QUARTERLY REVIEWS

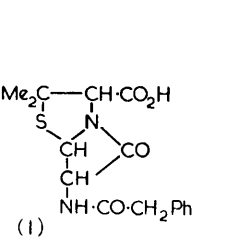
CHEMISTRY OF SOME NEWER ANTIBIOTICS

By N. G. Brink, Ph.D., and R. E. Harman, Ph.D.
(Merck, Sharp and Dohme Research Laboratories, Rahway, New Jersey)

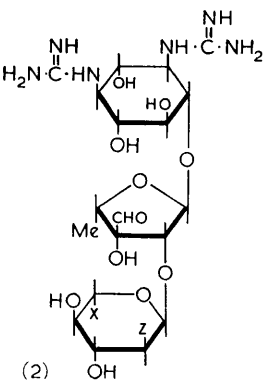
Penicillin made its appearance less than two decades ago as the first of a group of agents representative of a wholly new approach to the therapy of infectious diseases. In the ensuing years, the concepts of antibiosis and of antibiotics have become so familiar that it does not seem necessary to devote space here to historical background, to definitions, or even to a consideration of the place of antibiotics in modern medicine.

This Review, in keeping with its title, will confine itself almost entirely to the purely chemical aspects of the subject. Its primary objective will be to acquaint the reader with some of the types of molecules encountered among these biologically interesting substances, and to give a little insight into the degradative methods and synthetic studies that established their structures. A comprehensive treatment of the subject of this Review could well fill an entire volume, therefore many significant antibiotics have been omitted. Those chosen were selected primarily on the basis of chemical interest, although their demonstrated or potential usefulness and the nature of their biological action were also considered.

The wide diversity of structural types among antibiotics has been a challenge and a delight to those chemists whose task it has been to carry out the structural studies, and in some cases to synthesise the substances. Nature's infinite variety could hardly be better exemplified than in the marvellous array of atomic groupings represented both by the formulæ of



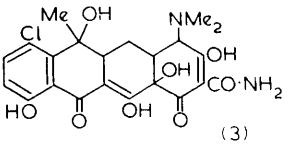
(1)



(2)

X = $CH_2 \cdot OH$
Z = NHMe

some of the less recent antibiotics—penicillin (1), streptomycin (2), chlorotetracycline (3), chloramphenicol (4), gramicidin-S (5)—and by those newer agents to be discussed below.



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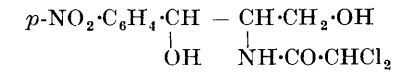


(4)

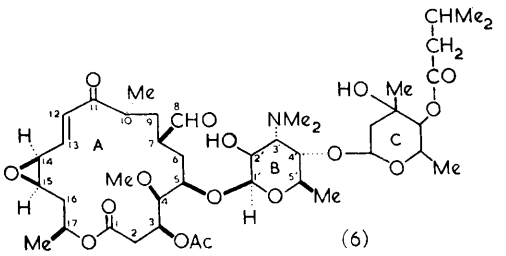
cyclo-(L-Valine-L-ornithine-L-leucine-D-phenylalanine-L-proline)$_2$

(5)

The macrolide antibiotics

Within less than ten years, a dozen or more members of a new and important class of antibiotics have been discovered. All are produced by various species of *Streptomyces*, and all have in common a many-membered, highly substituted lactone ring—hence the name, macrolide—to which is attached a dimethylamino-substituted sugar. In some of the macrolides the lactone ring is linked to one or two additional sugar residues. This group of substances is of interest because of its rapidly developing chemistry, because of the biogenetic implications in the structures involved, and because at least some members of the group are of demonstrated therapeutic usefulness.

Magnamycin.—The determination of the structure of magnamycin (6), chemically the most complex of those macrolide antibiotics that have been adequately characterised, was begun by workers in the Pfizer laboratories [1] and completed by Woodward and his collaborators.[2] In the early work on



(6)

A + B = Carimbose
B = Mycaminose
C = Mycarose

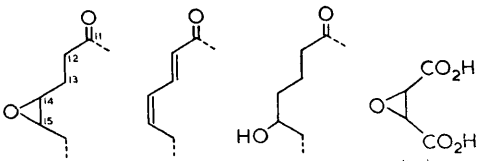
this antibiotic, the $\alpha\beta$-unsaturated carbonyl system was detected by spectroscopic studies, as were the hydroxyl groups and the carbonyl group of the

[1] Wagner, Hochstein, Murai, Messina, and Regna, *J. Amer. Chem. Soc.*, 1953, **75**, 4684. [2] Woodward, *Angew. Chem.*, 1957, **69**, 50.

lactone ring. Alkaline hydrolysis liberated acetic acid, *iso*valeric acid, and dimethylamine. Mild acid hydrolysis of magnamycin cleaved the glycosidic linkage between the two sugar moieties and released the *iso*valeryl derivative of mycarose (6, C), the nitrogen-free carbohydrate fragment. Mycaminose (6, B), the dimethylamino-sugar, was isolated after more drastic hydrolysis with acid. Methanolysis of magnamycin removed mycarose and yielded the crystalline base carimbose (6, A + B), $C_{30}H_{47}O_{12}N$. The instability of this and related compounds made it impossible to eliminate mycaminose and retain the central lactone ring intact, thus adding considerably to the difficulties of the determination of structure.

The observation that carimbose was distinctly more basic than the parent antibiotic suggested that in magnamycin the mycarose moiety was attached through an hydroxyl group adjacent to the dimethylamino-group. The pK_a shifts observed upon acetylation of magnamycin (from 7·0 to 6·0) and carimbose (from 8·3 to 5·4) indicated that the dimethylamino-group was flanked by two hydroxyl groups. This was confirmed and the exact point of attachment of mycarose placed at the 4′-position of the mycaminose ring by exhaustive methylation of magnamycin, followed by hydrolysis to yield the 2′-methyl derivative of the basic sugar.

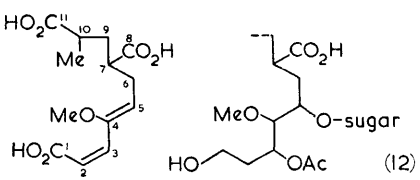
At this point magnamycin could be formulated as $C_{22}H_{31}O_8$-O-sugar. Four of the eight oxygen atoms of the unknown fragment were readily accounted for by the presence of a methoxyl, an acetyl, and an aldehyde group. Two more were shown to be in a lactone function, since a carboxyl group was generated by vigorous alkaline hydrolysis of magnamycin after reduction, first catalytic and then with borohydride. Existence of the difficultly reducible lactonic carbonyl group was required also to permit base-catalysed removal of a neighbouring proton, thus initiating consecutive elimination reactions which yielded a doubly unsaturated carboxylic acid (see below). The remaining two oxygen atoms were assigned to a structural moiety represented by (7) and corresponding eventually to $C_{(11)}$ to $C_{(15)}$ of magnamycin. This last conclusion was based upon : (*a*) mild reduction with iodide that removed one oxygen atom and gave a product identical with magnamycin B, a natural companion of magnamycin, whose absorption spectrum was characteristic of an $\alpha\beta : \gamma\delta$-doubly unsaturated carbonyl system (8) ; (*b*) mild catalytic hydrogenation, which added four hydrogen atoms to the molecule without loss of oxygen, but with formation of one new hydroxyl group (9) ; and (*c*) mild nitric acid oxidation from which ethylene oxide-*cis*-dicarboxylic acid (10) was isolated.



(7) (8) (9) (10)

A series of oxidative and hydrolytic experiments led to the isolation and characterisation of the tribasic acid (11) and hence to assignment of structure

for carbon atoms 1 to 11 of magnamycin. In order to establish the positions of the groups that had suffered elimination in the formation of (11), carimbose dimethyl acetal was subjected to vigorous reduction, mild hydrolysis, and then oxidation. Partial structure (12) was assigned to the product, since it yielded a 5-membered lactone only after vigorous hydrolysis had removed the amino-sugar. This series of experiments demonstrated that the *O*-acetyl group of magnamycin was at position 3, the glycosidic linkage at position 5, and the aldehyde group on $C_{(7)}$.



(11) (12) (13)

A further series of oxidative experiments on magnamycin B, partially represented by (8), revealed the structure at carbon atoms 11 to 17. Oxidation of magnamycin B followed by alkaline hydrolysis gave crotonic acid from carbon atoms 15, 16, 17, and the 17-methyl group. Tetrahydromagnamycin B was converted into the enol acetate (13) and ozonised, and *n*-octanoic acid isolated after reduction of the ozonide.

It is not possible to detail here the many reaction sequences or to reproduce the brilliant interpretation of the experimental results which led to the elucidation of the structure of magnamycin. Time devoted by the reader to perusal of the original article [2] could hardly be better spent.

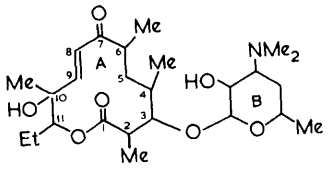
Methymycin and *neo*Methymycin.—Methymycin, $C_{25}H_{43}O_7N$, is structurally one of the simplest of the macrolide antibiotics, and its constitution (14) was the first of the group to be completely worked out.[3] The isomeric *neo*methymycin [4] isolated from the mother-liquors of methymycin is represented by formula (15). Acid-hydrolysis of these antibiotics cleaved the glycosidic linkages ; the aglycones were different, but both substances gave the same carbohydrate moiety. The sugar proved to be desosamine (14, B), which had been isolated previously from other macrolide antibiotics and whose structure was already known.[5]

An interesting feature of methymycin chemistry is the formation of spiroketals (16a, b, c). Thus, treatment of methynolide (14, A), $C_{17}H_{28}O_5$, the aglycone of methymycin, with methanol containing hydrogen chloride gave a compound $C_{18}H_{30}O_5$ (16a) in which hydroxyl and the conjugated carbonyl groups could no longer be detected in the infrared spectrum, but which now contained a methoxyl group. A similar product (16b) was formed when methymycin was treated with hydrochloric acid, and (16c) was generated from dihydromethynolide with great ease and rapidity when

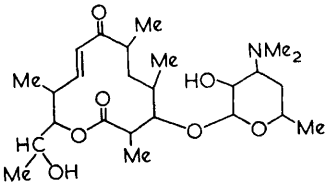
[3] Djerassi and Zderic, *J. Amer. Chem. Soc.*, 1956, **78**, 6390.

[4] Djerassi and Halpern, *ibid.*, 1957, **79**, 2022.

[5] Brockmann and Strufe, *Chem. Ber.*, 1953, **86**, 876 ; Clark, *Antibiotics and Chemotherapy*, 1953, **3**, 663.

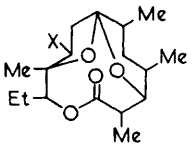


(14)



(15)

A = Methynolide
B = Desosamine



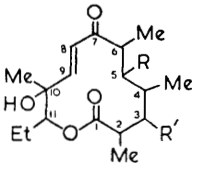
(16a) X = MeO
(16b) X = Cl
(16c) X = H

the reduced aglycone was placed in methanolic acid. It appears that although steric conditions are not favourable for spiroketal formation while the double bond is present, saturation of the bond by addition of methanol, hydrogen chloride, or hydrogen permits ready spiroketal formation between the carbonyl and the two hydroxyl groups. Spiroketals have not been observed in the *neo*methymycin series, nor would they be expected in view of the altered relationship of the hydroxyl groups to the carbonyl group.

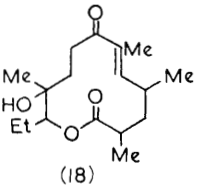
Pikromycin.—Pikromycin was the first of the macrolide antibiotics to be discovered.[6] Despite its rather good activity against a variety of Gram-positive micro-organisms, it has proved too toxic to be useful clinically. Pikromycin is very closely related chemically to the isomeric methymycin, but its exact structure has not yet been established. Extensive degradative studies both by Brockmann and his co-workers [7] in Germany and by Swiss workers [8] have led to agreement on formula (17a or b) for pikromycin.

Mild acid-hydrolysis [5] of pikromycin yielded desosamine (14 B ; pikrocinine) and the anhydroaglycone, kromycin, in which the newly introduced double bond has been shown to be in the 5 : 6-position. Dihydropikromycin, in which the 8 : 9-double bond was reduced, was readily cleaved, also with loss of water, to dihydrokromycin (18). Again, evidence was available to permit assigning the newly introduced double bond to the 5 : 6-position. Further information on the $C_{(1)}$—$C_{(7)}$-portion of the pikromycin lactone ring was provided by permanganate oxidation of the antibiotic and isolation [9] of the lactone $C_{10}H_{16}O_4$ (19). It is important to note, however, that the carboxyl of (19) could have originated in either $C_{(7)}$ of structure (17a) or $C_{(1)}$ of structure (17b). Of the two possible points of attachment of the sugar to the aglycone, $C_{(5)}$ seems somewhat favoured, both because of the location of the double bond at 5 : 6 in kromycin and dihydrokromycin, and because the extreme ease of removal of the sugar is suggestive of a linkage β to the carbonyl group. Should desosamine prove to be attached at $C_{(3)}$, pikromycin

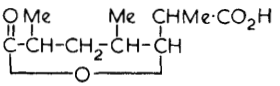
[6] Brockmann and Henkel, *Naturwiss.*, 1950, **37**, 138.
[7] Brockmann and Oster, *Chem. Ber.*, 1957, **90**, 605.
[8] Anliker and Gubler, *Helv. Chim. Acta*, 1957, **40**, 119, 1768.
[9] Anliker, Dvornik, Gubler, Heusser, and Prelog, *ibid.*, 1956, **39**, 1785.



(17a) R = Desosamine moiety
R′ = H
(17b) R = H
R′ = Desosamine moiety



(18)

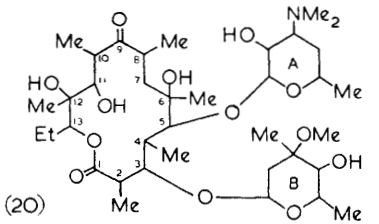


(19)

would have the same structure as methymycin (14), differing only stereochemically from that antibiotic.

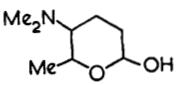
The elucidation of the structure of the rest of the pikromycin molecule came from other degradative studies. One key reaction sequence involved oxidation of dihydrokromycin (18) to yield propaldehyde (from $C_{(11)}$ and the ethyl group), lævulic acid (from $C_{(7)}$—$C_{(10)}$ and the 10-methyl group), and *meso*-αα′-dimethylglutaric acid (derived from $C_{(1)}$—$C_{(5)}$ and the 2- and 4-methyl groups).

Erythromycin.—One of the more complex macrolide antibiotics is erythromycin (20), $C_{37}H_{67}O_{13}N$. The constitution of this clinically useful antibiotic was established by studies carried out in the Lilly laboratories.[10] Erythromycin contains desosamine and in addition the 9-carbon sugar cladinose (20, B). Unlike the other members of this group, erythromycin does not possess a carbon–carbon double bond in the lactone ring. Erythromycin is inactivated under acid conditions, presumably because of irreversible spiroketal formation. It is of interest that erythromycin B, $C_{37}H_{67}O_{12}N$, a related antibiotic which differs from its companion only in the absence of the 12-hydroxyl group,[11] has the expected greater acid stability.



(20)

A = Desosamine
B = Cladinose



(21)

[10] Wiley, Gerzon, Flynn, Sigal, Weaver, Quark, Charwette, and Monahan, *J. Amer. Chem. Soc.*, 1957, **79**, 6062, and earlier papers.

[11] Wiley, Sigal, Weaver, Monahan, and Gerzon, *ibid.*, p. 6070.

Other Macrolide Antibiotics.—In addition to the members of this group already discussed, there are a number of representatives about which less is known, and reports of the discovery of new macrolide antibiotics continue to appear with some regularity.

Narbomycin, $C_{28}H_{47}O_7N$, in preliminary studies [12] showed the usual characteristics of these compounds : two *N*-methyl and six or more *C*-methyl groups, infrared absorption indicative of two carbonyl groups, one of which was conjugated, and hydrolysis to yield a basic sugar, in this case desosamine. Oxidation [9] gave the ten-carbon-atom lactone (19), also isolated from pikromycin [8] and methymycin.[3]

One of the newer antibiotics showing considerable clinical promise is oleandomycin, $C_{35}H_{63}O_{12}N$. It has been described [13] as a polyhydroxy-keto-lactone linked glycosidically to both desosamine and the new sugar L-oleandrose.

Other antibiotics which probably belong to the macrolide group include angolamycin,[14] miamycin,[15] and the spiramycins A, B, and C.[16] Hydrolysis of the spiramycins (foromacidines [17]) liberated propionic acid from C and acetic acid from B ; no volatile acid was obtained from A. Mycarose and mycaminose, the two carbohydrates isolated from hydrolytic degradation of magnamycin, have been obtained also from each of the spiramycins, along with a third sugar which has been assigned structure (21).

Stereochemistry.—Some progress has been made towards working out the stereochemistry of the macrolide antibiotics. For example, reference to formula (6) will show that magnamycin possesses 17 asymmetric carbon atoms and an asymmetrically substituted double bond, so that (6) can represent 262,144 stereoisomers. Infrared studies showed that the substituents on the double bond were in the *trans*-configuration ; the hydrogens of the ethylene oxide ring were known to be *cis* because of the isolation of ethylene oxide-*cis*-dicarboxylic acid ; nitric acid oxidation of magnamycin to yield L-(−)-methylsuccinic acid gave the configuration of the groups about $C_{(10)}$; and the stereochemistry at the 1′-, 2′-, 3′-, and 4′-positions of the mycosamine moiety was deduced from considerations of the relative basicity of the antibiotic and various pertinent derivatives, as well as from other points of chemical behaviour. As Woodward has mentioned,[2] this reduced the number of possible isomers to a mere 4096. Experimental evidence has also been acquired for the stereochemical configurations of most of the asymmetric centres of erythromycin.[10]

[12] Corbaz, Ettlinger, Gäumann, Keller, Kradolfer, Kyburz, Neipp, Prelog, Reusser, and Zähner, *Helv. Chim. Acta*, 1955, **38**, 935.

[13] Els, Murai, and Celmer, Abs. Papers, 130th Meeting, Amer. Chem. Soc., 1956, p. 15N.

[14] Corbaz, Ettlinger, Gäumann, Keller-Schierlein, Neipp, Prelog, Reusser, and Zähner, *Helv. Chim. Acta*, 1955, **38**, 1202.

[15] Schmitz, Misiek, Heinemann, Lein, and Hooper, *Antibiotics and Chemotherapy*, 1957, **7**, 37.

[16] Paul and Tchelitcheff, *Bull. Soc. chim. France*, 1957, 443, 734, 1059.

[17] Corbaz, Ettlinger, Gäumann, Keller-Schierlein, Kradolfer, Kyburz, Neipp, Prelog, Wettstein, and Zähner, *Helv. Chim. Acta*, 1956, **39**, 304.

It must be made clear that the points mentioned above refer to relative stereochemical configurations; some work, however, has been done on absolute stereochemistry. As has been mentioned, magnamycin was degraded to L-(−)-methylsuccinic acid, known to be related to L-glyceraldehyde, thus establishing the absolute configuration at $C_{(10)}$. A series of degradation reactions on *neo*methymycin gave the same methylsuccinic acid; in this case the asymmetric centre of the product corresponded to $C_{(4)}$ of the antibiotic (15). Since both *neo*methymycin and methymycin had been degraded *via* the C_{10}-lactone (19) to *meso*-$\alpha\alpha'$-dimethylglutaric acid, the absolute configurations at both $C_{(4)}$ and $C_{(6)}$ were established for these two antibiotics. The same conclusions were drawn for the configurations of the two corresponding asymmetric centres of pikromycin and narbomycin, since the lactone (19) was also obtained from these antibiotics.[18]

Biogenesis and the Propionate Rule.—The well-known "isoprene rule", advanced by Wallach in 1887, has been of great use in structural studies in the terpene field. In 1907 Collie [19] suggested that a number of naturally occurring aromatic compounds could have originated through condensations of chains built up of acetic acid units. Today the biosynthesis of fatty acids from acetate is also firmly established. A series of papers by Birch [20] has shown how helpful an "acetate rule" can be, within its proper limits, in attacking problems of the structures of natural products. With the elucidation of the constitutions of some of the macrolide antibiotics, a new "propionate rule" has come into being.

Several investigators [2, 21] have called attention to the likely participation of propionate units, as well as acetate fragments, in the biosynthesis of some of the branched long-chain aliphatic acids from tubercle bacilli. Now, in the lactone nucleus of erythromycin (20) there is at hand an example of a natural material built up with perfect regularity from seven three-carbon units. The aglycones of methymycin and pikromycin are constructed partly of propionic and partly of acetic acid units, but that of magnamycin seems to be made up almost entirely of acetate fragments.

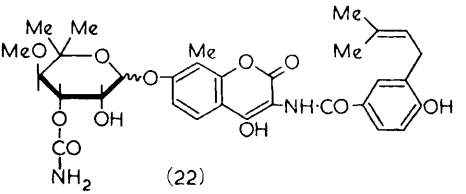
Woodward has pointed out [2] that biogenetic principles now becoming evident will limit the number of possible structures that members of this group can have, and should simplify the task of chemists occupied with such structural problems in the future.

Novobiocin

Novobiocin is a crystalline antibiotic produced by a *Streptomyces*. It is primarily effective against Gram-positive micro-organisms and has proved to be of considerable use clinically, especially in the treatment of infections caused by penicillin-resistant staphylococci. The structure of novobiocin,

[18] Djerassi and Halpern, *J. Amer. Chem. Soc.*, 1957, **79**, 3927.
[19] Collie, *J.*, 1907, **91**, 1806.
[20] Birch and Elliott, *Austral. J. Chem.*, 1956, **9**, 95, and earlier papers.
[21] Robinson, "The Structural Relations of Natural Products", Clarendon Press, London, 1955, p. 7; Woodward, *Angew. Chem.*, 1956, **68**, 19; Gerzon, Flynn, Sigal, Wiley, Monahan, and Quark, *J. Amer. Chem. Soc.*, 1956, **78**, 6396.

complete except for a single point of stereochemistry, is represented by formula (22). Studies which led to the elucidation of the constitution of



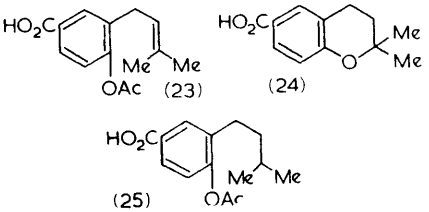
(22)

the antibiotic were carried out independently and the results reported more or less simultaneously by workers in the Merck [22] and the Upjohn [23] laboratories.

Novobiocin has the composition $C_{31}H_{36}O_{11}N_2$ and is a dibasic acid with pK_a' values of about 4·3 and 9·1. It possesses a methoxyl group, a mono-substituted amide group, and at least two *C*-methyl groups. The two acidic functions appeared to be enolic and phenolic in nature. Novobiocin was hydrogenated under mild conditions to the biologically active dihydro-derivative, apparently by saturation of an unconjugated double bond, since no change in the ultraviolet absorption spectrum was noted.

Preliminary degradative studies indicated that the antibiotic molecule was made up of a sugar attached to an aromatic heterocyclic moiety, which was in turn linked to a substituted benzoic acid in an A–B–C arrangement, and that reactions could be chosen to rupture either the A–B or the B–C link. As inspection of formula (22) would indicate, acid-alcoholysis liberated a sugar derivative. Less expectedly (see below), treatment of novobiocin with hot acetic anhydride cleaved the amide bond and released the substituted benzoic acid. It is convenient to consider separately the determination of the structures of the three parts. When these were elucidated, evidence was at hand to permit assignment of structure (22) to the parent substance.

The substituted benzoic acid moiety. Hot acetic anhydride cleaved [23] novobiocin to a large, carbohydrate-containing fragment of the composition $C_{23}H_{26}O_{10}N_2$ and a monobasic acid $C_{14}H_{16}O_4$ (23). Treatment of this acid (23) with osmium tetroxide and then periodate yielded acetone ; deacetylation gave a product with the properties of a substituted *p*-hydroxybenzoic



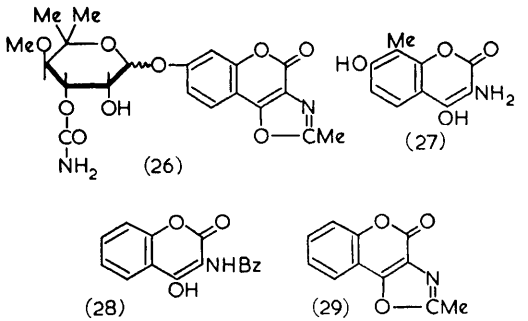
(23) (24) (25)

[22] Folkers *et al.*, *J. Amer. Chem. Soc.*, 1955, **77**, 6404 ; 1956, **78**, 1770, 2655, 4125 ; 1958, **80**, 137, 140.

[23] Hoeksema *et al.*, *Antibiotics and Chemotherapy*, 1956, **6**, 143 ; *J. Amer. Chem. Soc.*, 1955, **77**, 6710 ; 1956, **78**, 1072, 2019 ; 1957, **79**, 3789.

acid. The latter compound, heated in ethanolic hydrochloric acid, cyclised to the known 2 : 2-dimethylchroman-6-carboxylic acid (24), thus fixing the location of the pentenyl group on the benzene ring. 4-Acetoxy-3-*iso*pentylbenzoic acid (25) was obtained either by mild hydrogenation of acid (23) or by acetic anhydride cleavage of dihydronovobiocin.

The aromatic heterocyclic moiety. The product $C_{23}H_{26}O_{10}N_2$ (26) mentioned above was an optically active, neutral material which on treatment with methanolic hydrogen chloride yielded an optically inactive, amphoteric compound (27), $C_{10}H_9O_4N$. The reactions and properties of this compound indicated that it possessed an aromatic nucleus substituted with a methyl group, an amino-group, a phenolic hydroxyl group, and a strongly acidic enol. Because of the evident relation of compound (27) to 3-amino-4-hydroxycoumarin, model studies were undertaken. When 3-benzamido-4-hydroxycoumarin (28) was heated with acetic anhydride, the oxazole (29) was formed. This observation appeared to clarify the nature of degradation

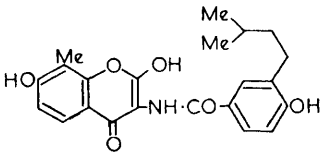


products (26) and (27) except for the location of the methyl substituent and the phenolic hydroxyl group. Alkali-fusion of the substituted coumarin (27) gave 2-methylresorcinol, and alkaline hydrolysis afforded 2 : 4-dihydroxy-*m*-toluic acid. With the substituents thus placed, the aromatic central moiety of novobiocin was assigned the indicated coumarin structure.

Another degradative route in a different laboratory [22] led to an independent elucidation of the structure of the central portion of the antibiotic. Alcoholysis of dihydronovobiocin gave dihydronovobiocic acid (30). The amide linkage therein was cleaved by reaction with a mixture of acetic acid, acetic anhydride, and hydrogen bromide, and the diacetylated aminohydroxycoumarin (31) was isolated. Deacetylation with acid converted the product (31) into the " aromatic " amine (27) which, as mentioned above, had been obtained elsewhere by methanolysis of the oxazole (26). Selective deacetylation of the product (31) gave the *N*-acetyl derivative (32). Both the products (27) and (32) could be reconverted by acetylation into the parent compound (31), showing that no deep-seated changes had occurred during either hydrolytic step.

The action of a mixture of hot acetic and hydrochloric acid on dihydronovobiocic acid (30) resulted in opening of the coumarin ring, with only a partial cleavage of the amide linkage, and products (33) and (34) were

obtained. Because of the aliphatic nature of the amino-group in (34) and its aromatic character in (27), it was evident that this group had originally been attached to the pyrone ring of the coumarin moiety. Clemmensen reduction of the amino-ketone (34) yielded the known 4-ethyl-2-methylresorcinol (35), establishing the relative positions of the substituents in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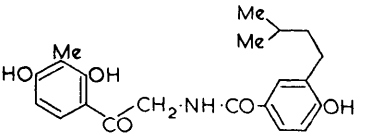
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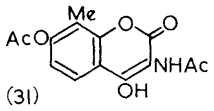
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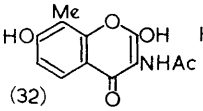
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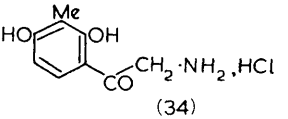
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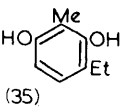

(35)

the earlier products of this series. The lack of ester-carbonyl absorption in the infrared spectra of both products (30) and (33) indicated that the benzoic acid moiety of novobiocin was attached to the coumarin structure through an amide linkage.

The correctness of the structure assigned to the central heterocyclic moiety of novobiocin has been confirmed by the synthesis [22] of dihydronovobiocic acid (30).

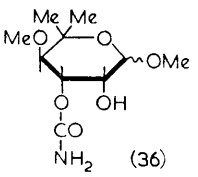
An interesting feature of novobiocin chemistry is the possibility of the existence of tautomeric forms in the central heterocyclic moiety, and this became evident during the degradative sequence just described.[22] The infrared spectrum of novobiocin (22) contained an absorption band at 5·92 μ attributed to the carbonyl group of the unsaturated lactone. Rather surprisingly, there was no corresponding carbonyl band in dihydronovobiocic acid (30), but with the conversion of this acid (30) into the diacetylated coumarin (31), the 5·92 μ band reappeared. Selective deacetylation of (31) by aqueous alkali resulted in a monoacetyl derivative (32) which showed no carbonyl absorption in the 5·5—6·0 μ region of its spectrum, although the band was restored upon reacetylation of (32) to (31). On the basis of these observations, novobiocin and the degradation product (31) were assigned the indicated coumarin structures, while dihydronovobiocic acid (30) and compound (32) have been represented as hydroxychromones. It is assumed that the chromone-carbonyl absorption band in the latter compounds has shifted to a longer wavelength where it is masked by the absorption of the amide group.

Structure of the sugar. Methanolysis of novobiocin gave an optically active, neutral product (36) * containing a carbamoyl group, two methoxyl groups, and one free hydroxyl group. The possible presence of a *gem.*-dimethyl structure was suggested from the Kuhn–Roth *C*-methyl value of

* Since configurations about the glycosidic carbon have not yet been assigned, structures (36) to (40) have been written to indicate that pure anomers of unknown configurations were used in the experimental work.

0·30. This compound, now designated methyl 3-*O*-carbamoylnovioside, was stable to periodate. After acid hydrolysis the resulting sugar, 3-*O*-carbamoylnoviose (37), consumed one equivalent of periodate with the formation of a mol. of formic acid. The original free hydroxyl group was thus located adjacent to the glycosidic carbon atom, *i.e.*, at position 2 (of the sugar molecule). Either alkaline hydrolysis or acid methanolysis of the sugar (36) gave carbon dioxide and ammonia, from the decomposition of the urethane structure, and a mixture of anomeric methyl noviosides (38). These gave acetone upon oxidation with chromic acid. The mixture consumed one mol. of periodate to give a dialdehyde which yielded glyoxal after mild hydrolysis. Acid hydrolysis of the methyl glycosides (38) afforded noviose (39), which reacted with two equivalents of periodate to form two mols. of formic acid. This series of experiments indicated that the two hydroxyl groups of compound (38) must have been at positions 2 and 3, and the original carbamoyl group at position 3. Further evidence bearing on the relation of the two groups at $C_{(2)}$ and $C_{(3)}$ was provided by the isolation of compound (40) from the methanolysis of the carbamoyl compound (36). This product, assigned its cyclic carbonate structure largely on the basis of analytical and infrared data, was converted by treatment with barium hydroxide into methyl novioside (38).

Another sequence of degradative reactions started with the conversion of the glycoside (36) *via* the diethyl mercaptal (41) into the 1-deoxy-derivative (42). Compound (42) was stable to periodate, but after removal of the carbamoyl group the new product took up one equivalent of periodate. From the reaction mixture were isolated acetaldehyde and, after further oxidation of the resulting aldehyde with bromine, the (−)-acid (43).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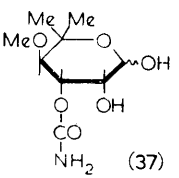
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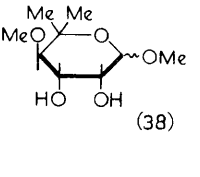
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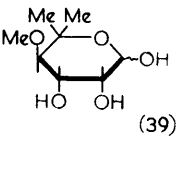
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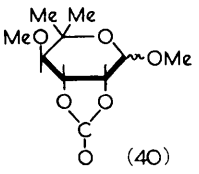
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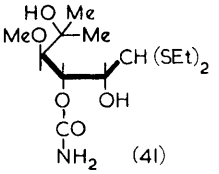
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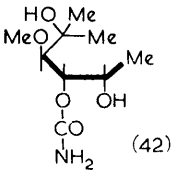
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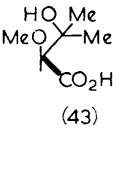

(43)

The stereochemistry of the groups at positions 2, 3, and 4 of the sugar moiety has been determined, based in part on the rules of optical rotation, and the configuration of L-lyxose has been assigned to the carbohydrate fragment.[24] Thus the structural studies on novobiocin are complete except

[24] Walton, Rodin, Stammer, Holly, and Folkers, *J. Amer. Chem. Soc.*, 1956, **78**, 5454.

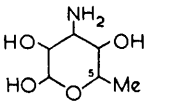
for one point—the configuration about the glycosidic carbon atom of the carbohydrate portion of the molecule.

Antifungal antibiotics

After the introduction of the broad-spectrum antibiotics, particularly the tetracyclines, clinicians have noted the occasional occurrence of severe and sometimes fatal super-infections by such organisms as the yeast *Candida albicans*. The important role of the fungi in these recently observed complications of antibiotic therapy has sparked interest in antifungal agents for clinical use in combination with a variety of antibacterial antibiotics.

Among antifungal agents whose chemistry has been reviewed recently [25] are such diverse structures as the tripyrrylmethene dye prodigiosin, the polypeptide fungistatin, the polyenyne mycomycin, the sulphur-containing thiolutin, the complex antimycin A, and gliotoxin. Most of these compounds are significantly active against bacteria and other micro-organisms, as well as yeasts and fungi. This Review will be concerned with a series of antibiotics, the antifungal polyenes,[26] which are active against a wide range of fungi and yeasts, but do not show antibacterial activity. This class of compound is further characterised by the ultraviolet spectra associated with the polyenic chromophores, by a blue-violet coloration with concentrated sulphuric acid, by low solubilities, and by sensitivity to light and air. Sub-groups of tetra-, penta-, hexa-, and hepta-enes are each characterised by a closely defined series of ultraviolet absorption maxima.

The heptaene amphotericin B occurs along with the tetraenic amphotericin A of lower activity against fungi. The biologically inactive perhydro-derivative was used in degradative studies [27] because of its more favourable solubility and stability. Amphotericin B has been assigned the tentative empirical formula $C_{46}H_{73}O_{20}N$; infrared absorption spectra and behaviour on potentiometric titration suggested the presence of a lactone group in the antibiotic. Acetolysis gave two crystalline substances which proved to be acetyl derivatives of an amino-sugar, mycosamine (44). This methylpentose possesses the D-configuration at position 5, since periodate cleavage of methyl *N*-ethylmycosaminide yielded D-methoxy-D′-methyldiglycollic aldehyde (45). The stereochemistry of the other asymmetric centres of mycosamine is unknown, and no further information on the nature of the remaining C_{40} moiety of amphotericin B has been reported. A similarity, and perhaps also a biogenetic relationship, of the polyenic nuclei of these antibiotics to the xanthophylls and carotenoids has been suggested.



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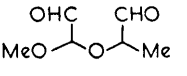


(45)

[25] Duggar and Singleton, *Ann. Rev. Biochem.*, 1953, **22**, 459 ; Binkley, *ibid.*, 1955, **24**, 597.

[26] Ball, Bessell, and Mortimer, *J. Gen. Microbiol.*, 1957, **17**, 96.

[27] Walters, Dutcher, and Wintersteiner, *J. Amer. Chem. Soc.*, 1957, **79**, 5076.

Fungichromin, a pentaene, and the hexaene fungichromatin, have been obtained from *Streptomyces cellulosæ*.[28] These are pale yellow, nitrogen-free polyenes; little has been reported about their structure. Filipin, $C_{30}H_{50}O_{10}$, from *S. filipinensis*, is another pentaene antifungal agent with properties common to this group of antibiotics.[29] Light and heat transform filipin into a new substance with neither the ultraviolet absorption nor the biological activity of the parent compound. In alcoholic solution, a crystalline transformation product $C_{30}H_{50}O_{11}$ is formed; this too is devoid of antifungal activity, but retains the pentaene ultraviolet absorption spectrum.

Anti-tumour antibiotics

Treatments that man has employed through the centuries in his efforts to cure cancer run the gamut from blood-letting to the use of diets of crab soup. The current experimental era, which dates from about 1900, was guided initially by the rapidly developing sciences of bacteriology and immunology. Recent promising advances in the chemotherapy of cancer, however, may be correlated with studies on cell-metabolism and metabolite-antagonists. Agents have been sought that would selectively inhibit the growth of, or actually destroy, malignant cells in the body either by inhibiting the synthesis of nucleic acids or by interfering with their utilisation at later stages of the metabolic process. In the most recent approach, metabolic products from fermentation broths have been screened directly for anti-tumour activity. Most of the agents discussed in this section, although primarily of interest for their activities against malignant tumours, also possess at least some degree of antibacterial activity. This antibacterial action has frequently been of aid in the isolation of the materials. On this basis, the inclusion of a few of these anti-tumour agents in the present Review seems justified.

The Actinomycins.—Since the isolation of actinomycin A by Waksman and Woodruff in 1940,[30] a number of closely related antibiotics produced by *Streptomyces* have been reported. These substances were observed to be highly active against Gram-positive bacteria, and somewhat less effective against Gram-negative organisms and fungi; they were later shown to possess a selective cytostatic effect on mammalian tissue. Although a German clinician [31] has described beneficial effects in patients with lymphatic cancers treated with actinomycin C, the extraordinary toxicity of these agents renders them of doubtful therapeutic value. Despite this, the hope remains that further search among the metabolic products of micro-organisms may lead to products of real utility in anti-tumour therapy.

Early studies on the actinomycins were hampered by the quality of the preparations; the original materials, judged homogeneous by classical

[28] Tytell, McCarthy, Fisher, Bolhofer, and Charney, " Antibiotics Annual ", Medical Encyclopedia, Inc., New York, 1954–5, p. 716.

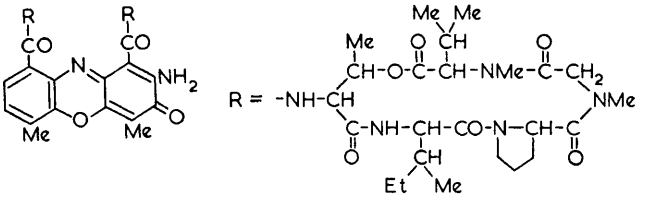
[29] Whitfield, Brock, Ammann, Gottlieb, and Carter, *J. Amer. Chem. Soc.*, 1955, **77**, 4799.

[30] Waksman and Woodruff, *Proc. Soc. Exp. Biol. Med.*, 1940, **45**, 609.

[31] Schulte, *Z. Krebsforsch.*, 1952, **58**, 500.

standards, were later shown to be mixtures. These were finally resolved by solvent-partition techniques.[32] To date, seven individual compounds have been characterised, and structures have been proposed for two. These consist of a polycyclic quinonoid chromophore linked to two cyclic peptide chains. Similarities in physical and chemical properties suggest that all the actinomycins may have the same chromophore and differ only in the nature of the peptide moieties. The elucidation of the structure of actinomycin C_3 by Brockmann and his collaborators [33] in Germany and of actinomycin D by Bullock and Johnson [34] in England required investigations in widely diverse areas of organic chemistry. The elegance of the work can only be suggested in the present Review.

Formula (46) has been provisionally proposed for actinomycin C_3. The bright red crystalline antibiotic appeared from early studies to be a very weak monoacidic base. A composition $C_{61}H_{90}O_{16}N_{12}$ was suggested by the results of elementary analyses, quantitative hydrogenation studies, and redox titrations. Quinone functionality was proposed from observations



(46)

both on the ready reduction of the antibiotic to the yellow dihydro-derivative and its ready reoxidation to the parent actinomycin, and on reductive acetylation to well-defined polyacetyl compounds. The presence of lactone or ester groups was indicated by the infrared spectrum of actinomycin C and by its consumption of alkali on titration at elevated temperature. Hydrolysis of the antibiotic gave a mixture of amino-acids ; a cyclic polypeptide structure was suggested by the absence in the parent compound of acidic or basic functionality other than the very weakly basic nuclear amino-group.

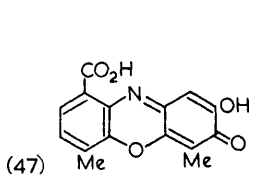
The lactone systems of actinomycin C_3 were hydrolysed in warm dilute methanolic alkali to give the dibasic actinomycinic acid C_3. Controlled acid-hydrolysis of this acid attacked the nuclear amino-group first and gave deaminoactinomycinic acid C_3. This was degraded further by acid to a series of deaminoactinocyl peptides, and finally, with loss of all of the amino-acid residues and of one carboxyl group, to the monocarboxy-compound actinocinin (47). The formation of actinocinin, either by the degradation sequence just mentioned or when carried out by direct vigorous acid hydrolysis of actinomycin C_3, was accompanied by further degradation by decarboxylation to 2-hydroxy-4 : 5-dimethylphenoxazin-3-one and by

[32] Roussos and Vining, *J.*, 1956, 2469.

[33] Brockmann *et al.*, *Angew. Chem.*, 1956, **68**, 70 ; *Chem. Ber.*, 1956, **89**, 1397, and earlier papers.

[34] Bullock and Johnson, *J.*, 1957, 3280, and earlier papers.

hydrolysis to 2 : 5-dihydroxytoluquinone (48). The structures of the decarboxylated phenoxazinone and of actinocinin itself were established by synthesis. The products were readily prepared by condensations of 2 : 5-dihydroxytoluquinone with 3-amino-2-hydroxytoluene and with 2-amino-3-hydroxy-4-methylbenzoic acid, respectively. Confirmation of the proposed relationship between actinomycin C_3 (46) and actinocinin (47) was secured through synthesis of (49), the dimethyl ester of actinocylbisglycine. This compound, prepared by oxidative self-condensation of 2-amino-3-hydroxy-4-methylbenzoylglycine methyl ester, was nearly identical with actinomycin in its colour reactions and ultraviolet absorption spectrum.



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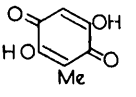

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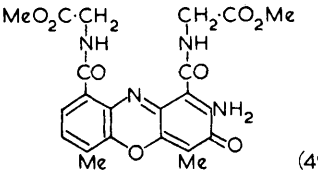
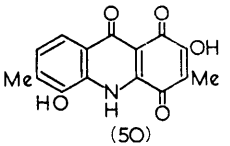


(49)

Under conditions of alkaline hydrolysis, rearrangement of the chromophoric structure occurred, and actinomycin C_3 yielded the constituent amino-acids and a fragment (50) designated depeptidoactinomycin. This rearrangement product has been obtained from all of the actinomycins that have been studied. Notable differences in acid stability, colour reactions, and absorption spectra indicated that depeptidoactinomycin was fundamentally different in structure from the actinomycin chromophore, and this was further demonstrated by the isolation of a dimethylacridine from (50) after zinc dust distillation. The acridone-1 : 4-quinone formulation of depeptidoactinomycin was confirmed by synthesis.

Studies on the identification and estimation of the amino-acids in hydrolysates of actinomycin C_3 indicated the presence of two mols. each of L-threonine, L-*N*-methylvaline, sarcosine, L-proline, and D-*alloiso*leucine. Hydrolysis of the methyl ketone obtained from actinomycinic acid C_3 (peptide lactones open) by a Dakin–West reaction failed to yield any *N*-methylvaline, and hence it was concluded that residues of this amino-acid must terminate each of the two opened peptide chains. Degradation of actinomycin C_3 with hydrazine gave somewhat more than one mol. of the dioxopiperazine from *N*-methylvaline and sarcosine, and this evidence was interpreted as suggestive of the adjacent disposition of these two amino-acids in the peptide chains. One characterised product of the controlled acid-hydrolysis of actinomycin C_3 was deaminoactinocylthreonine, in which only a threonine residue was linked to the nucleus ; thus it was evident that at least one of the peptide chains was linked to the chromophore through threonine. On the basis of these and other observations, Brockmann and his group have provisionally advanced a formulation of the peptide portion of actinomycin C as represented in (46), but they have indicated that some points of the structure of the peptide chains remain to be established.

Bullock and Johnson have established in somewhat more rigorous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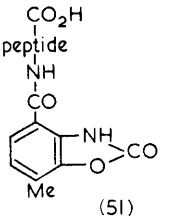
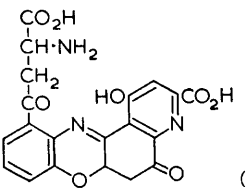

(51)

fashion [34] that actinomycin D has the same structure as that represented in (46) for actinomycin C_3, except that the two D-*alloiso*leucine residues have been replaced by D-valine. These investigators discovered that alkaline peroxide cleaved the chromophoric structure and permitted the separation of the two intact peptide chains, in each case with the original lactone ring open. One peptide was linked to 7-methylbenzoxazolone-4-carboxylic acid, as indicated in (51), and the other to oxalic acid.

Mild acid-hydrolysis of the peptide (51) selectively liberated *N*-methylvaline; the result of a Dakin–West degradation confirmed the conclusion that *N*-methylvaline was located at the carboxyl end of the chain. That sarcosine was attached to *N*-methylvaline was indicated by the isolation of the dioxopiperazine of these two amino-acids after high-vacuum pyrolysis of the peptide. Somewhat more drastic hydrolysis of the peptide (51) liberated proline, sarcosine, and *N*-methylvaline, only traces of valine, and no threonine. The remaining valine and some threonine were released on more prolonged hydrolysis. Since the peptide itself did not possess a free amino-group, it was thus possible to formulate the peptide chain as –NH–threonine–valine–proline–sarcosine–*N*-methylvaline–CO_2H. It was shown in similar fashion that the amino-acids of the second peptide chain, attached to oxalic acid, were arranged in the same way.

Before leaving the chemistry of the actinomycins, it is interesting to note that the unusual phenoxazin-3-one structure common to this group of antibiotics appears also in xanthommatin (52), isolated from the eyes of insects. According to Butenandt,[35] this compound is the prototype of a family of naturally occurring pigments of much importance in the field of chemical genetics. A series of substituted phenoxazin-3-ones has been synthesised; [36] the 2-amino- and the 2-*NN*-diethylglycylamino-derivative were reported to be highly bacteriostatic.


(52)

Azaserine.—The compound *O*-diazoacetyl-L-serine (53) was isolated from filtrates of a *Streptomyces* [37] and has been designated azaserine. It is moderately active against a number of bacteria and fungi, but is essentially without activity upon protozoa and viruses. It is quite effective against

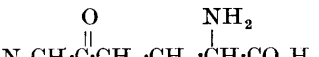
[35] Butenandt, *Angew. Chem.*, 1957, **69**, 16.

[36] Yuasa, *Chem. Abstr.*, 1954, **48**, 12900.

[37] Coffey, Hillegas, Knudsen, Koepsell, Oyaas, and Ehrlich, *Antibiotics and Chemotherapy*, 1954, **4**, 775.

some experimental mouse tumours. The light yellow-green, crystalline antibiotic was assigned diazo-ester functionality on the basis of its infrared spectrum and failure to react with hydroxylamine. Hydrolysis in hot 2N-formic acid gave L-serine and glycollic acid. Held in solution at pH 2 and room temperature, azaserine was converted into *O*-glycollyl-L-serine. Hydrogenation converted azaserine into *O*-acetyl-L-serine.[38] Several syntheses of azaserine have been recorded,[39] all involving the preparation and diazotisation of *O*-glycyl-L-serine. The D-serine analogue, similarly prepared, showed no anti-tumour activity. A series of publications [40] from Buchanan's laboratory on the mode of action of azaserine deals with its effects in the inhibition of purine synthesis by the enzyme systems of pigeon liver. Esters of serine with various substituted acids have been prepared,[41] as have also diazoacetyl esters of a number of hydroxy-amino-acids; none of these azaserine analogues has had significant anti-tumour activity.

(53)



(54)

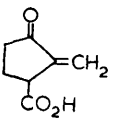
DON.—6-Diazo-5-oxo-L-norleucine (54), abbreviated DON, was also isolated from a *Streptomyces*.[42] Although only weakly active against bacteria and fungi, DON has a powerful inhibitory action upon an experimental mouse cancer.[42, 43]

Preliminary structural studies indicated that the antibiotic possessed the composition $C_6H_9O_3N_3$ and was an amino-acid with diazoketone functionality. Periodate cleavage gave L-glutamic acid, permitting the formulation of DON as 2- or 4-amino-6-diazo-5-oxohexanoic acid. Under conditions of the Wolff rearrangement, DON gave α-aminoadipic acid, establishing the correctness of structure (54). The compound was synthesised [44] from L-glutamic acid α-methyl ester by a series of reactions involving protection of the amino-group, conversion of the carboxyl group *via* the acid chloride into the diazo-ketone, and removal of the protective group.

Alazopeptin.—This new anti-tumour antibiotic isolated from an actinomycete has been only partially characterised.[45] It appears to be a structural analogue of azaserine and DON containing 1 mole of α-alanine and 2 moles of an amino-6-diazo-5-oxohexanoic acid.

[38] Fusari, Haskell, Frohardt, and Bartz, *J. Amer. Chem. Soc.*, 1954, **76**, 2881.
[39] Nicolaides, Westland, and Wittle, *ibid.*, p. 2887.
[40] Levenberg and Buchanan, *ibid.*, 1956, **78**, 504.
[41] DeWald, Behn, Moore, Morgan, and Renfrew, Abs. Papers, 129th Meeting, Amer. Chem. Soc., 1956, 16M.
[42] Ehrlich, Coffey, Fisher, Hillegas, Kohberger, Machamer, Rightsel, and Roegner, *Antibiotics and Chemotherapy*, 1956, **6**, 487.
[43] Clarke, Reilly, and Stock, Abs. Papers, 129th Meeting, Amer. Chem. Soc., 1956, p. 12M.
[44] DeWald and Moore, Abs. Papers, 129th Meeting, Amer. Chem. Soc., 1956, p. 13M.
[45] DeVoe, Rigler, Shay, Martin, Boyd, Backus, Mowat, and Bohonos, "Antibiotics Annual", Medical Encyclopedia, Inc., New York, 1956–7, p. 730.

Sarkomycin.—Much interest has been excited recently by the discovery [46] of a weakly antibacterial antibiotic which has been reported to have significant activity against an experimental tumour in mice and is being studied clinically in cases of inoperable malignancies.[47] Sarkomycin was isolated in Tokyo from the products of *Streptomyces erythrochromogenes*. The structure, 2-methylene-3-oxo*cyclo*pentanecarboxylic acid (55) was assigned on the basis of studies at the Bristol Laboratories.[48] Sarkomycin, obtained only as an oil of doubtful purity, had an absorption spectrum suggestive of the presence of carbon–carbon unsaturation and carbonyl and carboxyl groups. Hydrogenation destroyed the antibacterial, but not the anti-tumour, activity of sarkomycin and gave dihydrosarkomycin, a crystalline optically active keto-acid, $C_7H_{10}O_3$, which contained one *C*-methyl group. Wolff–Kishner reduction of dihydrosarkomycin and conversion into the amide gave a product that appeared to be a methyl*cyclo*pentanecarboxyamide. Ozonolysis of sarkomycin gave formaldehyde, establishing the exocyclic nature of the double bond. Upon destructive distillation of the antibiotic, the double bond shifted into conjugation with the carboxyl group, and a compound identified as 2-methyl-3-oxo*cyclo*pent-1-enecarboxylic acid was obtained. From this and other evidence it was concluded that the chief active constituent of sarkomycin possessed structure (55).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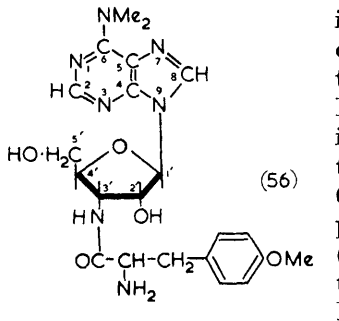
The synthesis of the (±)-isomer of sarkomycin by a Mannich reaction on ethyl 3-oxo*cyclo*pentanecarboxylate and subsequent thermal elimination of the β-amino-group and hydrolysis of the ester has been reported.[49]

Puromycin.—Despite possession of a considerable range of antibacterial activity, puromycin has primarily been of interest because of its action against infections caused by various protozoa and for its ability to inhibit an experimental cancer in mice. Degradative studies on puromycin, discovered in the Lederle Laboratories in 1952, led to the announcement [50] of its structure as 6-dimethylamino-9-[3′-deoxy-3′-(*p*-methoxy-L-phenylalanylamino)-β-D-ribofuranosyl]purine (56) in 1953. The work on the structure of the antibiotic has been recently reviewed by Binkley.[25] Some of the synthetic studies by Baker and his collaborators [51] will be presented here, since they contribute


(56)

[46] Umezawa, Yamamoto, Takeuchi, Osata, Okami, Yamaoka, Okuda, Nitta, Yagishita, Utahara, and Umezawa, *Antibiotics and Chemotherapy*, 1954, **4**, 514.

[47] Ishiyama, *J. Antibiotics (Japan)*, 1954, **7**, *A*, 82 ; 1955, **8**, *A*, 57 ; Fujii, Onizawa, Shima, Okuyama, and Okamoto, *ibid.*, p. 83.

[48] Hooper, Cheney, Cron, Fardig, Johnson, Johnson, Palermiti, Schmitz, and Wheatley, *Antibiotics and Chemotherapy*, 1955, **5**, 585.

[49] Tiki, *Bull. Chem. Soc. Japan*, 1957, **30**, 450.

[50] Waller, Fryth, Hutchings, and Williams, *J. Amer. Chem. Soc.*, 1953, **75**, 2025.

[51] Baker *et al.*, *ibid.*, 1955, **77**, 5911, and earlier papers.

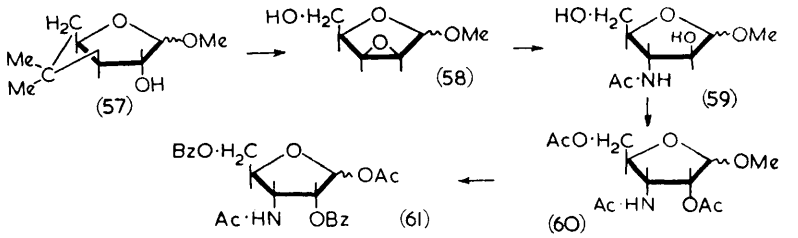
significantly to our fund of chemical knowledge and our understanding of structure–activity relations.

The 6-dimethylaminopurine moiety was constructed without difficulty by a series of well-established reactions starting with thiourea and cyanoacetic ester. In model studies, condensation of α-acetobromoglucose with the chloromercuri-derivative of 6-dimethylaminopurine or its 2 : 8-bismethylthio-derivative gave nucleosides which on the basis of their ultraviolet absorption spectra were 7- rather than 9-glucosides. Fortunately, coupling of the chloromercuri-derivative of the 2-methylthio-substituted purine with the acetohalogeno-sugar yielded the desired purine 9-glucoside.

Further model studies were required to establish conditions for elaboration of the purine 9-glycosides of amino-sugars, which had not previously been synthesised. It was found that the required bromo-sugar derivative was not stable under the coupling conditions, but that α-acetochloro-D-glucosamine could be condensed successfully with the methylthio-purine. The product on reductive desulphurisation and *O*-deacetylation gave the expected *N*-acetyl nucleoside.

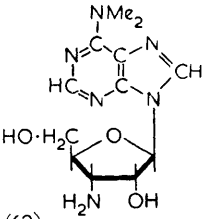
The starting material for the preparation of the ribose fragment required for the synthesis of puromycin was the 3 : 5-*iso*propylidene derivative of methyl D-xylofuranoside* (57). This was methanesulphonylated, the *iso*propylidene group was removed and the product treated with sodium methoxide, to form methyl 2 : 3-anhydro-D-lyxofuranoside (58). Oxide cleavage with ammonia was followed by *N*-acetylation to give 3-acetamido-3-deoxy-D-arabofuranoside (59). In the final series of reactions involving an inversion of stereochemical configuration, the arabinose derivative (59) was methanesulphonylated, and both sulphonyl groups displaced by the action of sodium acetate in hot " Cellosolve ", to yield the 3-acetamido-2 : 5-di-*O*-acetyl-3-deoxy-D-ribofuranoside (60). This was then converted into an anomeric mixture of the desired 3-acetamido-1-*O*-acetyl-2 : 5-di-*O*-benzoyl-3-deoxy-D-ribofuranosides (61).

Preparation of the chloro-sugar was carried out concurrently with nucleoside coupling by treating the titanium tetrachloride complex of the 1-acetylribofuranoside (61) with the chloromercuri-derivative of 6-dimethylamino-2-methylthiopurine in refluxing ethylene dichloride solution. The crude nucleoside so produced was desulphurised, debenzoylated, and de-*N*-acetylated, to yield the aminonucleoside (62) that had been obtained



* The anomers were separated by fractional distillation and carried separately through the synthesis.

previously by degradation of puromycin. Reaction of the aminonucleoside with the mixed anhydride (63) of *N*-benzyloxycarbonyl-*p*-methoxy-L-phenylalanine and ethyl hydrogen carbonate, followed by removal of the benzyloxycarbonyl group by hydrogenolysis, gave puromycin.



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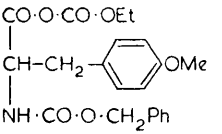
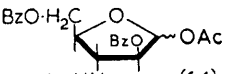


(63)

The α-anomer of the aminonucleoside (62) was synthesised by Baker and Schaub by a reaction sequence planned on their postulate that in this type of coupling the bulky purine moiety would always enter the sugar ring from the side opposite the group at $C_{(2)}$, regardless of the relative configurations at $C_{(1)}$ and $C_{(2)}$. Reactions similar to those outlined above were used to prepare the arabofuranoside (64). The nucleoside condensation proceeded normally; desulphurisation and debenzoylation gave the product (65), the 2′-epimer of the α-anomer of the *N*-acetyl derivative of (62). Inversion at 2′ was effected by methanesulphonylation and displacement by acetate ion.



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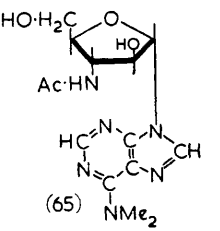


(65)

This summary of the work of Baker's group must suffice to indicate the excellence of their efforts in the field. Their further contributions include synthesis of the 2′-epimer of the aminonucleoside (62), use of the *N*-phthaloyl blocking group in the preparation of other aminonucleosides, discovery of an unusual degradation of the purine ring system, and syntheses of puromycin analogues which lack the sugar amino-group. Analogues of the aminonucleoside (62) which have variations in the alkyl groups on the pyrimidine nitrogen have also been made.[52]

Numerous publications deal with the physiological activity of puromycin and the many related compounds that have been prepared and studied. The interesting structure–activity relations can only be briefly indicated here. The amino-acid-free aminonucleoside (62) was highly effective against the mouse cancer and three to four times as active as puromycin

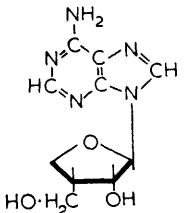
[52] Goldman, Marsico, and Angier, *J. Amer. Chem. Soc.*, 1956, **78**, 4173.

against the protozoan *Trypanosoma equiperdum*. However, in contrast to the original antibiotic, it was almost devoid of activity toward the dysentery pathogen, *Endamœba histolytica*, and inactive against bacteria. Interestingly, its α-anomer showed neither of the types of activity exhibited by the aminonucleoside itself.

Most of the derivatives of puromycin have significant anti-tumour activity in mice. Of the several amino-acid analogues prepared by Baker *et al.*, increased activity has been observed upon replacement of the *p*-methoxyphenylalanyl moiety of the parent compound by L-phenylalanyl, glycyl, leucyl, and glycyl-*p*-methoxy-L-phenylalanyl residues.[53] It was noted that the D-phenylalanyl analogue was inactive. The *p*-methoxy-group of puromycin, although apparently not necessary for anti-tumour activity, does have a specific function against *E. histolytica*, since the L-phenylalanyl analogue was devoid of action against this protozoan.[54]

Other Nucleoside Antibiotics.—Cordycepin (66) was isolated [55] from the mould *Cordyceps militaris* (Linn.) Link. It is active against strains of *B. subtilis* and an avian tubercle bacillus, but is not tumour-inhibitory. Acid hydrolysis gave adenine and a sugar, (−)-cordycepose, $C_5H_{10}O_4$; a 9-purine linkage was indicated by the ultraviolet spectrum. Formation of an osazone from cordycepose eliminated the possibility of a 2-deoxy-structure, and formulation as a 3-deoxypentose was suggested by the stability of the parent antibiotic to periodic acid. Cordycepose was oxidised with bromine to a lactone $C_5H_8O_4$ whose phenylhydrazide differed from those produced similarly from the four stereoisomeric straight-chain 3-deoxyaldopentoses. The usual structure assigned to the sugar on the basis of these data was confirmed by synthesis.[56]

Nebularine (67) was isolated [57] by Swedish investigators from the mushroom *Agaricus* (*Clytocybe*) *nebularis* Batsch. It is active against mycobacteria and is highly toxic in mice, but at high dilution attacks cancerous cells preferentially. The structure assigned to nebularine on the basis of degradative evidence has been confirmed by synthesis.[58]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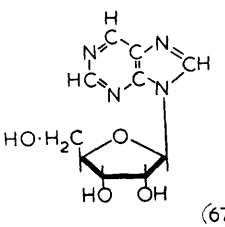
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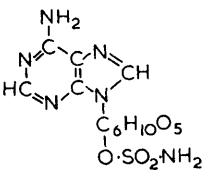
(68)

[53] Bennett, Halliday, Oleson, and Williams, "Antibiotics Annual", Medical Encyclopedia, Inc., New York, 1954–5, p. 756.

[54] Bond, Sherman, and Taylor, "Antibiotics Annual", Medical Encyclopedia, Inc., New York, 1954–5, p. 751.

[55] Bentley, Cunningham, and Spring, *J.*, 1951, 2301.

[56] Raphael and Roxburgh, *Chem. and Ind.*, 1953, 1034.

[57] Löfgren, Lüning, and Hedström, *Acta Chem. Scand.*, 1954, **8**, 670.

[58] Brown and Weliky, *J. Biol. Chem.*, 1953, **204**, 1019.

A recently reported product of a *Streptomyces*, nucleocidin, possesses broad-spectrum antibacterial activity and is also phenomenally active against *T. equiperdum* in mice, having 4000 times the potency of puromycin against this pathogen. Degradative studies [59] have suggested a sulphamic ester structure for nucleocidin, as indicated by the partial formula (68).

[59] Waller, Patrick, Fulmor, and Meyer, *J. Amer. Chem. Soc.*, 1957, **79**, 1011.