187. *The Structure of the Antimycin-A Complex.*

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On the basis of degradative evidence, structure (I) is proposed for the antibiotic complex antimycin-A, produced by *Streptomyces kitazawaensis*. The individual components of the complex differ in the nature of the alkyl (R = n-C_4H_9, n-C_6H_{13}) and acyl (R′ = CHMeEt, CH_2·$CHMe_2$, $CHMe_2$) substituents present. A preliminary account [1] of this work has been published.

Antimycin-A, the antibiotic responsible for the antifungal activity of certain *Streptomyces* species, was first isolated crystalline in 1949 from an unidentified *Streptomycete*.[2] Although apparently of no medical importance the substance has proved to have considerable biochemical use in connection with electron-transport processes because of its specific inhibitory action on certain of the enzymes involved.[3,4] Analytical figures and melting points of the antibiotic varied with the source and the method of purification, and the suggestion that antimycin-A was a mixture of chemically closely related substances was verified by paper-chromatographic resolution into at least four components.[4] More recently,[5] counter-current distribution has separated at least three definite components: the major constituent, antimycin-A_1, $C_{28}H_{40}O_9N_2$, the isomeric antimycin-A_2, and antimycin-A_3, $C_{26}H_{36}O_9N_2$. Blastmycin, $C_{26}H_{36}O_9N_2$, isolated by Japanese workers from *S. blastmyceticus*,[6] resembled the antimycins in its chemical properties, and more specifically antimycin-A_3 in its physical properties.[7]

In a preliminary communication [1] we advanced the structure (I; R = n-C_4H_9, n-C_6H_{13}; R′ = CHMeEt, CH_2·$CHMe_2$, or $CHMe_2$) for the antimycin-A complex. We here describe the evidence leading to this formula and confirming the identity of blastmycin with antimycin-A_3. Strong, van Tamelen, and their co-workers [8] have independently presented evidence supporting structure (I).

Very mild treatment of antimycin-A with aqueous alkali at room temperature yielded [9,10] a neutral fragment $C_{16}H_{28}O_4$, formic acid, and antimycic acid, $C_{11}H_{14}O_5N_2$, for which the proposed structure [11] (II) was confirmed by synthesis.[12] Since the antibiotic contained a free phenolic hydroxyl group and was non-basic, the presence of an *N*-formyl group was inferred.[9]

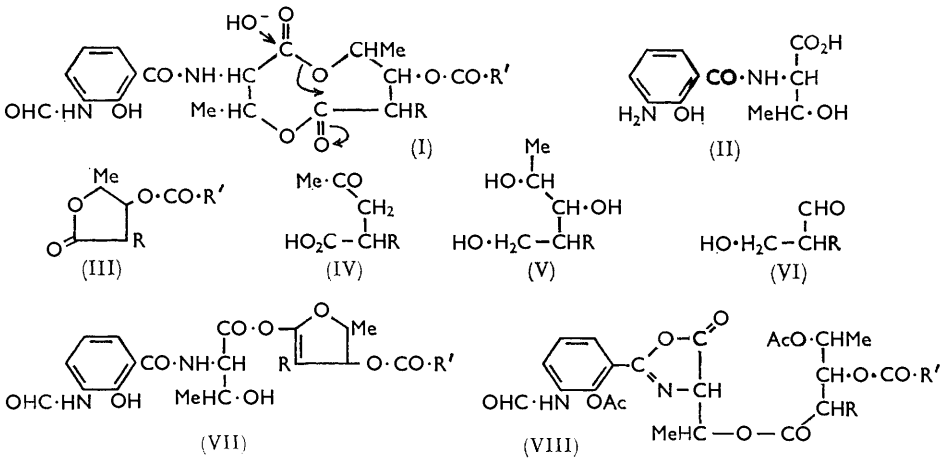
We examined this question by reduction of antimycin-A with a quantity of lithium aluminium hydride insufficient for complete reduction of the carbonyl functions present [3 mol.; structure (I) requires 3·25 mol.], wishing to reduce selectively the ester and formanilide groupings in preference to the salicylamide, which could then be hydrolysed to yield the methylaminosalicylic acid. There is some evidence [13] that esters can be selectively reduced in the presence of amides, but the preferential reduction of different amides themselves with this reagent has not been described. Alkaline hydrolysis of the basic reduction product gave in low yield 3-methylaminosalicylic acid, identical with an authentic sample, verifying the presence of the formanilide grouping in the antibiotic. While this

[1] Birch, Cameron, Harada, and Rickards, *Proc. Chem. Soc.*, 1960, 22.
[2] Dunshee, Leben, Keitt, and Strong, *J. Amer. Chem. Soc.*, 1949, **71**, 2436.
[3] Lockwood, Leben, and Keitt, *Phytopath.*, 1954, **44**, 438.
[4] Strong, "Topics in Microbial Chemistry," Squibb Lectures on Chemistry of Microbial Products, Wiley, New York, 1958, Vol. I, p. 1.
[5] Liu and Strong, *J. Amer. Chem. Soc.*, 1959, **81**, 4387.
[6] Watanabe, Tanaka, Fukuhara, Miyairi, Yonehara, and Umezawa, *J. Antibiotics, A*, 1957, **10**, 39.
[7] Yonehara and Takeuchi, *J. Antibiotics*, A, 1958, **11**, 254.
[8] Strong, Dickie, Loomans, van Tamelen, and Dewey, *J. Amer. Chem. Soc.*, 1960, **82**, 1513.
[9] Tener, Bumpus, Dunshee, and Strong, *J. Amer. Chem. Soc.*, 1953, **75**, 1100.
[10] van Tamelen, Strong, and Quarck, *J. Amer. Chem. Soc.*, 1959, **81**, 750.
[11] Tener, van Tamelen, and Strong, *J. Amer. Chem. Soc.*, 1953, **75**, 3623.
[12] Okumura, Masumura, and Horie, *J. Amer. Chem. Soc.*, 1959, **81**, 5215.
[13] Gaylord, "Reduction with Complex Metal Hydrides," Interscience Publ. Inc., New York, 1956, p. 556.

work was in progress, the isolation of blastmycic acid (*ar-N*-formylantimycic acid) from blastmycin [7] and also from antimycin-A [14] was reported, providing further confirmation.

3-Methylaminosalicylic acid was synthesised by methylation of 3-aminosalicylic acid with alkaline dimethyl sulphate, and isolated by chromatography as its methyl ester. No *O*-alkyl or dialkyl product was formed in this reaction, in accord with similar observations [15] on the methylation of 4-aminosalicylic acid. The required 3-aminosalicylic acid was prepared by a new route. In order to avoid the difficulty of separating the desired 3-nitro- from the more abundant 5-nitro-salicylic acid produced on nitration, salicylic acid was first brominated,[16] yielding almost entirely the 5-bromo-derivative. Nitration [17] then gave in good yield 5-bromo-3-nitrosalicylic acid, which was simultaneously reduced and debrominated with Raney alloy in alkali [18] to yield 3-aminosalicylic acid.

The neutral fragment formed on mild alkaline hydrolysis of antimycin-A showed infrared absorption (in CS_2) at 1788 and 1748 cm.$^{-1}$ compatible with the β-acyloxy-γ-lactone structure (as in III) proposed by van Tamelen, Strong, and Quarck [10] on the basis of reactions of model compounds and further alkaline hydrolysis to a mixture of acids whose major components were (+)-α-methylbutyric acid and an unidentified keto-acid, $C_{11}H_{20}O_3$. Blastmycinone, the corresponding degradation product of blastmycin which consists essentially of antimycin-A_3, was formulated by Yonehara and Takeuchi [7] as (III; R = n-C_4H_9, R′ = CH_2·$CHMe_2$) on degradative evidence, while, after completion of the present work, the neutral fragment from purified antimycin-A_1 was shown [14] to differ only in having R = n-C_6H_{13}. Vapour-phase chromatography of the neutral fragment resolved at least two components, confirming the earlier evidence of inhomogeneity [9] and indicating that the structural variations responsible for the different components of the antimycin-A complex resided in this portion of the molecule. Vigorous alkaline hydrolysis of the lactonic ester mixture (III) afforded a keto-acid fraction (IV), purified through the



semicarbazone and resolvable by paper chromatography of the ammonium salts [19] or vapour-phase chromatography of the methyl esters into two components present in comparable amounts, together with volatile acids identified by paper chromatography as branched-chain C_5 acids containing a trace of isobutyric acid.

[14] Liu, van Tamelen, and Strong, *J. Amer. Chem. Soc.*, 1960, **82**, 1652.
[15] Rosdahl, Swed. P. 133,598; *Chem. Abs.*, 1952, **46**, 10,204.
[16] Hewitt, Kenner, and Silk, *J.*, 1904, **85**, 1228.
[17] Lillman and Grothmann, *Ber.*, 1884, **17**, 2729.
[18] Cf. Schwenk, Papa, Whitman, and Ginsberg, *J. Org. Chem.*, 1944, **9**, 1.
[19] Reid and Lederer, *Biochem. J.*, 1951, **50**, 60.

Reduction, with lithium aluminium hydride, of antimycin-A or the neutral hydrolysis fragment gave the triol mixture (V), together with a volatile isopentyl alcohol fraction (discussed below). Oxidation of the triol mixture with unbuffered periodate yielded acetaldehyde as the only volatile aldehyde, and the aldol mixture (VI). While the aldol readily formed a 2,4-dinitrophenylhydrazone, its infrared spectrum ($\nu_{max.}$ in CS_2 3570, 3370, 1714 cm.$^{-1}$) showed only weak carbonyl absorption in accord with its expected existence [20] in the dimeric 1,3-dioxan form. Extended oxidation of the triol (V) with periodate in the presence of potassium hydrogen carbonate afforded in high yield a mixture of acids, shown by paper and vapour-phase chromatography to consist chiefly of n-valeric and n-heptanoic acid, together with small quantities of acetic and branched-chain C_5 acids arising from oxidation of residual acetaldehyde and isopentyl alcohols contaminating the triol.* While the oxidation of cyclic 1,3-diketones and aliphatic dicarbonyl compounds of the type R·CO·CHR'·COR'', where R = OH or H, by periodate is well known,[21] no appreciable reaction has previously been observed with aldols.[22] These results confirm the β-acyloxy-γ-lactone structure (as in III) for the neutral fragment from hydrolysis of antimycin-A, and define the variation in alkyl substitution (R = n-C_4H_9, n-C_6H_{13}) present in antimycin-A (I) and its degradation products (III—VI).

Saponification of the lactone (III) from the antimycin-A complex had previously yielded (+)-α-methylbutyric acid, small discrepancies in physical properties of the acid being attributed to partial racemisation during hydrolysis.[9] Similar degradation of blastmycin afforded β-methylbutyric acid,[7] later isolated also from purified antimycin-A_1,[14] the major component of the antibiotic complex. In our hands the *p*-phenylphenacyl ester of the volatile acid obtained on saponification of the lactone (III) appeared to be a mixture. The volatile alcohol fraction, from lithium aluminium hydride reduction of antimycin (I) or the lactone (III) could not be resolved on vapour-phase chromatography but corresponded to isopentyl alcohol. The alcohols yielded an inhomogeneous 3,5-dinitrobenzoate, m. p. 53—61°, $[\alpha]_D$ +1·5°. White and Ratchford [23] report m. p. 83—84°, $[\alpha]_D$ +4·9° for the derivative of L-2-methylbutanol; the racemic derivative [24] had m. p. 70—70·5°; isopentyl 3,5-dinitrobenzoate had m. p. 63·7—64·5°. The volatile alcohol mixture is therefore either partially racemised *L*-2-methylbutanol or a mixture of this alcohol with 3-methylbutanol in the ratio 3 : 7 (calc. from optical rotations). We prefer the latter hypothesis since the infrared spectrum of the alcohols (R'·CH_2·OH) ($\nu_{max.}$ as liquid film 1388, 1373 cm.$^{-1}$) indicates the presence of a large proportion of *gem*-dimethyl component and is almost identical with that of commercial " isoamyl alcohol," $[\alpha]_D$ −1·0°. These results, together with the detection of a trace of isobutyric acid on hydrolysis (mentioned above), define the variation in the acyloxy-substituents (R' = CHMeEt, CH_2·$CHMe_2$, $CHMe_2$) in the lactone (III) and antimycin-A (I). Other natural products are known in which a similar mixture of side chains occurs.[25]

The various components of the antibiotic complex thus differ in the nature of the substituents R and R' in the hydrolysis product (III), and it remains to determine the mode of linkage in antimycin-A between (III) and the other primary cleavage product (II). The infrared spectrum of antimycin-A has absorption maxima (in CCl_4) at 3435 (NH), 1752 (ester), 1712 (*N*-formyl; cf. methyl 3-formamidosalicylate which has amide absorption at 1708 cm.$^{-1}$ in CS_2), 1646 (H-bonded aromatic amide), and 1518 cm.$^{-1}$ (amide), indicating

* Before we had completed work on this oxidation Dr. van Tamelen informed us that his independent work demonstrated the presence of an n-C_6H_{13} chain in antimycin-A_1.

[20] Owen, *Ann. Reports*, 1944, **41**, 139.
[21] Wolfrom and Bobbitt, *J. Amer. Chem. Soc.*, 1956, **78**, 2489.
[22] Cf. Bose, Foster, and Stephens, *J.*, 1959, 3314.
[23] White and Ratchford, *J. Amer. Chem. Soc.*, 1949, **71**, 1136; cf. Crombie and Harper, *J.*, 1950, 2685.
[24] Reichstein, *Helv. Chim. Acta*, 1926, **9**, 799.
[25] Birch, " Progress in the Chemistry of Organic Natural Products," Springer, Vienna, 1957, Vol. XIV, p. 200.

the absence of a γ-lactone structure. The relative stability of the antibiotic in acidic conditions [4] renders unlikely the presence of ketal functions, or enolic linkages as have been proposed by American and Japanese workers in structures (as in VII) for antimycin-A [4] and blastmycin [7] respectively. Such formulæ were advanced to account for the lack of acidic functions other than a phenolic hydroxyl group, and for the extreme ease of formation of the γ-lactonic ester which is directly extractable from the alkaline hydrolysis medium without prior acidification. We propose the nine-membered dilactone ring structure (I; R = n-C_4H_9, n-C_6H_{13}; R′ = CHMeEt, CH_2·$CHMe_2$, $CHMe_2$). In the presence of base, transannular displacement occurs as indicated, assisted by compression in the medium ring [26] and juxtaposition of the appropriate oxygen and carbonyl functions. Similar nitrogen–carbonyl interaction has been observed,[27] and it is at a maximum in a nine-membered ring.

Japanese workers [7] treated blastmycin, now formulated as (I; R = n-C_4H_9, R′ = CH_2·$CHMe_2$), with acetic anhydride and pyridine to yield a compound $C_{30}H_{40}O_{11}N_2$, described on the basis of their structure (VII; R = n-C_4H_9; R′ = CH_2·$CHMe_2$) as *OO*-diacetylblastmycin although conclusive proof of the degree of acetylation was lacking. Since structure (I) does not admit an *OO*-diacetyl derivative, we examined the reaction of the antimycin-A complex with [*carbonyl*-^{14}C]acetic anhydride, finding that the product contained radioactivity corresponding to two acetyl residues. This radio-tracer approach enabled accurate acetyl determinations to be made in a case where conventional methods, such as saponification to volatile acids and *C*-methyl determination, were unreliable in view of the numerous interfering structures present. Mild alkaline hydrolysis of the diacetyl derivative afforded the lactonic ester (III) in high yield, excluding an oxazolone-type structure (VIII) which might result from rupture of the dilactone ring during acetylation (cf. the similar behaviour of some α-acylamino-esters [28]). The diacetyl compound showed infrared maxima (in CCl_4) at 3430 (NH), 1782 (phenolic OAc), 1752, 1734 (ester), 1714 (*N*-Ac) and 1677 cm.$^{-1}$ (aromatic amide), and ultraviolet absorption ($\lambda_{max.}$ 271 mμ; log ε 3·02) resembling that of benzamide [29] ($\lambda_{max.}$ 265; log ε 2·7); and on the basis of these spectra and reactions of model compounds this product is formulated as *ON*-diacetyldeformylantimycin-A, in which the *N*-formyl group of the antibiotic has been displaced by acetyl.

The lability of such *N*-formyl groups is well known. Nef [30] observed almost quantitative formation of acetanilide from formanilide and acetic anhydride, but his conditions were rather more vigorous than those used in the present work, where only unchanged formanilide was recovered. The presence of an *o*-hydroxyl group facilitates the displacement. *o*-Formamidophenol, a closer model for antimycin-A, yielded *o*-acetamidophenyl acetate together with a trace of *o*-acetamidophenol (resulting possibly from hydrolysis of the diacetyl compound during crystallisation from water); the *O*-methyl ether of antimycin-A is reported [8] unchanged on attempted acetylation. Replacement and migration of acyl substituents in *o*-aminophenols is extensive and complex.[31] In the case of antimycin-A the reaction does not appear to proceed through an *N*-acetyl-*N*-formyl stage which undergoes hydrolysis on crystallisation, since the same product is obtained under anhydrous conditions.

Experimental

Ultraviolet spectra were measured for EtOH solutions. Light petroleum refers to the fraction of b. p. 60—80°. Paper chromatograms were run on Whatman No. 1 paper. Radioactivity was assayed as described by Birch *et al.*[32]

[26] Cf. Prelog, in " Perspectives in Organic Chemistry," Interscience Publ. Inc., London, 1956, p. 96.
[27] Leonard, Fox, Oki, and Chiavarelli, *J. Amer. Chem. Soc.*, 1954, **76**, 630.
[28] Cornforth, in " Heterocyclic Compounds," ed. Elderfield, Wiley, New York, 1957, Vol. V, p. 340.
[29] Ley and Specker, *Ber.*, 1939, **72**, 199.
[30] Nef, *Annalen*, 1892, **270**, 278.
[31] Bell, *J.*, 1931, 2962.
[32] Birch, Massy-Westropp, Rickards, and Smith, *J.*, 1958, 360.

Antimycin-A.—The antibiotic complex, produced by a strain of *Streptomyces kitazawaensis*, had m. p. 143°, $\lambda_{max.}$ 229, 254 (infl.), 319 mμ (log ε 4·51, 4·02, 3·78), $\nu_{max.}$ (in CCl_4) 3435, 1752, 1712, 1646, 1518 cm.$^{-1}$.

Action of Alkali on Antimycin-A.—Hydrolysis [9] of antimycin-A (1·00 g.) with aqueous sodium hydroxide gave antimycic acid (170 mg.), m. p. 219—220° (decomp.) (from ethanol), $\nu_{max.}$ (in Nujol) 3348, 3220, 2590, 1659, 1628, 1599, 1578, 1539 cm.$^{-1}$, and the lactonic ester mixture (III) (450 mg.), a colourless oil, $\nu_{max.}$ (in CS_2) 1789, 1748 cm.$^{-1}$. Vapour-phase chromatography of the latter on a polyester column indicated the presence of at least two components.

Reduction of Antimycin-A by Lithium Aluminium Hydride.—Antimycin-A (1·0 g., 1·85 mmoles) in tetrahydrofuran (25 ml.) was added rapidly to a stirred, ice-cold suspension of lithium aluminium hydride (210 mg., 5·54 mmoles) in tetrahydrofuran (25 ml.). After 30 min. the mixture was brought to room temperature, kept there for 30 min., then refluxed briefly. The cooled mixture was acidified with 2N-hydrochloric acid and extracted with chloroform. The extracts, after being washed with aqueous potassium hydroxide, yielded a colourless oil (380 mg.) [still showing some carbonyl absorption ($\nu_{max.}$ in CCl_4 1769 cm.$^{-1}$)] which on further reduction by lithium aluminium hydride gave the triol (V).

The acidic aqueous liquors were neutralised, and the solution and precipitated inorganic solid were extracted separately with chloroform. The recovered oil (80 mg.), in saturated aqueous sodium hydroxide (3 ml.), was refluxed for 2 hr. under nitrogen. The solution was adjusted to pH 8—9 and washed with chloroform, then acidified (pH 3—4) and extracted with ether to yield 3-methylaminosalicylic acid (20 mg.), sublimed at 180°/10^{-2} mm., m. p. and mixed m. p. 209° (decomp.). Paper chromatography,[33] as the free acid, R_F 0·41 in methanol–pentyl alcohol–benzene–water (35 : 17·5 : 35 : 12·5), and as the ammonium salt, R_F 0·66 in methanol–pentyl alcohol–benzene–4% ammonia (35 : 17·5 : 35 : 12·5), confirmed the identity, spots being detected with ferric chloride or bisdiazotised benzidine reagents.

Brief treatment of the acid (13 mg.) with diazomethane in ether gave the ester (9 mg.) which, purified by chromatography on "Florisil" and crystallisation from light petroleum, had m. p. 54°, $\nu_{max.}$ (in CCl_4) 3474, 2820, 1678 cm.$^{-1}$, identical (mixed m. p. and infrared spectrum) with authentic methyl 3-methylaminosalicylate, m. p. 56—57·5°.

3-*Aminosalicylic Acid*.—To 5-bromo-3-nitrosalicylic acid [16,17] (280 mg.) in 5% aqueous sodium hydroxide (50 ml.) was added Raney alloy (2 g.) portionwise with stirring during 1·5 hr. After a further 30 min., the mixture was benzoylated under Schotten–Baumann conditions. The ether-extracted material obtained after acidification was steam-distilled to completion in the presence of 18N-sulphuric acid (5 ml.). The residual solution was filtered, extracted with ether, and adjusted to pH 3—4. Extraction with ethyl acetate afforded 3-aminosalicylic acid (70 mg.), m. p. 240° (decomp.) after sublimation at 180°/10^{-2} mm., identical {mixed m. p., R_F,[33] and infrared spectrum [(in Nujol) 2600, 1664sh, 1638, 1561 cm.$^{-1}$)]} with the product obtained by hydrogenating 3-nitrosalicylic acid in methanol in the presence of Adams catalyst.

Attempted extraction of the amino-acid from the acidified reduction mixture without benzoylation was unsuccessful.

Methyl 3-*Methylaminosalicylate*.—To 3-aminosalicylic acid (500 mg.) in 0·1N-aqueous sodium hydroxide (60 ml.), stirred at 80° under nitrogen, was added in 1·5 hr. dimethyl sulphate (900 mg.), with additional alkali as required. After acidification to pH 4, ether-extraction gave a solid (327 mg.), m. p. 223—236° after sublimation at 180°/10^{-2} mm. Paper chromatography [33] of this material showed the presence of 3-amino- and 3-methylamino-salicylic acids (R_F 0·25 and 0·41 respectively as the acids, 0·51 and 0·66 as the ammonium salts).

Crystallisation failed to resolve the mixture, which was treated with ethereal diazomethane; the bicarbonate-insoluble material (283 mg.) was chromatographed on "Florisil." Elution with pentane containing ether (1%) yielded *methyl* 3-*methylaminosalicylate* (54 mg.), m. p. 56—57·5° (from light petroleum). This gave a red-violet ferric test (Found: C, 59·3; H, 6·0. $C_9H_{11}NO_3$ requires C, 59·7; H, 6·1%). Elution with pentane–ether (4 : 1) gave methyl 3-aminosalicylate, long needles (from light petroleum), m. p. 87—88° (Tener *et al.*[11] record m. p. 85—85·5°), $\nu_{max.}$ (in CCl_4) 3502, 3418, 1681 cm.$^{-1}$.

3-*Methylaminosalicylic Acid*.—Methyl 3-methylaminosalicylate (5 mg.) in 10% aqueous sodium hydroxide (5 ml.) was refluxed for 2 hr. under nitrogen. The solution was extracted with ether at pH 8—9 and again at pH 4, the latter extract affording 3-methylaminosalicylic acid (4 mg.), m. p. 207° (decomp.) after sublimation at 180°/10^{-2} mm.

[33] Ekman, *Acta Chem. Scand.*, 1948, **2**, 383.

Methyl 3-Formamidosalicylate.—3-Aminosalicylic acid (100 mg.) and 90% formic acid (1 ml.) were refluxed 10 min., diluted with water (3 ml.), and refrigerated. The filtered 3-formamidosalicylic acid had m. p. 215—220° (Zahn [34] records m. p. 215°). Brief treatment with ethereal diazomethane afforded *methyl 3-formamidosalicylate*, colourless needles, m. p. 126·5—127·5° (from light petroleum; charcoal), giving a violet ferric test (Found: C, 55·0; H, 4·4; N, 7·6. $C_9H_9NO_4$ requires C, 55·4; H, 4·6; N, 7·2%), $\nu_{max.}$ (in CS_2) 3408, 1708, 1676 cm.$^{-1}$. Paper chromatography [33] in methanol–pentyl alcohol–benzene–water (35 : 17·5 : 35 : 12·5) gave R_F 0·33.

Hydrolysis of the Lactonic Ester Mixture (III).—The ester mixture (III) (330 mg.) in 10% aqueous sodium hydroxide (5·5 ml.) was refluxed for 2 hr. Acidification and ether-extraction gave mixed acids (325 mg.). Paper chromatography as the ammonium salts [19] identified isobutyric (R_F 0·38, a trace) and a branched C_5 acid (0·53), while two further components (R_F 0·68, 0·78) were identical with the keto-acids (IV) obtained on hydrolysis of the semicarbazone mixture (see below). Vapour-phase chromatography on " polyester " and " silicone oil " columns of the acids as their methyl esters resolved three main components, identical in retention times with methyl isovalerate and the methyl esters of the keto-acids (IV).

Water (5 ml.) was added, the acid mixture distilled, and the distillate extracted with ether. The *p*-phenylphenacyl ester, m. p. 72·5—75·5° (from ethanol or light petroleum), obtained on treatment of the neutralised volatile acids with *p*-phenylphenacyl bromide in the usual way, could not be obtained homogeneous.

The aqueous, less volatile residue gave with semicarbazide a mixture (90 mg.), m. p. 132—138° (from ethanol), of the semicarbazones of the keto-acids (IV). Hydrolysis of this semicarbazone mixture (10 mg.) with warm N-hydrochloric acid (5 ml.) for 30 min. and ether-extraction afforded the keto-acids (IV), methylated with diazomethane to the keto-ester mixture, $\nu_{max.}$ (in CS_2) 1740, 1727 cm.$^{-1}$.

Reduction of the Lactonic Ester Mixture (III).—The lactonic ester mixture (III) (940 mg.) in ether (10 ml.) was added dropwise to lithium aluminium hydride (500 mg.) suspended in ether (10 ml.). After being kept overnight, the mixture was refluxed for 2 hr. The excess of reagent was destroyed, and the mixture acidified and extracted with ether. The clear oil (503 mg.) obtained on careful recovery was fractionated by short-path distillation; the short-chain alcohols were collected up to 130°/150 mm.; the triols (V) (333 mg.), $\nu_{max.}$ (in $CHCl_3$) 3560, 3385 cm.$^{-1}$, distilled at 130°/10^{-3} mm. as a colourless syrup.

The fraction of b. p. 130°/150 mm., $\nu_{max.}$ (liquid film) 3340, 1388, 1373 cm.$^{-1}$ {cf. commercial " isoamyl alcohol," $[\alpha]_D$ −1·0°, $\nu_{max.}$ (liquid film) 3332, 1387, 1371 cm.$^{-1}$} corresponded to isopentyl alcohol on vapour-phase chromatography and gave a 3,5-dinitrobenzoate mixture (275 mg.), plates, m. p. 50·5—56·5° after chromatography on " Florisil " and crystallisation from light petroleum, $[\alpha]_D^{22}$ +1·5° (6·7% in acetone) (Found: C, 51·4; H, 4·9; N, 10·1. Calc. for $C_{12}H_{14}N_2O_6$: C, 51·1; H, 5·0; N, 9·9%).

Periodate Oxidation of the Triol Mixture (V).—(i) To the triol mixture (V) (333 mg.) in dioxan (5 ml.) and water (5 ml.) was added aqueous 0·04N-sodium periodate (90 ml.), a nitrogen stream carrying volatile products into dinitrophenylhydrazine reagent. After 1 hr., utilisation of periodate corresponded to 0·95 oxygen atom per mole of triol. The precipitated 2,4-dinitrophenylhydrazone (30 mg.), after chromatography on alumina and crystallisation from methanol, had m. p. 162—164·5°, undepressed by acetaldehyde 2,4-dinitrophenylhydrazone, m. p. 164°. Comparison of infrared spectra and R_F values on paper chromatography in decalin–dimethylformamide [35] confirmed the identity.

Extraction of the aqueous oxidation solution with pentane afforded the aldol mixture (VI) (154 mg.), $\nu_{max.}$ (in CS_2) 3570, 3370, 1714 cm.$^{-1}$, with no significant ultraviolet absorption. The aldol (VI) (81 mg.) with Brady's reagent at room temperature gave a 2,4-dinitrophenylhydrazone which after filtration in ether through a short column of alumina (Peter Spence, Grade " H ") showed two spots on paper chromatography, one of R_F 0·10, the aldol derivative, the second, of R_F 0·83, probably a dehydration product thereof. After chromatography on alumina in ether, and crystallisation from ethyl acetate–light petroleum, the 2,4-dinitrophenylhydrazone of the aldol mixture (VI) was obtained as yellow crystals (82 mg.), m. p. 82·5—89·5°, $\nu_{max.}$ (in $CHCl_3$) 3595, 3452, 3322, 1623, and 1597 cm.$^{-1}$ (Found: C, 53·0; H, 6·4; N, 16·8. Calc. for $C_{15}H_{22}N_4O_5$: C, 53·2; H, 6·5; N, 16·6%.

[34] Zahn, *J. prakt. Chem.*, 1900, **61**, 532.
[35] Horner and Kirmse, *Annalen*, 1955, **597**, 48.

(ii) To the triol mixture (V) (30 mg.) in dioxan (2 ml.) was added aqueous 0·041N-sodium periodate (98 ml.). After utilisation of 1 mol. of periodate as in (i), no further uptake occurred during 20 hr. Further oxidation occurred on addition of sodium hydrogen carbonate (2 g.), and was complete 24 hr. later, when a total of 7 mol. of periodate had been utilised. Ether extracted neutral materials (4 mg.), then acidification and re-extraction gave a mixture of acids (19 mg.) in which acetic, n-valeric, and heptanoic acid were identified by paper chromatography,[19] as the ammonium salts (R_F 0·11, 0·56, and 0·70 respectively). Vapour-phase chromatography of the methylated acid mixture confirmed the presence of n-valeric and heptanoic acid, in addition to isovaleric acid.

ON-*Diacetyldeformylantimycin-A*.—Antimycin-A (50 mg.) was treated with acetic anhydride–pyridine (10 : 1; 1·8 ml.) at room temperature for 20 hr.,[7] yielding ON-*diacetyldeformylantimycin-A* (48 mg.), m. p. 134—138° (from aqueous ethanol) (Found: C, 60·5; H, 6·7; N, 5·0. $C_{29}H_{40}N_2O_{10}$ requires C, 60·4; H, 6·9; N, 4·9%). The derivative, $\nu_{max.}$ (in CCl_4) 3430, 1782, 1752, 1734, 1714, 1677 cm.$^{-1}$, $\lambda_{max.}$ 271 mμ (log ε 3·02), gave a negative ferric test.

Acetylation of antimycin-A and isolation of the product under anhydrous conditions by evaporation of the excess of reagents afforded the same diacetyl derivative (m. p. and infrared spectrum).

Acetylation of aniline with [*carbonyl*-^{14}C]acetic anhydride, prepared[36] from sodium [1-^{14}C]-acetate (15 μC) diluted to 13 g., gave acetanilide, m. p. 113—115° (Found: r.m.a. × 10^{-4}, 4·05). Acetylation of antimycin-A with this anhydride gave diacetyldeformylantimycin-A (Found: r.m.a. × 10^{-4}, 7·57. 2Ac require 8·10).

The diacetyl derivative (100 mg.) was shaken with 5% aqueous sodium hydroxide (1 ml.) for 10 min. Extraction with pentane gave the lactonic ester mixture (III) (35 mg., 79%), identified by infrared spectrum. Acidification and extraction with ether afforded a viscous intractable oil (43 mg.) giving a deep violet ferric test.

Acetylation of Model Compounds.—(i) Acetylation of 2-formamidophenol (62 mg.) with acetic anhydride–pyridine (10 : 1; 7 ml.) at room temperature for 20 hr., and dilution of the mixture with water, gave crystals (64 mg.), m. p. 114—125° (from water). Crystallisation from light petroleum gave the major, more soluble fraction as colourless needles, m. p. 122—123° after sublimation at 85°/0·1 mm., identified by mixed m. p. and infrared spectrum [$\nu_{max.}$ (in $CHCl_3$) 1772, 1694 cm.$^{-1}$] as *o*-acetamidophenyl acetate (prepared by similar acetylation of *o*-aminophenol). The less soluble fraction, m. p. 201—203°, gave a brown ferric test, and was identical (mixed m. p.) with *o*-acetamidophenol, m. p. 203·5° (from water), prepared by refluxing *o*-acetamidophenyl acetate (10 mg.) in 0·1N-aqueous potassium hydroxide (0·5 ml.) for 5 min.

(ii) Similar treatment of formanilide (100 mg.) with acetic anhydride–pyridine (9 ml.) yielded only starting material (45 mg.), m. p. and mixed m. p. 45—47°.

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[36] Shantz and Rittenberg, *J. Amer. Chem. Soc.*, 1946, **68**, 210.