acid (IV) and its lactone by cleavage of the tetrahydrofuran ring with a solution of potassium iodide in 95% phosphoric acid. The crude mixture containing IV and its lactone was heated at reflux for sixteen hours with 2 moles of thiourea and 1.5 moles of hydrobromic acid (34%) and the resultant reaction mixture was hydrolyzed with 0.5 *N* sodium hydroxide at 100° for 15 minutes. The solution was acidified and extracted with chloroform. The chloroform solution was then treated with a slight excess of aqueous potassium iodide–iodine solution, chloroform was removed by distillation and the residue was purified by chromatography on silicic acid to yield a yellow oil, neutralization equivalent = 208. The biological activity of the oil was approximately 20% that of protogen-A for *Tetrahymena geleii* and a species of *Corynebacterium*.[4] Oxidation of the oil with *t*-butyl hydroperoxide yielded a second biologically active compound with properties closely similar to those of protogen-B as measured by paper chromatography, solvent distribution and infrared studies. This compound gave a crystalline S-benzylthiuronium salt, m.p. 143 to 144°, calculated for $C_{16}H_{24}N_2S_3O_3$: C, 49.45; H, 6.23; N, 7.21; S, 24.76; found C, 49.81; H, 6.30; N, 7.31; S, 25.39; C-methyl, negative. The position of the secondary sulfur atom cannot be stated unequivocally, as migration of the hydroxyl group has been shown to occur in aliphatic hydroxy-acids when treated with heat and acid.[5]

(4) E. L. R. Stokstad, *et. al.*, *Proc. Soc. Exp. Biol. Med.*, **74**, 571 (1950).

(5) E. E. Blaine and A. Kohler, *Compt. rend.*, **148**, 1772 (1909).

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THE BIOSYNTHESIS OF SQUALENE AND CHOLESTEROL[1]

Sir:

Squalene from shark liver oil was shown to be a dihydrotriterpene in 1926.[2,3] Although the structure of cholesterol was then only incompletely known, the suggestion was made that squalene might be an intermediate in steroid biosynthesis.[2,4] Balance studies which were carried out gave conflicting results.[5,6] Work carried out with isotopic tracers during recent years has demonstrated that acetate is the principal carbon source of cholesterol.[7,8] The distribution of acetate carbon which was found in the cholesterol molecule, led to the suggestion that cholesterol biosynthesis might proceed via the condensation of isoprenoid units.[9] The data were also compatible with a cyclization of squalene to cholesterol as proposed by Robinson.[10]

It has now been shown that squalene is synthesized biologically from acetate, that squalene is absorbed from the gut, and that carbon from labeled squalene is efficiently incorporated into cholesterol. Rat tissues do not contain detectable quantities of squalene. However, when the hydrocarbon is fed, a small amount can subsequently be recovered from the liver and intestinal tract. Rats received in their diet 0.5 g. of squalene and 0.54 millicurie of 1-C^{14} acetate (0.125 g.) per 100 g. rat per day for two days. The combined nonsaponifiable fractions of the livers and intestinal tracts were chromatographed on alumina and "washed out" with normal cholesterol. This yielded 35 mg. of hydrocarbon, having a specific activity of 2080 c.p.m. A portion was diluted with purified natural squalene[11] and two isomeric hexahydrochlorides,[2] m.p. 108° and 144° were prepared. Corrected for dilution, these derivatives had specific activities of 2120 c.p.m. and 2040 c.p.m., respectively. This demonstrated that all of the radioactivity of the hydrocarbon fraction resided in the squalene. The remainder of this C^{14} squalene was fed to mice at a level of 10 micromoles of squalene per animal per day for two days. Cholesterol and fatty acids were isolated from tissues. Data from one of two identical experiments are shown in Table I.

TABLE I

FEEDING OF C^{14} SQUALENE, 2080 C.P.M.,[a] TO MICE

1. Liver and gut	C^{14}, c.p.m.	% of squalene C recovered	RIC[b]
Cholesterol digitonide	132[c]	4.2	6.4
Cholesterol dibromide	131		
Fatty acids	<2		
2. Carcass and viscera			
Crude steroids	34		2.1
Cholesterol digitonide	43[c]	3.9	
Cholesterol dibromide	42		
Fatty acids	0		
		8.1	

[a] All C^{14} values expressed as c.p.m. of infinitely thick $BaCO_3$ samples. [b] RIC = (c.p.m. of cholesterol/c.p.m. of squalene fed) × 100. [c] Calculated for free cholesterol.

Comparison with earlier results indicates that the utilization of squalene carbon for cholesterol formation is 10–20 times as efficient as that of acetate.[7,8] It is also more than three times as efficient as that of isovaleric acid,[12] until now the most efficient carbon source of cholesterol. The percentage recovery of squalene carbon in cholesterol is based on the total amount fed. Since squalene is not quantitatively absorbed from the gut,[5] this figure (8%) represents a minimal value. The insignificant isotope concentration in the fatty acids precludes the possibility that squalene was

(1) Supported by a grant from the Life Insurance Medical Research Fund.

(2) I. M. Heilbron, E. D. Kamm and W. M. Owens, *J. Chem. Soc.*, 1630 (1926).

(3) I. M. Heilbron, T. P. Hilditch and E. D. Kamm, *ibid.*, 3131 (1926).

(4) H. J. Channon, *Biochem. J.*, **20**, 400 (1926).

(5) H. J. Channon and G. R. Tristram, *ibid.*, **31**, 738 (1937).

(6) T. Kimizuka, *J. Biochem. (Japan)*, **27**, 469 (1938).

(7) K. Bloch and D. Rittenberg, *J. Biol. Chem.*, **145**, 625 (1942).

(8) H. N. Little and K. Bloch, *ibid.*, **183**, 33 (1950).

(9) J. Würsch, R. L. Huang and K. Bloch, *ibid.*, in press.

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

(11) Generously supplied by Dr. Stanley Ames, Distillation Products Company.

(12) I. Zabin and K. Bloch, *J. Biol. Chem.*, **185**, 131 (1950).

converted to cholesterol by way of 2-carbon intermediates.

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THE STRUCTURE OF THE ANTIBIOTIC MYCOMYCIN

Sir:

We have deduced the structure of the highly unstable antibiotic mycomycin[1] as 3,5,7,8-tridecatetraene-10,12-diynoic acid (I).

$$HC{\equiv}C{-}C{\equiv}CH{=}C{=}CHCH{=}CH{-}CH{=}CHCH_2CO_2H$$

Mycomycin undergoes an unusual rearrangement in normal aqueous potassium hydroxide at 27° involving an allene to acetylene isomerization accompanied by migration of existing acetylenic bonds. The rearranged acid, isomycomycin, has been assigned the structure 3,5-tridecadiene-7,9,11-triynoic acid (II).

$$CH_3{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2{-}CO_2H$$

I crystallizes in colorless needles from methylene chloride at −40°; m.p. 75° (dec. explosively) and $[\alpha]^{25}D$ −130° (*c*, 0.4, ethanol). (*Anal.* Calcd. for $C_{13}H_{10}O_2$: C, 78.76; H, 5.08; one C-methyl, 7.6; neut. equiv., 198; Found: C, 78.17; H, 5.36; C-methyl (Kuhn–Roth), 0.48; neut. equiv., 200). Ultraviolet absorption in diethyl ether: $\lambda^{m\mu}_{infl.}$ 256, ϵ 35,000; $\lambda^{m\mu}_{max.}$ 267, ϵ 61,000; $\lambda^{m\mu}_{max.}$ 281, ϵ 67,000.

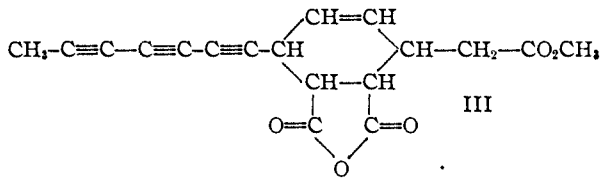
II crystallizes in colorless needles from ether–hexane, decomposes slowly above 140° and is optically inactive. (*Anal.* Found: C, 78.87; H, 5.43; C-methyl (Kuhn–Roth), 9.6; neut. equiv., 198). Ultraviolet absorption in diethyl ether: $\lambda^{m\mu}_{infl.}$ 246, ϵ 24,000; $\lambda^{m\mu}_{max.}$ 257.5, ϵ 58,000; $\lambda^{m\mu}_{max.}$ 267, ϵ 110,000; $\lambda^{m\mu}_{max.}$ 287.5, ϵ 14,000; $\lambda^{m\mu}_{max.}$ 305.5, ϵ 27,000; $\lambda^{m\mu}_{max.}$ 324, ϵ 41,000; $\lambda^{m\mu}_{max.}$ 347, ϵ 34,000.

Complete hydrogenation of I and II requires eight moles of hydrogen, quantitatively yielding *n*-tridecanoic acid, unequivocally identified by comparisons with an authentic sample.[2]

The infrared spectrum of I in dioxane has characteristic bands near 3180, 2200, 1930 and 1730 cm.$^{-1}$ attributed to ≡C—H, disubstituted —C≡C—, —CH=C=CH— and unconjugated —CO_2H[2a] functions, respectively. I reacts with acetylenic hydrogen reagents such as alcoholic silver nitrate. In view of the linear nature of the reduction product, the high order of optical activity of I can only be reconciled with its allenic function. The fine structure spacing ($\Delta\nu'$, 1900 cm.$^{-1}$) of the ultraviolet absorption maxima of I gives evidence that the two recognized acetylenic bonds are in conjugation.[3] The remaining two units of unsaturation are believed to be a conjugated diene, conjugated with the allenic group but not with the carboxyl group.

The location of the observed long wave length ultraviolet absorption maximum of I at 281 mμ limits the length of contributing conjugated multiple bonds to three units, part of which may be acetylenic.[4] The allene bond in the proposed 7,8-position of I performs a unique chromophoric role whereby the Δ^7 bond and the Δ^8 bond are conjugated with the 3,5-diene and 10,12-diyne, respectively, while the central carbon atom of the allene serves as an electronic insulator between the two resulting conjugated systems.[5] The two effectively isolated chromophores, each totaling three units of conjugation, explain the observed general light absorption zone of I.

The infrared spectrum of II in dioxane exhibits characteristic bands at 2200 cm.$^{-1}$ and 1730 cm.$^{-1}$ attributed to disubstituted —C≡C— and unconjugated —CO_2H,[2a] respectively. Monosubstituted acetylenic and allenic bands are absent. II analyzes for one C-methyl group, whereas I possesses none. II does not react with alcoholic silver nitrate, substantiating the absence of a ≡C—H function. The ultraviolet light absorption properties of II prove to be very similar to those recorded for compounds containing a conjugated dienetriyne grouping.[6,7] II reacts with ethereal diazomethane to form a methyl ester, crystallized from ether–hexane as colorless needles, m.p. 69–70°. A Diels–Alder reaction of the methyl ester of II with maleic anhydride gives a monoaddition product (III), crystallized from acetone–hexane as colorless plates, m.p. 177–178° (dec.). (*Anal.* Calcd. for $C_{18}H_{14}O_5$: C, 69.67; H, 4.55; CH_3O, 10.00. Found: C, 69.59; H, 4.64; CH_3O, 10.89.) Ultraviolet light absorption in methanol: $\lambda^{m\mu}_{infl.}$ 215, ϵ 82,000; $\lambda^{m\mu}_{max.}$ 272.5, ϵ 450; $\lambda^{m\mu}_{max.}$ 289, ϵ 430; $\lambda^{m\mu}_{max.}$ 310, ϵ 170. These light absorption properties of III



III

bear striking resemblance to the unique light absorption behavior of the conjugated triacetylene grouping when it is the sole contributing chromo-

(1) E. A. Johnson and K. L. Burdon, *J. Bact.*, **54**, 281 (1947).

(2) Obtained through the courtesy of Dr. H. J. Harwood of Armour and Company.

(2a) In a comparison of infrared spectra determined in dioxane solution, the location of the C=O stretching frequency exhibited by I and II near 1730 cm.$^{-1}$ is the same as the corresponding band of their respective perhydro derivatives and authentic *n*-tridecanoic acid. If I and/or II contained a conjugated carboxyl group, this band would be expected to occur at a measurably lower frequency than the corresponding saturated derivative.

(3) T. Brunn, C. J. Haug and N. A. Sorensen, *Acta Chem. Scand.*, **4**, 850 (1950).

(4) I. M. Heilbron, E. R. H. Jones and R. A. Raphael, *J. Chem. Soc.*, 268 (1943); I. M. Heilbron, E. R. H. Jones and F. Sondheimer, *ibid.*, 1586 (1947).

(5) In a somewhat analogous situation, the ultraviolet light absorption of tetraphenylallene ($\lambda^{m\mu}_{max.}$ 267, ϵ 12,000) corresponds to that of 1,1-diphenylethylene ($\lambda^{m\mu}_{max.}$ 250, ϵ 11,000) and not to *trans,trans*-diphenyl-1,3-butadiene ($\lambda^{m\mu}_{max.}$ 328, ϵ 56,000).

(6) K. Stavholt and N. A. Sorensen, *Acta Chem. Scand.*, **4**, 1567 (1950).

(7) E. R. H. Jones, M. C. Whiting, J. B. Armitage, C. L. Cook and N. Entwistle, *Nature*, **168**, 900 (1951).