A sulfur containing traizolopyrimidine has been reported to inhibit the growth of *Streptococcus faecalis*[8] at very low concentration.

The 8-aza analog of 6-mercaptopurine has been prepared as follows:

Eight grams of 7-hydroxy-1-v-triazolo(d)pyrimidine[9] is added to a solution of 15.0 g. of phosphorus pentasulfide in 233 ml. of boiling pyridine.[10] A clear solution results which begins to deposit crystals as refluxing is continued. After refluxing three to four hours the hot mixture is poured into 400 ml. of boiling water and boiled for a few minutes. Since the product may exist in both oxidized and reduced forms in the same way as cystine–cysteine, care is taken to avoid exposure to atmospheric oxidation. The hot mixture is filtered. The crystals are dissolved in 500 ml. of hot 0.06 *M* KSH solution. The solution is boiled about five minutes, acidified with acetic acid and cooled. The crystals are dissolved in boiling water. The solution is treated with activated charcoal and filtered. The crystals which separate on cooling are dried and treated with hot methanol. The white crystals, 0.9 g. (10%), which separate on chilling the methanol darken about 262° and decompose suddenly about 272°. By paper chromatography using a solvent containing 300 ml. of butanol, 60 ml. of water and 3.6 ml. of glacial acetic acid the R_f is found to be 0.72. The ultraviolet absorption spectrum of a methanol solution shows maxima at 245, 265 and 312 and minima at 235, 260 and 280. Data covering the inhibitory effect of the compound on various tumors are to be published elsewhere. *Anal.* Calcd. for $C_4H_3N_5S$: C, 31.37; H, 1.97. Found[11]: C, 31.60; H, 1.84.

We wish to express our thanks to Dr. Howard Skipper and his associates, of the Southern Research Institute, for their encouragement and for carrying out screening tests. We are indebted to Dr. Gertrude Elion and Dr. George Hitchings of Wellcome Research Laboratories for their interest and for measuring the ultraviolet absorption.

(8) C. T. Bahner, H. A. Rutter, Jr., and J. R. Totter, *J. Tenn. Acad. Sci.*, **27**, 179 (1952).

(9) R. O. Roblin, *et al.*, ref. 8.

(10) *Cf.* E. Klingsberg and D. Papa, THIS JOURNAL, **73**, 4988 (1951).

(11) Analyses by Dr. Harry W. Galbraith, Galbraith Laboratories, Knoxville, Tennessee.

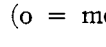
DEPARTMENT OF CHEMISTRY
CARSON-NEWMAN COLLEGE
JEFFERSON CITY, TENNESSEE

A Study of the Mechanism of Conversion of Acetate to Cholesterol *via* Squalene[1]

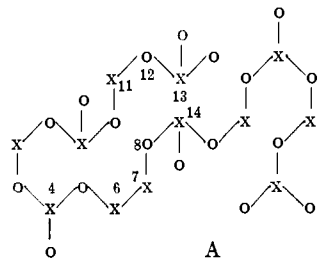
BY WILLIAM G. DAUBEN AND K. H. TAKEMURA

RECEIVED AUGUST 24, 1953

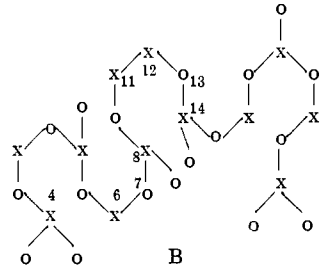
The role of squalene in the biosynthetic mechanism of formation of cholesterol from acetate has received attention in the past year[2,3] and two schemes for its direct cyclization to a steroidal nucleus have been postulated.[4-6] To date, the method employed to evaluate the likelihood of each scheme has been to degrade the labeled cholesterol molecule which has been formed by incubation of labeled acetate with liver slices. Although no degradation experiments have, as yet, been performed with squalene, it has been assumed that the isoprenoid units from which it may have been derived contain 3 methyl groups and 2 carboxyl groups of acetate arranged as shown:



(o = methyl of acetate, x = carboxyl of acetate). The unique character of squalene is its symmetry since it can be viewed as being composed of two sesquiterpenic units linked head-to-head while each sesquiterpenic unit is composed of three isoprenoid units combined in a head-to-tail fashion. Such an arrangement when coupled with the above postulated isotope distribution of the isoprenoid unit requires that the central two carbon atoms of the chain be derived from carboxyl groups of acetate and it is only at this point that two similarly labeled carbon atoms are in a juxtaposition; the remainder of the chain alternates between methyl carbon and carboxyl carbon. With this symmetry feature in mind, the distribution of the labeled atoms in a cholesterol molecule derived by cyclization of squalene following the two previously postulated schemes can be examined.



A



B

It is seen that route A places the two carboxyl carbon atoms under discussion in a juxtaposition at C_6 and C_7 while in route B it occurs at C_{11} and C_{12}. Recently, Cornforth, Hunter and Popjak[7] reported that C_6 is derived from a carboxyl carbon atom, as expected in both schemes, and we should like to report the determination of C_7.

Cholesterol (I), which had been derived from carboxyl-labeled acetate, was converted into cholesteryl chloride[8] with thionyl chloride and the reduction of the chloride by sodium and amyl alcohol[9] yielded cholest-5-ene (II). Oxidation of II with *t*-butyl chromate[10] gave rise to 7-keto-cholest-5-ene

(1) This work was supported by a grant from the Atomic Energy Commission.

(2) W. G. Dauben, H. L. Bradlow, N. K. Freeman, D. Kritchevsky and M. Kirk, THIS JOURNAL, **74**, 4321 (1952); G. M. Tompkins, I. L. Chaikoff, W. G. Dauben, H. L. Bradlow and P. A. Srere, *ibid.*, **74**, 6145 (1952); G. M. Tompkins, I. L. Chaikoff and W. G. Dauben, *J. Biol. Chem.*, **202**, 487 (1953).

(3) R. C. Langdon and K. Bloch, *ibid.*, **200**, 129, 135 (1953).

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

(5) R. B. Woodward and K. Bloch, THIS JOURNAL, **75**, 2023 (1953).

(6) W. G. Dauben, S. Abraham, S. Hotta, I. L. Chaikoff, H. L. Bradlow and A. H. Soloway, *ibid.*, **75**, 3038 (1953).

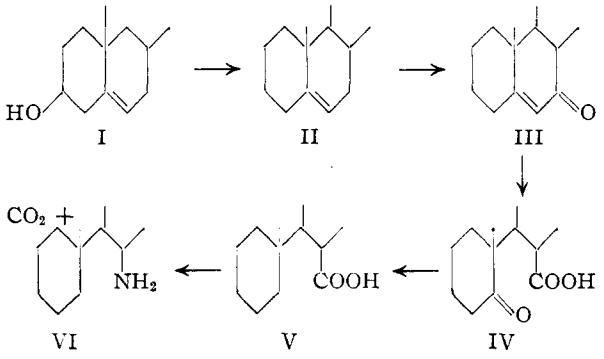
(7) J. W. Cornforth, G. D. Hunter and G. Popjak, *Biochem. J.*, **54**, 590, 597 (1953).

(8) O. Diels and P. Blumberg, *Ber.*, **44**, 2847 (1911).

(9) J. Mauthner and W. Suida, *Monatsh.*, **15**, 85 (1894).

(10) R. V. Oppenauer and H. Oberrauch, *Anal. asoc. quim. argentina*, **37**, 246 (1949).

(III)[11] which upon ozonization was transformed into 6-nor-5,7-seco-5-keto-7-carboxycholestane (IV). When the seco-acid IV was subjected to Wolff–Kishner reduction, the desketo-acid V was obtained. The acid V was allowed to react with



hydrazoic acid under the conditions of a Schmidt reaction and the evolved carbon dioxide (from the original C_7 of cholesterol) was collected in the usual manner and the amine isolated as a crystalline acetyl derivative. The carbon dioxide was found to contain no C^{14} and thus proves that C_7 of cholesterol is derived from a methyl carbon of acetate. This result, when taken together with the knowledge that C_6 comes from a carboxyl carbon of acetate,[7] rules out C_6 and C_7 as the position of symmetry and negates scheme A for the direct utilization of squalene in cholesterol biosynthesis. Although this result and the previously published work[5,6] which indicated that C_{13} is derived from a methyl carbon atom fit the distribution of labeled atoms postulated by scheme B, they do not yield unequivocal proof of the tetracyclic-triterpenoid type ring closure.

Experimental[12]

Cholesterol.—The cholesterol obtained by incubation of carboxyl-labeled acetate with liver slices (through the courtesy of Drs. I. L. Chaikoff and G. M. Tompkins) was highly diluted with purified cholesterol and recrystallized, m.p. 148.0–149.0°, specific activity: 14.7 cts./min./mg. $BaCO_3$, 33.1 c./min./mg. $BaCO_3$ (cor.).[13]

Cholesteryl Chloride.—A mixture of 3.600 g. of labeled cholesterol and 10 ml. of purified thionyl chloride was allowed to stand at room temperature for two hours. The excess reagent was removed under reduced pressure at 35–40°, 10 ml. of dry benzene added and the solution again concentrated to dryness. The residual dark yellow crystals were dissolved in 12 ml. of hot, dry acetone and first cooled at ice-bath temperature and finally at −70°. The solid was filtered and dried, yield 3.642 g. (94.5%), m.p. 93.0–95.5° (lit.[8] 96°).

Cholest-5-ene.—To a boiling solution of 3.64 g. of cholesteryl chloride in 80 ml. of isoamyl alcohol there was added 6.3 g. of sodium. After all the metal had reacted, the solution was cooled and then diluted with water. The mixture was thoroughly extracted with ether, the ethereal solution washed successively with water, dilute hydrochloric acid, water, sodium bicarbonate, water and finally saturated sodium chloride solution. After drying over anhydrous sodium sulfate, the ethereal solution was concentrated under reduced pressure on a steam-bath and the residue recrystallized from methanol, yield 3.21 g. (96.5%), m.p. 89.5–91.2° (lit.[9] 89–90°).

7-Keto-cholest-5-ene.—Cholest-5-ene (3.21 g.) was oxidized with *t*-butyl chromate following the procedure of Heusler and Wettstein[14] for the preparation of 3,17-diacetoxy-7-keto-androst-5-ene. The crude oily oxidation product was chromatographed on alumina. Elution with hexane yielded 1.13 g. of recovered cholest-5-ene while ether–hexane (10–90) gave 0.879 g. (41% based on recovered starting material) of 7-keto-cholest-5-ene, m.p. 125–129° (lit.[11] 124–125°).

6-Nor-5,7-seco-5-keto-7-carboxycholestane.—Ozone (0.5 mmole/min.) was passed through an ice-cold solution of 879 mg. of 7-keto-cholest-5-ene in 100 ml. of ethyl acetate–acetic acid (50-50 by volume) for 20 minutes. Hydrogen peroxide (10 ml., 3%) was added to the reaction mixture and the solution allowed to stand overnight at room temperature. The mixture was then warmed on a steam-bath for 30 minutes and the solvents removed under reduced pressure (steam-bath temperature). The residue was dissolved in ether and extracted thoroughly with dilute aqueous-methanolic potassium hydroxide solution. Acidification of the alkaline extract gave white crystals which were filtered, washed with water and dried. The acid was recrystallized from ether–hexane, yield 403 mg. (43.5%), m.p. 180–186°. One further recrystallization from methanol–water yielded material melting from 184–185°. The acid was sublimed in high vacuum before analysis. The specific activity was 13.7 cts./min./mg. $BaCO_3$, 32.3 cts./min./mg. $BaCO_3$ (cor.).[15]

Anal. Calcd. for $C_{26}H_{44}O_3$: C, 77.17; H, 10.96. Found: C, 76.92; H, 10.75.

6-Nor-5,7-seco-7-carboxycholestane.—A mixture of 655 mg. of keto-acid, 1.65 g. of sodium hydroxide, 1.50 ml. of 85% hydrazine hydrate and 15 ml. of diethylene glycol was refluxed for one hour (bath 135°). The condenser was removed and the vapors allowed to escape until an internal temperature of 195 (bath 215°) was reached. After replacing the condenser, the mixture was heated for 5.5 hour and the cooled mixture was diluted with water (150 ml.) and acidified with concentrated hydrochloric acid. By extraction with ether, 652 mg. of a dark yellow oil was isolated. This material was treated with excess diazomethane, the resulting mixture dissolved in hexane and chromatographed on alumina (15 g.). From the hexane eluate, 205 mg. (31.4%) of crystalline ester was isolated, m.p. 75–78°. Recrystallization from methanol gave crystals melting from 80.5–81.0°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.14; H, 11.95. Found: C, 80.48; H, 11.76.

The ester was hydrolyzed by refluxing for 4 hours, under a nitrogen atmosphere, in ethylene glycol containing 5% potassium hydroxide. The white crystalline acid was recrystallized from methanol, m.p. 129.0–130.2°.

Anal. Calcd. for $C_{26}H_{46}O_2$: C, 80.02; H, 11.87. Found: C, 79.70; H, 11.69.

6,7-Bisnor-5,8-seco-8-acetylaminocholestane.—A mixture of 192 mg. of seco-acid, 4 ml. of chloroform and 1.5 ml. of concentrated sulfuric acid was treated with 65 mg. of sodium azide, in portions, while cooling in an ice-bath (stirring). The mixture was stirred at ice-bath temperature for one hour and at room temperature for 17 hours. The reaction flask was swept with nitrogen during all these operations and the carbon dioxide liberated was collected in 3 ml. of carbon dioxide-free 1 *N* sodium hydroxide. The barium carbonate was precipitated by addition of 6 ml. of carbon dioxide-free 1 *N* ammonium chloride followed by 6 ml. of carbon dioxide-free 1 *M* barium chloride, yield 91 mg. (94%), specific activity: 0 cts./min./mg. $BaCO_3$.

The reaction mixture was diluted with ice and the chloroform layer separated, washed successively with water, dilute sodium bicarbonate, water and saturated sodium chloride. The chloroform solution was filtered through alumina, the

(11) A. Windaus, *Ber.*, **53**, 488 (1920); A. Windaus and E. Kirchner, *ibid.*, **53**, 614 (1920).

(12) All analyses were performed by the Microanalytical Laboratory of the Department of Chemistry, University of California. The radioactivity determinations were conducted as previously described (*Anal. Chem.*, **19**, 828 (1947)). All melting points are corrected.

(13) The corrected value has been calculated on the basis that in the original cholesterol there are 15 methyl and 12 carboxyl groups; such a value will give the specific activity of the carbon atoms derived from carboxyl groups after correcting for the dilution due to the methyl derived carbon atoms.

(14) K. Heusler and A. Wettstein, *Helv. Chim. Acta*, **35**, 284 (1952).

(15) The correction factor employed for this compound was 26/11 since the carbon atom lost upon ozonization has been shown to be derived from a carboxyl carbon atom.[7] The agreement of the corrected specific activity further confirms this assumption.

chloroform evaporated and the residual oil allowed to stand 18 hours with 5 ml. of acetic anhydride. The reaction mixture was diluted with water, extracted with chloroform and processed in the usual manner. The product was recrystallized from ethanol–water, yield 128 mg. (65%), m.p. 185–187°, specific activity: 13.2 cts./min./mg. $BaCO_3$, 32.3 cts./min./mg. $BaCO_3$ (cor.).[16]

Anal. Calcd. for $C_{27}H_{49}ON$: C, 80.33; H, 12.23. Found: C, 80.23; H, 12.16.

(16) The correction factor employed was 27/11 allowing for the methyl carbon lost and the two atoms of the acetyl derivative.

CHEMICAL LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

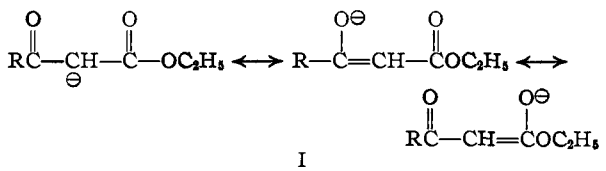
The Infrared Spectra of Enolate Ions

BY MYRON L. BENDER AND JOHN FIGUERAS

RECEIVED JULY 20, 1953

While studying the addition of sodium alkoxide to various esters,[1] the reactions of sodium ethoxide with ethyl acetoacetate, ethyl benzoylacetate and diethyl malonate in ethanol solution were investigated. These reactions did not result in addition to the carbonyl group[1] but rather in the production of enolate ions in all cases. The infrared spectra of the enolate ions exhibited a new band characteristic of the enolate anion as well as the absence of the original ester carbonyl and keto carbonyl absorption bands. These changes were not found in the system, ethyl levulinate and sodium ethoxide, which would not be expected to form an enolate ion. In addition, enolate ions were prepared in ethanol solution from the reaction of sodium ethoxide with acetylacetone and 2-nitropropane.

In the case of the β-keto esters, the enolate ion shows the disappearance of both the keto and ester carbonyl bands which indicates that the resonance hydrid of the enolate ion includes contributors involving the negative charge on the ester oxygen as well as the keto oxygen and the α-carbon as shown in I.



I

(1) M. L. Bender, THIS JOURNAL, **75**, 5986 (1953).

The enolate ion from diethyl malonate was formed only to the extent of 30–35% when equimolar quantities of sodium ethoxide and diethyl malonate were mixed, whereas the β-keto esters formed enolate ions under identical conditions to the extent of 95–100%. These results are in conformity with the relative stabilities of the resulting enolate ions. This is the first instance of the determination of the infrared spectra of enolate anions.[2,3]

Experimental

Materials.—Ethanol and sodium ethoxide in ethanol were prepared as described previously.[1] Ethyl acetoacetate ($n^{20}D$ 1.4198), ethyl benzoylacetate ($n^{20}D$ 1.5290), diethyl malonate ($n^{20}D$ 1.4143), acetylacetone ($n^{20}D$ 1.4511) and 2-nitropropane ($n^{20}D$ 1.3941) were Eastman Kodak Co. products which were fractionated before use.

Infrared Spectra.—Infrared spectra were determined by use of a Perkin-Elmer Model 21 Double Beam Recording Infrared Spectrophotometer. Matched sealed liquid absorption cells approximately 0.1 mm. in thickness were employed. Spectra of all compounds were determined in the region from 1200–2000 cm.$^{-1}$ using approximately 0.1 *M* solutions in absolute ethanol. Sodium ethoxide in ethanol was added to the compounds in equimolar amounts and the spectra of the resulting anions were determined. Table I indicates the absorption bands in the carbonyl region before and after the addition of ethoxide ion.

TABLE I

INFRARED ABSORPTION BANDS IN THE CARBONYL REGION OF SOME COMPOUNDS AND THEIR CORRESPONDING ENOLATE IONS IN ETHANOL

	Original bands (cm.$^{-1}$)[a]				
Compound	Ester C=O	Ketone C=O	Chelated conjugated C=O	Conjugated C=C	Enolate ion bands
Ethyl acetoacetate	1741 (s)[e]	1715 (s)	1650 (w)	1630 (w)[b]	1662 (1649, 1629) (s)
Ethyl benzoylacetate	1739 (m)	1686 (m)[c]			1656 (s)
Diethyl malonate	1740 (s)[d]				1666 (m)
Ethyl levulinate	1726 (s)	1721 (s)			
Acetylacetone		1726 (w)	1615 (s)[b]		1604 (s)
2-Nitropropane	1550 (s) (nitro)				1604 (s)

[a] These values are reliable to ±2 cm.$^{-1}$. [b] Compare assignments of R. S. Rasmussen and R. B. Brattain, THIS JOURNAL, **71**, 1093 (1949); N. J. Leonard, H. S. Gutowsky, W. J. Middleton and E. M. Peterson, *ibid.*, **74**, 4070 (1952). [c] The keto group is conjugated with the benzene ring as in acetophenone. [d] This band is unusually broad (25 cm.$^{-1}$). [e] Relative intensity symbols determined by per cent. transmission as follows: vw, 95–100; w, 90–95, m, 60–90; s, 20–60; vs, 5–20; vvs, 0–5.

(2) C. Duval, R. Freymann and J. Lecomte, *Compt. rend.*, **231**, 272 (1950); *Bull. soc. chim. France*, 106 (1952), reported the infrared spectra of several metal chelates of acetylacetone. They observed no normal carbonyl band and suggested that the metal acetylacetonates exist solely in the enolic form, that the C=O and C=C bands may undergo large shifts because of perturbations and that in general the infrared spectra of the acetylacetonates resemble those of complexes rather than those of salts.

(3) M. Kubota, *J. Chem. Soc. Japan*, **62**, 214 (1941), reported the ultraviolet absorption spectra of the sodium salts of acetylacetone and ethyl acetoacetate in alcohol solution. He observed a shift from the parent compounds toward the longer wave lengths.

DEPARTMENT OF CHEMISTRY
ILLINOIS INSTITUTE OF TECHNOLOGY
CHICAGO, ILLINOIS

Aryloxyketones

BY CHARLES K. BRADSHER, FRANCES C. BROWN AND R. JACK GRANTHAM

RECEIVED AUGUST 17, 1953

In view of the reported fungistatic activity of pentachlorophenoxyethanol[1] and related compounds, it seemed of interest to prepare and test some aryl-

(1) C. W. MacMullen (to Röhm and Haas), U. S. Patent 2,416,263; Felton and McLaughlin, *J. Org. Chem.*, **12**, 298 (1947).