

TABLE I

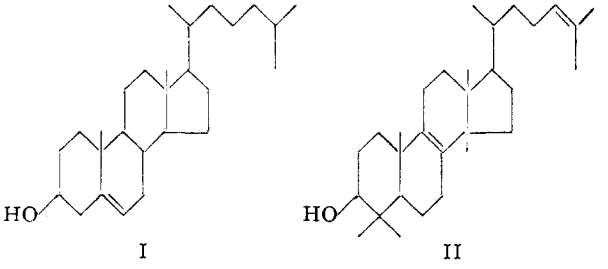
CHEMICAL DEGRADATION OF STEROID SYNTHESIZED FROM METHYL-LABELED ACETATE C^{14}, C.P.M.[a]

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.

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.



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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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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RECEIVED MARCH 24, 1953

(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).