51. *Studies in Relation to Biosynthesis. Part XXV.* A Preliminary Study of the Antimycin-A Complex.*

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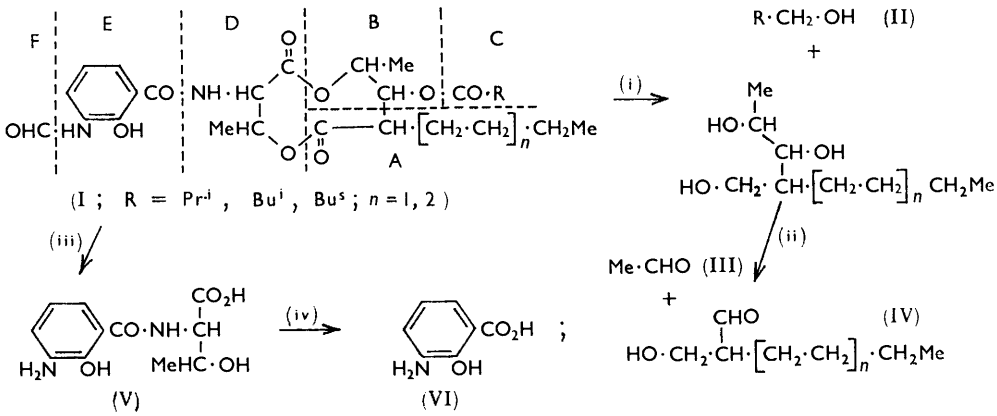
A preliminary study has been made of the incorporation of [1-^{14}C]acetic acid and [2-^{14}C]pyruvic acid into the antimycin-A complex produced by *Streptomyces kitazawaensis*. The significance of the results is discussed, and the probable relationship of the biosynthetic routes to the branched aliphatic portion of this antibiotic and to the mould tetronic acids is indicated.

The structure (I) has been established [1,2] for the antibiotic complex antimycin-A, a metabolite of certain *Streptomyces* species. In the light of present knowledge of biosynthesis the threonine (D) and the C_1-unit (F) in this structure are evident. The branched-chain substituents in (C) are presumably related in origin to the amino-acids valine, leucine, and isoleucine, and the straight-chain fragment (A) to the fatty acids, especially in view of the mixture of C_4- and C_6-chains present, first proved by van Tamelen.[3] The two units of major biosynthetic interest are the aminosalicylic acid ring (E) and the branched-chain portion (A—B), and we present here some studies bearing mainly on the latter.

The work has been hampered by low production of antimycin-A by the *S. kitazawaensis* strain, and by extremely low incorporations of [^{14}C]-labelled precursors from the complex medium used. Owing to the low activities involved, and moreover to the mixed nature of the antibiotic complex and some of the degradation products, the results are not as quantitatively significant as we would desire. However, a general picture does emerge.



(i) $LiAlH_4$ reduction. (ii) $NaIO_4$ oxidation. (iii) Mild NaOH hydrolysis. (iv) Vigorous NaOH hydrolysis.

The Table gives the pattern of incorporation of [1-^{14}C]acetic acid and [2-^{14}C]pyruvic acid (incorporation 0·002% and 0·02%, respectively) into antimycin-A, which was degraded by the reactions shown in the chart. A fermentation was also carried out in the presence of [^{14}C]formic acid, but incorporation into the antibiotic was negligible. Where substituent variation occurs, mean molecular weights are calculated on the basis of a mixture of three parts of antimycin-A_1 (I; R = Bu^i; $n = 2$) to one part of antimycin-A_3 (I;

* Part XXIV, *J.*, 1961, 3128.

[1] Birch, Cameron, Harada, and Rickards, *Proc. Chem. Soc.*, 1960, 22; Birch, Cameron, Harada, and Rickards, *J.*, 1961, 889.

[2] van Tamelen, Dickie, Loomans, Dewey, and Strong, *J. Amer. Chem. Soc.*, 1960, **82**, 1513; Strong, Dickie, Loomans, van Tamelen, and Dewey, *J. Amer. Chem. Soc.*, 1961, **83**, 1639.

[3] van Tamelen, personal communication.

R = Bu^{i}; $n = 1$).[4] All samples were assayed as the crystalline derivatives already described.[1]

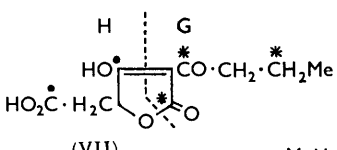
Fragment A.—Inspection of the formulæ of the components of the antimycin-A complex indicates an origin from C_2-units through the acetate–malonate pathway.[5] Incorporation of [1-^{14}C]acetic acid measured in the aldol mixture (IV), is significant although small. Lack of material prevented further degradation. The virtual non-incorporation of [2-^{14}C]-pyruvic acid is somewhat surprising in view of its expected biological conversion into [1-^{14}C]acetyl-coenzyme A, but this is probably due to the particular fermentation conditions.

	[1-^{14}C]Acetic acid		[2-^{14}C]Pyruvic acid	
Compound	r.m.a.*	r.m.a. (% of antimycin)	r.m.a.*	r.m.a. (% of antimycin)
Antimycin-A (I)	15,960	100	23,450	100
Alcohols, R·CH$_2$·OH (II)	12,550	78·6	16,500	70·5
Acetaldehyde (III)	200	1·2	3630	15·5
Acetaldehyde (Me)	—	—	0	0
Acetaldehyde (C=O)	—	—	2560	10·9
Aldols (IV)	2610	16·4	230	1·0
Antimycic acid (V)	2160	13·5	3175	13·5
Aminosalicylic acid (VI)	0	0	1715	7·3
Threonine †	2160	13·5	1460	6·2

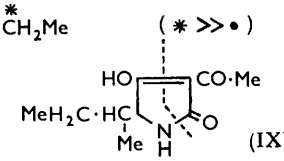
* Relative molar activity (cf. Birch, Massy-Westropp, Rickards, and Smith, *J.*, 1958, 360).
† Estimated by difference.

Fragment B.—The relatively high incorporation of [2-^{14}C]pyruvic acid compared with that of acetic acid into this fragment as measured in the acetaldehyde (III), indicates that the former is utilised as a unit. In support, degradation of the acetaldehyde, by Kuhn–Roth oxidation of its 2,4-dinitrophenylhydrazone and Schmidt degradation of the resulting lithium acetate, showed the methyl group to be completely inactive and most of the activity to be in the carbonyl group. [The discrepancy (30%) between the observed activity of the acetaldehyde (III) and that of the barium carbonate representing its carbonyl-carbon atom is attributed to accidental contamination by aerial carbon dioxide.]

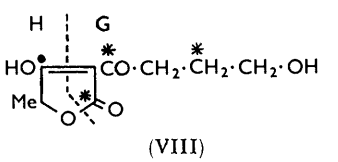
The closest structural analogues of this branched (A–B) section of antimycin-A which have been investigated biochemically are the mould tetronic acids.[6] Tracer studies[7] of the formation in *Penicillium charlesii* of carlosic and carolic acid (VII and VIII, respectively, in which the labelling pattern from [1-^{14}C]acetate is shown) show that portion (G) of these structures arises directly from acetic acid, while (H) is less heavily labelled and probably originates in an intermediate of the citric acid cycle, such as malic or oxaloacetic acid, with a decarboxylation stage in the case of (VIII). Similarly, biosynthesis of tenuazonic acid (IX) in *Alternaria tenuis* involves acetic acid and L-isoleucine.[8]



(VII)



(IX)



(VIII)

The present results indicate a close biosynthetic relation between the tetronic acids, formed in general by condensation of an acetate-derived β-keto-acid (which, moreover,

[4] Harada, Usu, and Asai, *J. Antibiotics* (*Japan*), A, 1958, **11**, 32; Liu and Strong, *J. Amer. Chem. Soc.*, 1959, **81**, 4387.
[5] Cf. Stumpf, *Ann. Rev. Biochem.*, 1960, **29**, 282.
[6] Haynes and Plimmer, *Quart. Rev.*, 1960, **14**, 292; Birkinshaw and Samant, *Biochem. J.*, 1960, **74**, 369.
[7] Lybing and Reio, *Acta Chem. Scand.*, 1958, **12**, 1575.
[8] Stickings and Townsend, *Biochem. J.*, 1960, **74**, 36P.

varies in chain length from C_2 to C_8) with an α-hydroxy-, α-keto-, or α-amino-acid, and the (A–B) fragment of the antimycin-A complex. The α-substituted acid involved here could be pyruvic acid itself, its reduction product lactic acid, or its carboxylation product oxaloacetic acid with subsequent decarboxylation.

Fragment C.—Most of the activity in the antimycin-A preparations derived from both [1-^{14}C]acetic acid and [2-^{14}C]pyruvic acid is found in the side-chain ester mixture (C) (assayed as R·CH_2·OH 3,5-dinitrobenzoate). Acetate and pyruvate are known to be precursors of the amino-acids valine, leucine, and isoleucine,[9] and the antimycin esters are probably derived from these amino-acids or from their immediate precursors, the corresponding α-keto-acids.[10] Fractional crystallisation of the mixed 3,5-dinitrobenozates obtained from the [2-^{14}C]pyruvate experiment indicated that the major component, the 3-methylbutanol derivative, is considerably less radioactive than the minor components, 2-methylbutanol and 2-methylpropanol.

Fragment D.—The threonine moiety was labelled both by [1-^{14}C]acetic acid and [2-^{14}C]pyruvic acid, in accord with well-established biogenetic pathways involving oxaloacetate, aspartate, and homoserine.[11]

Fragment E.—This aminosalicylic acid ring, unusual among natural products, is clearly not derived from acetic acid [cf. Table 1, compound (VI)], but may arise by the shikimic acid route to aromatic rings, which is known [12] to involve pyruvic acid.

Experimental

Labelled antimycin-A was degraded by our published methods.[1] Radioactivity was assayed as described by Birch *et al.*[13]

[^{14}C]*Antimycin-A.—Streptomyces hitazawaensis* Harada and Tanaka was cultured on a medium containing starch, vegetable protein, soyabean extracts, and inorganic salts. After 3½ days' growth, sodium [^{14}C]formate (300 μc), sodium [1-^{14}C]acetate (500 μc), or sodium [2-^{14}C]pyruvate (100 μc) in water (20 ml.) was added to the culture (1 l.). Cultures were harvested after 5 days' growth, the yield of antimycin-A being ~15 mg./l. To inactive antimycin-A in acetone (minimum vol.) was added a concentrated light petroleum (b. p. 60—80°) extract of the filtered culture medium. The crystalline product was collected after refrigeration, and this carrier extraction repeated twice. The combined material (500 mg.) was recrystallised from acetone–light petroleum (b. p. 60—80°) to constant activity.

Degradation of Acetaldehyde 2,4-Dinitrophenylhydrazone.—The derivative (38 mg.) was subjected to Kuhn–Roth oxidation. The resulting acetic acid (82% yield) was degraded by the Schmidt procedure [14] to carbon dioxide and methylamine, which were assayed as barium carbonate and *N*-methyl-2,4-dinitroaniline, respectively.

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Department of Chemistry, University of Manchester.
Kyowa Fermentation Industry Co., Tokyo, Japan. [*Received, July 17th,* 1961.]

[9] Kamin and Handler, *Ann. Rev. Biochem.*, 1957, **26**, 451; Knox and Behrman, *ibid.*, 1959, **28**, 245.
[10] Meister, "Biochemistry of the Amino Acids," Academic Press, New York, 1957, p. 296.
[11] Ref. 10, p. 279.
[12] Davis, *Arch. Biochem. Biophys.*, 1958, **78**, 497, and references therein.
[13] Birch, Massy-Westropp, Rickards, and Smith, *J.*, 1958, 360.
[14] Phares, *Arch. Biochem. Biophys.*, 1951, **33**, 173.