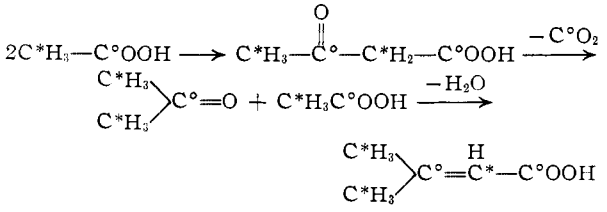
mechanism suggested by Würsch, *et al.*, in Scheme I for β-DMA synthesis.[7]

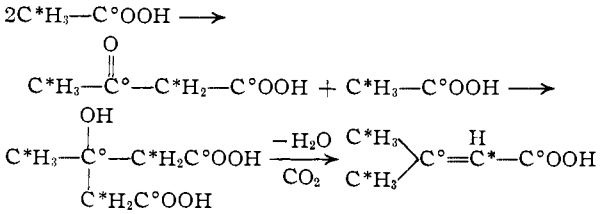
SCHEME I



° = Acetate-COOH; * = Acetate $CH_3$

However the reactions of Scheme II would explain the incorporation of carboxyl labeled acetoacetate.

SCHEME II

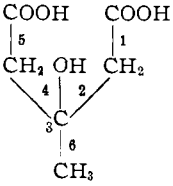


It is assumed that these acids are probably in the form of acyl CoA derivatives.[8,9]

β-Hydroxy-β-methylglutaric acid[10] (β-HMG) occurs naturally[11,12] and might be expected to decarboxylate asymmetrically.[13] Upon feeding to animals it appears to be incorporated directly into cholesterol.[14] To test the validity of Scheme II, 10 μM. of $C^{14}H_3$-COOH (s.a. = $5.0 \times 10^5$ c.p.m./μM. acetate)[15] + 50 μM. β-HMG were added to 21.4 ml. of incubation mixture containing rat liver homogenate and ATP and DPN prepared as previously described.[4] Incubation was for three hours at 38° with 100% $O_2$ in large Warburg cups. The mixture was made alkaline with KOH (final concentration 0.17 *N*) 0.3 mM. of β-HMG was added as carrier, and allowed to stand for half an hour at room temperature. It was acidified with $H_2SO_4$, then mixed with Celite and extracted with ether for four hours. The ether extract was steam distilled and β-HMG was isolated and purified from the non-volatile fraction by separation on Dowex-1 (formate form) with 0.1 *N* formic acid as eluant.

The β-HMG contained considerable activity. Its purity was established by the coincidence of the titration and radioactivity curves on Dowex-1 and on a Celite column with butanol and chloroform as solvent. Also, no depression of the melting point occurred on admixture with an authentic sample. β-HMG was degraded in the following manner. A Schmidt reaction gave carbons 1 and 5 as $CO_2$. Dehydration with $H_2SO_4$ followed by $KMnO_4$ oxidation gives acetoacetic acid which was degraded further to acetate (carbons 3 and 6) and formate (carbons 2 and 4).[16] Acetate and formate were isolated and identified by partition chromatography. Formate was oxidized by HgO. Acetate was degraded by pyrolysis of the barium salt. The distribution of isotope found is shown in Table I.

TABLE I

| β-HMG | c.p.m./mM. $C^{15}$ |
|---|---|
|  | 500 |
| | 11100 |
| | 250 |
| | 10500 |
| Total oxidation calculated | 5650 |
| found | 6050 |

It will be noted that it parallels that found in β-DMA.[5] These results offer support for Scheme II and indicate that β-DMA and β-HMG may be precursors in the synthesis of cholesterol. They suggest that acetoacetic acid or its CoA derivative may condense with acetyl CoA in a manner analogous to the condensation of oxalacetate and acetyl CoA to form citric acid. These results do not rule out Scheme I nor the possibility that β-HMG may also be formed by $CO_2$ fixation with β-DMA.[8]

The author wishes to acknowledge the technical assistance of Miss Lillian Brown.

(7) M. Blecher (*Fed. Proc.*, **13**, 184 (1954)), also R. W. Chen, *et al.* (*J. Biol. Chem.*, **205**, 383 (1953)) however, present evidence indicating that acetoacetic acid is equilibrated with two carbon units prior to incorporation into cholesterol.

(8) W. G. Robinson, B. K. Bachawat and M. J. Coon, *Fed. Proc.*, **13**, 281, 1954.

(9) H. Klein and F. Lipmann, *J. Biol. Chem.*, **203**, 101 (1953).

(10) While this work was in progress we found that a similar mechanism was suggested by Bloch in a Harvey Lecture delivered December 18, 1952, but published in February, 1954 (Academic Press, Inc., New York, N. Y., 1954).

(11) R. Adams and B. L. Van Duuren, THIS JOURNAL, **75**, 2377 (1953).

(12) H. J. Klosterman and F. Smith, *ibid.*, **76**, 1229 (1954).

(13) D. W. Racusen and S. Aranoff, *Arch. Biochem. Biophys.*, **34**, 218 (1951).

(14) L. C. Clark, I. Harary, O. Reiss and K. Bloch, *Fed. Proc.*, **13**, 192 (1954).

(15) β-HMG counted as carbon dioxide in gas phase counter,

(16) S. Weinhouse and R. Millington, *J. Biol. Chem.*, **181**, 645 (1949).

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## THE STEREOCHEMISTRY OF SQUALENE. A NEW METHOD FOR THE DETERMINATION OF *CIS-TRANS* ISOMERISM[1]

*Sir:*

When X-ray diffraction patterns are taken of single crystals of urea or thiourea adducts, continuous layer lines appear in addition to the sharp spots produced by the host. The distance between these lines is a function of the length of the adducted molecule. Such lines have been reported.[2,3,4]

This property was used to measure the length of aliphatic molecules and their olefinic derivatives to see what shortening effect a *cis* or *trans* double bond has on the length. Results for urea adducts of a series of $C_{18}$ acids are summarized in Table I.

(1) This investigation was supported in part by the Medical Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-411.

(2) A. E. Smith, *Acta Crystallographica*, **5**, 224 (1952).

(3) W. Borchert, *Heidelberger Beiträge z. Min. u. Pet.*, **3**, 124 (1952).

(4) F. Laves and N. Nicolaides, Abstr. of Am. Cryst. Assoc. Meeting, pp. 16–17 (1952).

TABLE I

| Acid[a] | Double bonds | Length of dimeric acid, Å. | Shortening,[b] Å. | Length of *c*-axis of urea host, Å. |
|---|---|---|---|---|
| Stearic | None | 50.11 ± 0.20 | | 11.03 ± 0.02 |
| Oleic | 1 *cis* $\Delta^{9,10}$ | 48.15 ± 0.20 | 0.88 ± 0.10 | 11.00 ± 0.02 |
| Linoleic | 2 *cis* $\Delta^{9,10,12,13}$ | 46.65 ± 0.20 | 0.87 ± 0.05 | 11.00 ± 0.02 |
| Elaidic | 1 *trans* $\Delta^{9,10}$ | 49.75 ± 0.20 | 0.18 ± 0.10 | 11.02 ± 0.02 |
| Linolelaidic | 2 *trans* $\Delta^{9,10,12,13}$ | 49.30 ± 0.20 | 0.20 ± 0.05 | 11.03 ± 0.02 |

[a] Acids of high purity were kindly provided by the Research Division of Armour & Co., General Mills, Inc., and the Hormel Institute. [b] Shortening = stearic acid dimer length − unsaturated fatty acid dimer length/2 × number of double bonds in the molecule.

These data clearly show that an isolated *trans* double bond shortens a molecule 0.19 Å., whereas an isolated *cis* double bond shortens it 0.88 Å.

Squalene has a total of six double bonds. The four middle ones can have the main chain substituents either *cis* or *trans* with respect to each other. Assuming that in the thiourea adducts, squalene and fully hydrogenated squalene stretch out their maximum normal length in the thiourea framework as the fatty acids do in urea adducts, and assuming that the same shortening effect for isolated *cis* or *trans* double bonds applies, a comparison of the lengths of these two molecules should indicate the number of *cis* or *trans* double bonds present (Table II).

TABLE II

| | $n^{25}D$ | Length, Å. | *c*-Axis, Å. |
|---|---|---|---|
| Hydrogenated squalene | 1.4509 | 31.33 ± 0.15 | 12.54 ± 0.02 |
| Squalene | 1.4941 | 30.60 ± 0.15 | 12.56 ± 0.02 |

The shortening for six *trans* double bonds would be 1.1 ± 0.3 Å., whereas the shortening for one *cis* and five *trans* double bonds would be 1.8 ± 0.3 Å. Since the measured shortening was only 0.73 ± 0.30, natural squalene must be the all-*trans* isomer.

Without further stereochemical change, all-*trans* squalene could cyclize[6] to form the required all-*trans* fused rings A to B to C to D of cholesterol.[6]

The difference between the length, measured by X-rays, of an isoprene unit in rubber (one *cis* double bond), and that of β-gutta-percha (one *trans* double bond) is 0.67 Å.[7] This is identical to the difference in length found by us between a *cis* and a *trans* double bond in the chain of an adducted molecule (0.88 − 0.19 = 0.69 Å.).

Although the method was used here to differentiate *cis* and *trans* isomers, it could be applied to any structural problem where the length of an adducted molecule is discriminating. The method is being further investigated. Details and further applications will be reported later.

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(5) R. B. Woodward and K. Bloch, THIS JOURNAL, **75**, 2023 (1953); see also R. G. Langdon and K. Bloch, *J. Biol. Chem.*, **200**, 135 (1953); W. G. Dauben and K. H. Takemura, THIS JOURNAL, **75**, 6302 (1953).

(6) R. B. Turner in "Natural Products Related to Phenanthrene," L. F. Fieser and Mary Fieser, 3rd ed. Reinhold Publ. Corp., New York, N. Y., p. 620.

(7) *c*-Axis (rubber) minus 2 *c*-axis (β-gutta-percha)/2; C. W. Bunn, *Proc. Roy. Soc.* (*London*), **180**, 40 (1942).

## SALT EFFECTS AND ION-PAIRS IN SOLVOLYSIS[1]

*Sir:*

We have observed some striking salt effects on rates of acetolysis of a number of benzenesulfonates which yield carbonium ions with bridged structures. The addition of, *e.g.*, lithium perchlorate in low concentrations in acetolysis of, *e.g.*, 2,4-dimethoxyphenylethyl or 3-anisyl-2-butyl *p*-bromobenzenesulfonate (ROBs) gives a two-stage effect: (i) an initial very steep rise in the first-order titrimetric rate constant, $k_t$, from the value in the absence of salt, $k_t^\circ$; (ii) a subsequent small increase in $k_t$, nearly perfectly linear in salt concentration, $[LiClO_4]$. A short extrapolation of the linear part of the plot of $k_t$ *vs.* $[LiClO_4]$ to zero $[LiClO_4]$ yields the intercept $k^\circ_{ext.}$. The linear part of the plot may be expressed by: $k_t = k^\circ_{ext.}(1 + b[LiClO_4])$. The behavior of any system may be characterized by the ratio $k^\circ_{ext.}/k^\circ_t$, the slope $b$ and the $[LiClO_4]$ where $k_t$ is midway between $k^\circ_t$ and $k^\circ_{ext.}$, namely $[LiClO_4]_{1/2}$. Sample data are illustrated:

| Compound | °C. | $k^\circ_{ext.}/k^\circ_t$ | $[LiClO_4]_{1/2}$ | *b* |
|---|---|---|---|---|
| (*p*-$CH_3OC_6H_4$)($CH_3$)$_2$-$CCH_2OTs$ | 25 | 1.00 | | 16 |
| *threo*-$CH_3CH(C_6H_5)$-$CH(OTs)CH_3$ | 50 | 1.00 | | 37 |
| *exo*-NorbornylOBs | 25 | 1.00 | | 37 |
| *threo*-$CH_3CH(C_6H_4$-$OCH_3$-*p*)$CH(OBs)$-$CH_3$ | 25 | 2.59 | $2.3 \times 10^{-3}$ | 22 |
| *erythro*-$CH_3CH(C_6H_4$-$OCH_3$-*p*)$CH(OBs)$-$CH_3$ | 25 | 3.08 | $3.8 \times 10^{-3}$ | 19 |
| $CH_3CH(C_6H_4OCH_3$-*p*)-$CH_2OTs$ | 50 | 2.5 | $3.2 \times 10^{-3}$ | 24 |
| Cholesteryl OTs | 50 | 2.3 | $4 \times 10^{-5}$ | 28 |
| 2,4-$(MeO)_2C_6H_3CH_2$-$CH_2OBs$ | 50 | 2.2 | $6 \times 10^{-5}$ | 13 |

The lithium perchlorate is evidently involved in altering ion-pair return.[2] For example, only the shallow linear salt effect is observed with *p*-methoxyneophyl OTs, a system where the observed $k_t$ is equal to the total ionization rate, $k_1$. However, the presence of ion-pair return is not a sufficient condition for the appearance of the steep salt effect, for it is absent with the norbornyl and 3-phenyl-2-butyl systems.[2b]

With the *threo*-3-anisyl-2-butyl and 2-anisyl-1-propyl systems, $k_1$ has been obtained by polarimetric[2b] or other kinetic methods[2c] and these follow

(1) Sponsored by the Office of Ordnance Research, U. S. Army.

(2) *E.g.*, S. Winstein, *et al.*, THIS JOURNAL, (a) **73**, 1958 (1951); (b) **74**, 2165 (1952); (c) **74**, 2171 (1952).