# *NOMENCLATURE OF THE ACTINOMYCINS**

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Actinomycin, discovered by Waksman and Woodruff in 1940,[1] was the first antibiotic to be isolated in crystalline form from a culture of an actinomycete (*Streptomyces antibioticus*). Waksman *et al.*[2] found that it was a red-pigmented substance, highly active against Gram-positive bacteria and, to a lesser degree, Gram-negative organisms; it proved to be extremely toxic to experimental animals. Waksman and Tishler[3] reported that actinomycin was optically active ($[\alpha]_D^{25} = -320° \pm 5°$), possessing a molecular weight of about 768–1,000, and gave a melting point of 250° C. It exhibited characteristic absorption in the visible (450 m$\mu$) and ultraviolet (between 230 and 250 m$\mu$) regions. On the basis of the analytical data, an approximate empirical formula, $C_{41}H_{56}O_{11}N_9$, was suggested. Actinomycin was thought to be a polycyclic nitrogen compound, possessing a quinonoid system. Initially, it was believed that actinomycin represented two active substances, which were designated A and B. Subsequently it was discovered that the B fraction consisted of lipoidal material contaminated with small quantities of A, and the recognition of an actinomycin B was dropped. The homogeneity of actinomycin A was based on the constant optical rotation and absorption spectra obtained when preparations of the antibiotic were crystallized repeatedly from acetone and from ethyl acetate.

Further studies[4] revealed the fact that actinomycin is produced by different species of actinomycetes belonging largely to the genus *Streptomyces*. Since then, numerous investigators have reported the isolation of actinomycin or actinomycin-like substances from different cultures of *Streptomyces*.[5, 6, 7, 8]

A crystalline preparation, isolated by Lehr and Berger[9] and designated "Antibiotic X-45," exhibited activity against bacteria and fungi and toxicity in mice very similar to that of actinomycin A. The color, melting point, optical rotation, and absorption spectrum of this preparation were in good agreement with the published data for actinomycin A. However, the elementary analyses (carbon value) and the molecular weights of these preparations appeared to differ. Dalgliesh and Todd[10] re-examined "Antibiotic X-45" and designated it actinomycin B. While some discrepancies existed between the analytical values of the two preparations, there was a remarkable degree of coincidence in their chemical and physical properties and, in particular, in their infrared spectra.[11] Vigorous acid hydrolysis of actinomycin B gave five amino acids: sarcosine, D-valine, L-threonine, L-proline, and *N*-methyl-L-valine.[12] On hydrolyzing a preparation of actinomycin A, the same five amino acids were found. The conclusion was reached that the two were probably identical.

Soon after, Brockmann and Grubhofer[13, 14] isolated from a culture of *S. chrysomallus* another actinomycin which they designated as C. It appeared to be different from A and B on the basis of crystal form, melting point, solubility, color reaction in ethanolic alkali, infrared spectrum (between 1500 and 700 cm.$^{-1}$), analytical composition (especially the lower nitrogen content), and the nature of the amino acids present. Hydrolyzates of actinomycin C contained the six amino acids: D-valine, sarcosine, L-threonine, L-proline, *N*-methyl-L-valine, and D-alloisoleucine.

As a result of screening various cultures for new actinomycin preparations, Brockmann and Pfennig[15] and Brockmann *et al.*[16] reported the isolation of a crystalline product that differed from actinomycin C in certain physical and chemical properties and that gave, on hydrolysis, the same amino acids as actinomycin B. It was concluded that this antibiotic was very similar to B; nevertheless, it was labeled "actinomycin X." Shortly thereafter, Brockmann[17] and Brockmann and Gröne[18] reported the isolation of another actinomycin preparation, which was considered to be similar to actinomycin A but was given the designation "actinomycin I."

Beginning in 1953, a renewed search for actinomycin-producing strains was conducted in our laboratories, resulting in the isolation from cultures of *S. parvullus* of still another crystalline substance which was named "actinomycin D."[19]

In addition to the actinomycins listed above, the alphabetical designations AA, AC, J, and M have been employed by several investigators to name other preparations isolated by them.

*Multiple Nature of the Actinomycins.*—Although actinomycins A, B, and C had been obtained as crystalline substances and were considered to be homogeneous compounds by criteria such as constancy of melting point and optical rotation after repeated recrystallization, it was soon realized that actinomycin C was not a single chemical compound but a mixture of very similar substances.[14] This conclusion was based on the fact that actinomycin C, prepared at different times, exhibited different characteristics (e.g., solubility in absolute alcohol, analytical values). By means of countercurrent distribution and circular paper chromatography, it was possible to separate actinomycin C into three distinct components, which were designated $C_1$, $C_2$, and $C_3$.[15, 20, 21] Subsequently, by countercurrent distribution and partition chromatography on cellulose columns, Brockmann and Gröne[22] also demonstrated the presence of two other components, present in trace amounts, which they named $C_{0a}$ and $C_0$. The chemical and physical properties of the various components are given in Table 1. Unfortunately, little biological information was presented.

Employing the same techniques of countercurrent distribution, circular paper chromatography, and cellulose column chromatography, Brockmann *et al.*[16, 18, 21, 22] separated actinomycin X into a number of components, designated $X_{0a}$, $X_0$, $X_1$, $X_{1a}$, $X_2$, $X_3$, and $X_4$. Only $X_1$ and $X_2$ were obtained in crystalline form, the latter representing 80–90 per cent of the complex. Brockmann and Pampus[23] reported briefly on the isolation of a crystalline fraction from $X_0$ which they named $X_{0\beta}$. The remaining components were present in trace quantities and have not been studied further (Table 1). It was also reported that $X_{0\beta}$ was four to five times less active against *Staphylococcus aureus* than $X_1$ and $X_2$.

Actinomycin I was also shown to be a mixture of several components, designated $I_{0a}$, $I_0$, $I_1$, $I_2$, and $I_3$. Only actinomycin $I_0$ and $I_1$ have been obtained in crystalline form, the latter making up more than 80 per cent of the complex.[22] No information was presented concerning their biological activity (Table 1).

Brockmann and Gröne[18] also examined samples of the actinomycin A studied by Waksman and Tishler[3] and the actinomycin B studied by Dalgliesh and Todd.[11, 12] They reported that actinomycins A and I were identical on the basis of their amino acid content, melting point, and behavior to circular paper chromatography.

TABLE 1

PROPERTIES OF PURE ACTINOMYCINS*

| Actinomycin Complex | Actinomycin Component | Crystal Form | Melting Point† (°C.) | Specific Rotation‡ | Specific Extinction§ | Amino Acid Composition (Moles/Mole of Actinomycin Mol. Wt. 1,200) | | | | | | | Relative Percentage of Components in Actinomycin Complex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | L-Threonine | Sarcosine | L-Proline | D-Valine | N-CH$_3$-L-Valine | D-Allo-Isoleucine | Hydroxy Proline | |
| C | $C_1$ | Bipyramids, prisms, or needles | 241–243 | $[\alpha]_D^{20} = -349° \pm 10°$ | 20.5 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | $C_0$, $C_{0a}$ } 2.0<br>$C_1$, 18.1 |
| | $C_2$ | Same as $C_1$ | 237–239 | $[\alpha]_D^{19} = -325° \pm 10°$ | 19.9 | 2 | 2 | 2 | 1 | 2 | 1 | 0 | $C_2$, 45.7 |
| | $C_3$ | Same as $C_1$ | 232–235 | $[\alpha]_D^{19} = -321° \pm 10°$ | 18.8 | 2 | 2 | 2 | 0 | 2 | 2 | 0 | $C_3$, 34.4 |
| X | $X_{0\beta}$ | Yellow-red needles | 245–247 | $-261° \pm 10°$ | 17.1 | + | + | + | + | + | 0 | + | $X_{0a}$, $X_0$ } 5.1 |
| | $X_1$ | Same as $C_1$ | 241–242 | $[\alpha]_D^{20} = -309° \pm 10°$ | 17.3 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | $X_1$, 5.1<br>$X_{1a}$, trace |
| | $X_2$ | Rhombic plates | 244–246 | $[\alpha]_D^{19} = -341° \pm 10°$ | 18.6 | 2 | 2 | 1 | 2 | 2 | 0 | 0 | $X_2$, 88.6<br>$X_3$, 0.5<br>$X_4$, 0.5 |
| I | $I_0$ | Same as $C_1$ | 242–243 | $[\alpha]_D^{20} = -314° \pm 10°$ | 20.5 | + | + | + | + | + | 0 | 0 | $I_{0a}$, 0.5<br>$I_0$, 7.3 |
| | $I_1$ | Same as $C_1$ | 240–242 | $[\alpha]_D^{20} = -353° \pm 10°$ | 20.7 | + | + | + | + | + | 0 | 0 | $I_1$, 86.5<br>$I_2$, 5.6<br>$I_3$, 0.5 |

* See Brockman and Gröne, *Naturwissenschaften*, **41**, 65, 1954, and *Chem. Ber.*, **87**, 1036, 1954; Brockman and Pampus, *Angew. Chem.*, **67**, 519, 1955.
† Kofler-Block.
‡ In methanol
§ At 445 mμ in methanol.

Actinomycins B and X behaved exactly alike on chromatograms, and the agreement in their melting points, amino acid contents, and other properties led to the conclusion that they were also identical. Subsequently, two crystalline components (named $B_1$ and $B_2$) were isolated from the B mixture and found to be identical with $X_1$ and $X_2$.[22]

Brockmann and Gröne[22] thus reported a total of 17 fractions from the actinomycins C, X, and I. Thirteen of these components were found to be different, namely, $C_{0a}$, $C_0$, $C_1$, $C_2$, $C_3$, $X_{0a}$, $X_0$, $X_{1a}$, $X_1$, $X_2$, $X_3$, $X_4$, and $I_0$; three were similar, namely, $C_{0a} = I_{0a}$, $C_1 = I_1$, and $C_2 = I_2$; it was also suggested that $C_3$ could be identical with $I_3$, although this was not certain.

The confusion in the actinomycin nomenclature had now attained the highest degree. Not only were actinomycins A, B, C, D, X, etc., reported, but each of these was found to be a mixture of closely related chemical components; it further appeared that each component was different from most of the others.

To ascertain whether such a multiplicity of actinomycins actually existed and to attempt a rationalization of the highly complicated actinomycin nomenclature, Vining and Waksman[24] and Gregory *et al.*[25] used paper chromatography for the separation of the various crystalline actinomycin preparations. They demonstrated that A, B, X, and C were mixtures of a number of components, whereas D was essentially a homogeneous substance. They also showed that the $C_1$ of actinomycin C and the D component were present in the A, B, and X preparations and that B and X actinomycins consisted essentially of the same components in slightly different proportions. Actinomycin A was very similar to B and X, whereas two of the components of C, namely, $C_2$ and $C_3$, were unique and were not found in any of the other preparations.

Roussos and Vining[26] subsequently corroborated and extended these initial findings. By means of circular paper chromatography and partition chromatography on powdered cellulose columns, they separated seven pure components from actinomycins A, B, and D. An examination of their chemical properties (Table 2) revealed that the actinomycin component $A_I$ is equivalent to $B_I$, that $A_{IV} = B_{IV} = D_{IV}$, and that $A_V = B_V$. A study of their biological properties fully confirmed these conclusions.[27] On the basis of these results, Roussos and Vining suggested that the naturally produced actinomycins A, B, X, and D are made up of the same components and that the differences observed may be attributed to the relative amounts of each produced by the respective *Streptomyces* species.

*Composition of Actinomycin A.*—An examination of the various samples of actinomycin A isolated in our earlier studies (1940–42) and of the more recent preparations, obtained from the same culture of *S. antibioticus*, revealed the fact that during the initial isolation and purification of actinomycin A on alumina[3] one of its components ($A_I$) was apparently lost. Such a sample of actinomycin A was sent to Brockmann for his examination. On the basis of paper chromatograms, Brockmann and Gröne[18] considered it to be a homogeneous substance and at first claimed that it was similar to, if not identical with, actinomycin I; later, however, they concluded that it was similar to the $I_1$ component.[22]

We have also examined the same A material but concluded that it was a mixture rather than a homogeneous entity. The components present corresponded in Rf value with those of B, though the relative amounts of each differed in the two

TABLE 2

PROPERTIES OF PURE ACTINOMYCINS*

| Actinomycin Complex | Actinomycin Component | Crystal Form | Melting Point† (° C.) | Specific Rotation‡ | Specific Extinction | Amino Acid Composition (Moles/Mole of Actinomycin of Mol. Wt. 1,200) | | | | | Relative Percentage of Components in Complex |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | L-Threonine | Sarcosine | L-Proline | D-Valine | *N*-$CH_3$-L-Valine | |
| A | $A_I$ | Plates | 237–238 | ... | 18.0 | 2 | 2 | 1 | 2 | 2 | $A_I$, 6.6<br>$A_{II}$, 2.9 |
| | $A_{IV}$ | Prisms | 235–236 | −261° | 19.2 | 2 | 2 | 2 | 2 | 2 | $A_{III}$, tr.<br>$A_{IV}$, 66.7 |
| | $A_V$ | Fine Needles | 245–246.5 | −323° | 19.6 | 2 | 2 | 1 | 2 | 2 | $A_V$, 23.8<br>$A_{VI}$, tr. |
| B | $B_I$ | Plates | 237.5–238 | −235° | 17.8 | 2 | 2 | 1 | 2 | 2 | $B_I$, 9.6<br>$B_{II}$, tr. |
| | $B_{IV}$ | Prisms | 235.5–236.5 | −268° | 18.8 | 2 | 2 | 2 | 2 | 2 | $B_{III}$, tr.<br>$B_{IV}$, 28.1 |
| | $B_V$ | Fine Needles | 246–246.5 | −320 | 19.2 | 2 | 2 | 1 | 2 | 2 | $B_V$, 59.3<br>$B_{VI}$, 3.1 |
| D | $D_{IV}$ | Prisms | 235.5–236.5 | −262° | 19.6 | 2 | 2 | 2 | 2 | 2 | $D_{IV}$, 100<br>$D_I$, $D_{II}$, $D_{III}$, $D_V$ in trace amounts |

* See Roussos and Vining, *J. Chem. Soc.*, p. 2469, 1956.
† Kofler hot plate.
‡ $[\alpha]_D^{23}$ (C, 0.25 in 95 per cent EtOH).

samples. Moreover, an $A_1$ component was lacking.[24-26] Since Brockmann and co-workers had reported that there were differences between I and X(B) in their behavior to paper chromatography in certain solvent systems as well as in the properties of the isolated pure components of each complex, the two sets of results appeared to be in conflict. It was concluded by Roussos and Vining[26] that Brockmann's earlier paper chromatographic evidence was probably in error and

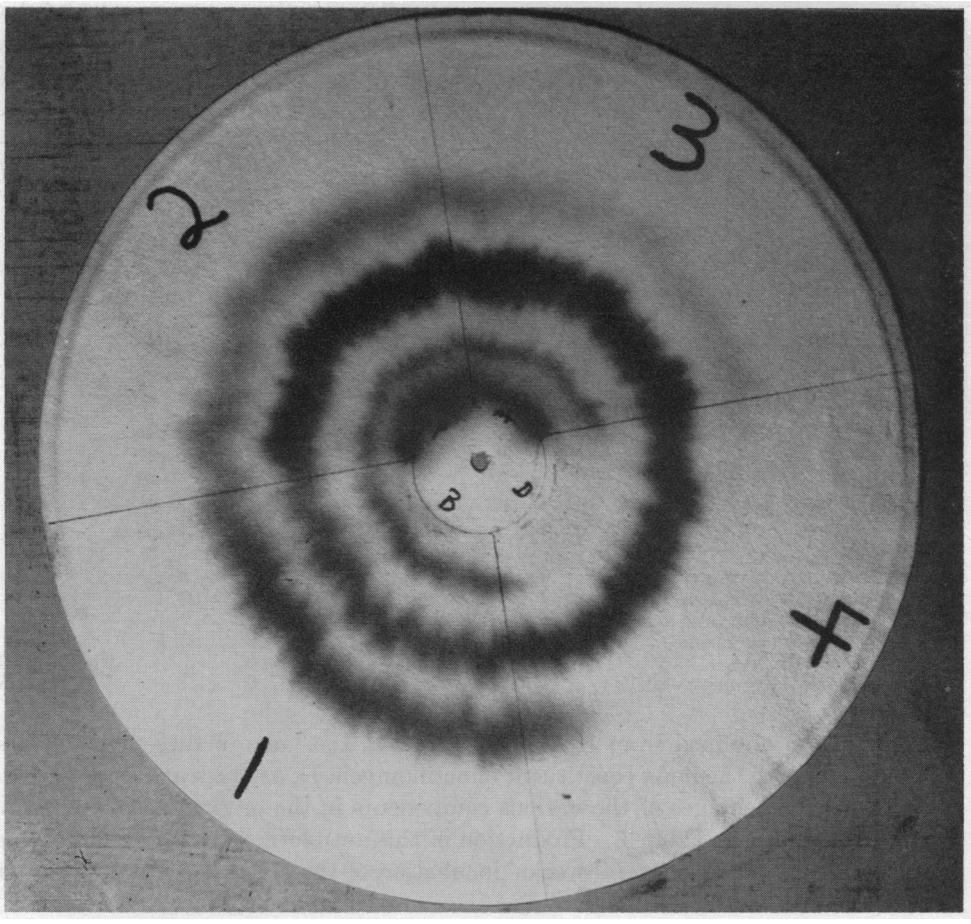


FIG. 1.—Circular paper chromatographs of actinomycin A, B, and D preparations. The system was composed of equal parts of a 10 per cent aqueous solution of sodium-*ortho*-cresotinate, and a mixture of ethyl acetate and *n*-dibutyl ether (2:1). *1*, actinomycin B; *2*, actinomycin A produced in 1948; *3*, actinomycin A produced in 1940; *4*, actinomycin D.

that, in fact, actinomycin I was different from A. The evidence for a close relationship between I and C seemed more plausible.[22]

In 1953, in order to carry out additional comparative studies with actinomycin A, we prepared fresh lots of this actinomycin employing the original culture of *s. antibioticus*, from which actinomycin A was first isolated in 1940. We found that the material produced could be considered as a B-type complex. While these results were quite unexpected, it was believed possible that the difference in the

process of cultivation of the organism employed in 1940 (surface process) and in 1953 (shaken process) might ifluence the composition of the actinomycin thus produced.

Since some questions have arisen concerning the composition of the actinomycin A complex, due, on the one hand, to the loss of component $A_1$ through purification[3] and, on the other, to the production of a B-type complex[25] in 1955, we decided to enlarge upon these investigations to resolve these discrepancies.

It has been possible to obtain from several different laboratories samples of the actinomycin A produced in our laboratory in 1940 and in 1948, and placed at that time at the disposal of various investigators in those laboratories. By means of circular paper chromatography, we were now able to show that actinomycin A preparations consisted of several components. By the use of different solvent systems, these components were found to be identical with those present in a sample of

TABLE 3

RELATIVE PERCENTAGES OF VARIOUS COMPONENTS OF ACTINOMYCIN A PREPARATIONS

| | Relative Percentage of Components | |
|---|---|---|
| Component | Actinomycin A Produced in 1940 | Actinomycin A Produced in 1948 |
| $A_I$ | 7.9 | 7.0 |
| $A_{IV}$ | 84.6 | 71.7 |
| $A_V$ | 7.5 | 24.1 |

TABLE 4

INFLUENCE OF NITROGEN SOURCE ON COMPOSITION OF ACTINOMYCIN COMPLEX

| | | Relative Percentage of Components | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Organism | Nitrogen Source | I | II | III | IV | V | VI | VII |
| *S. antibioticus* | L-glutamic acid | 6.2 | 2.1 | 2.8 | 80.4 | 8.4 | ... | .. |
| *S. antibioticus* | L-threonine | 7.1 | 2.4 | 2.9 | 29.2 | 58.4 | ... | .. |
| *S. antibioticus* | L-glutamic acid plus sarcosine | 7.1 | 24.6 | 35.6 | 27.2 | 5.5 | ... | .. |
| *S. chrysomallus* | $KNO_3$ | .. | ... | .. | 10.0 | | 60.0 | 40.0 |
| *S chrysomallus* | Glycine | .. | ... | .. | 7.0 | | 43.2 | 49.8 |

actinomycin B obtained from Hoffmann-LaRoche, Inc.; one of these components was identical with the main constituent of actinomycin D, as illustrated in Figure 1. The relative percentages of the various components in the original actinomycin A complex are given in Table 3. Production of this antibiotic was carried out under static conditions during several weeks' incubation of the culture, a procedure used in 1940.

Recent studies with another strain of *S. antibioticus* grown under both static and shaken conditions have revealed that, with increased time of incubation, there occurs in the actinomycin complex a continuous rise in the relative percentage of component $A_{IV}$ to 70–80 per cent and even more, and a concomitant drop of $A_I$ to 5 per cent and of $A_V$ to 10–20 per cent. Purification of such actinomycin A preparations was accompanied by a selective loss of $A_I$ (adherence to impurities on the alumina column, discarding certain fractions of the eluate), which thus gradually resulted in an actinomycin A lacking $A_I$. This conceivably occurred during the purification procedure employed by Waksman and Tishler[3]; the actinomycin A thus obtained in crystalline form consisted essentially of $A_{IV}$ and $A_V$ components.

The explanation for the production of a B-type complex by *S. antibioticus* in

1953 resides solely in the poor yields of the actinomycin. This culture, regardless of the medium used, was now unable to form more than a microgram or two of actinomycin per milliliter of broth. Recently it was found that cultures of *S. antibioticus* are able to form a B-type complex during the early stages of actinomycin production (0–30 $\mu g/m_1$) and later an A-type complex (30–150 $\mu_2$/ml), when grown on complex as well as on chemically defined media.[28, 29] The actinomycin extracted from a broth during the early stages of growth or when the antibiotic yield was extremely low was a B-type mixture.

*Effect of Age of Culture and Composition of Medium.*—Further studies of the course of biosynthesis of actinomycin yielded much information that has a bearing

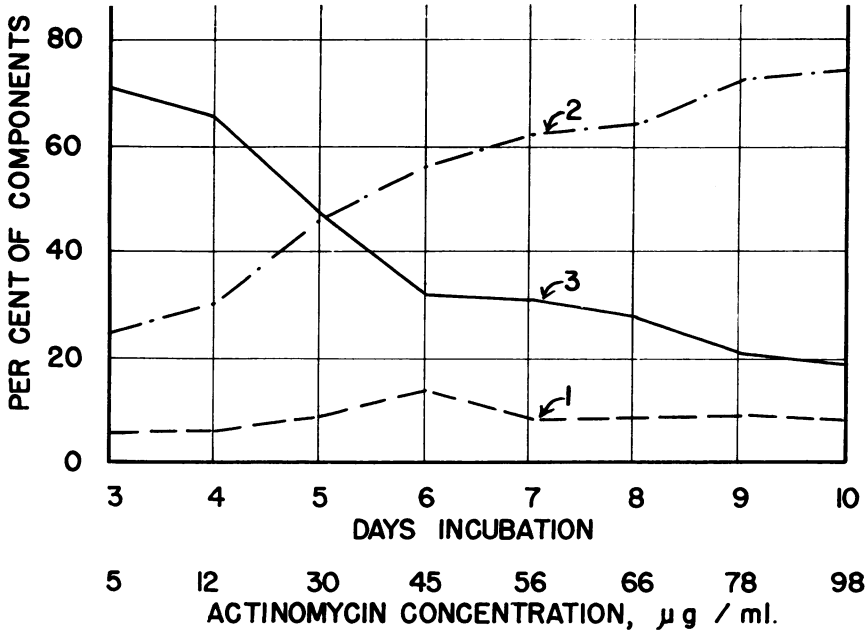


FIG. 2.—Relative percentages of the components in an actinomycin preparation produced by *S. antibioticus* 3720, growing on a galactose–glutamic acid–mineral salts medium. *1*, component $A_I$; *2*, component $A_{IV}$; *3*, component $A_V$.

upon the question of the nomenclature of the actinomycins. This information may be summarized briefly as follows:

During the course of actinomycin production in various complex and in chemically defined media by several strains of *S. antibioticus*, the proportion of the various components in the actinomycin preparations varied from day to day, so that a single component might represent 30 per cent of the actinomycin at one time and 70 per cent at another (Fig. 2). This was also true of other *Streptomyces* species producing actinomycin.[29]

The nature of the actinomycin formed by the various organisms also depended to a considerable extent on the nitrogen supply in the medium.[29] When threonine was present, *S. antibioticus* produced only the B-type complex, but when glutamic acid was supplied as the sole nitrogen source, the B type was first produced and then the A type. *Streptomyces* species 3723 (Streptomyces X-45 of Lehr and Berger)

produced a B-type actinomycin on many complex media, but, when supplied with glutamic acid, glycine, or $KNO_3$ in a chemically defined medium, the A type was formed. The actinomycin C producer, *S. chrysomallus*, gave a C-type complex which contained $C_2$ as the major component when $KNO_3$ was the source of nitrogen, but $C_3$ was the major constituent when glycine was the sole nitrogen source.

*Discussion.*—On the basis of the various chemical, biological, and microbiological studies presented in this paper, it is proposed that a revision of the existing actinomycin nomenclature be made.

Since a single *Streptomyces* culture is capable of producing mixtures of actinomycins with widely varying proportions of components, depending almost as much on the nutrient medium used and the age at which the culture is harvested as on the strain of organism, the use of symbols to designate either the number, the type, or the proportion of components present is certain to result in hopeless confusion. Moreover, the precise composition of the mixture would not be clearly indicated.

We now know that actinomycin complexes A, B, X, and D consist of the same chemical components but differ in the relative proportions of each present.[26] Actinomycin A (1940) actually represents the primary complex, which possesses the different components found in the actinomycins B, X, and D. Moreover, actinomycin $C_1$ is identical with component IV found in the above complexes. These results have been confirmed and extended by Brockmann‡, who has shown by circular paper chromatography that $A_I = B_I = X_{0\beta}$; $A_{IV} = B_{IV} = D_{IV} = C_1 = I_1 = X_1$; and $A_V = B_V = X_2$. Actinomycin $C_2$ and $C_3$ remain the only other clearly defined chemical entities which have been sufficiently characterized.

Corbaz *et al.*[30] concluded that there were three actinomycin types, namely, C, I, and X. They also stated that they clearly recognized the priority of the A and B complexes; however, they promptly proceeded to ignore such precedence. Brockmann[31] also recognizes only the existence of the actinomycin complexes C, I, and X. On the basis of the data obtained by Brockmann and reported here, as well as our own findings, it would be more proper to state that A = B = X and that D represents only a component ($A_{IV}$) found in the actinomycin A complex.

The relationship of actinomycin complex I to complexes A and C has never been clearly settled. Published paper chromatograms of I are not sufficiently well defined, so that it is impossible to decide what its components are related to. Brockmann has indicated, at one time or another, that I = A; $I_1$ = A; $C_{0a} = I_{0a}$, $C_1 = I_1$, $C_2 = I_2$; $C_1 = I_1 = A_{IV}$, and, in a recent publication,[31] I = D. If I is nearly homogeneous but possesses additional components in small concentrations, it may be similar to an A complex harvested after prolonged incubation of the culture (Table 3); or it may represent an A complex which has lost certain components through purification, or it may be similar to actinomycin D. It may also be a C complex, with $C_1$ as major component. In this case it should contain D-alloisoleucine. Data published on the amino acid content of the I complex and the pure components $I_0$ and $I_1$ indicate that it is absent, but if $I_2$ and $I_3$ are equivalent to $C_2$ and $C_3$ respectively and represent only about 6 per cent of the complex, it is not unlikely that its presence may have been overlooked on the chromatogram. In view of the results of Brockmann and Gröne[22] quoted above, it appears possible that I may bear the same relationship to C as A does to B and X.

The actinomycin preparations AA, AC,[32, 33] M,[34] and J[7, 35, 36] have been inade-

quately studied, and it is not entirely certain whether they represent new or previously described actinomycin complexes or components. For example, actinomycin AA and AC are fractions obtained by Sarlet through alumina chromatography of an actinomycin preparation. The amino acid content of the material was identical with that of actinomycin C; the amino acid composition of the fractions suggested that AA = $A_{IV}$ ($C_1$) and AC = $C_2$. Actinomycin M was examined in our laboratory and found to be of the B type. Until the nature of these preparations is more completely known, we propose that their designations be abolished.

Trace components designated as $C_{0a}$, $X_{0a}$, $X_4$, etc.,[22] may represent either actinomycin components or degradation products derived therefrom. The amount of these substances isolated in crystalline form has been negligible, and consequently little or no information is available concerning their chemical and biological properties. Until more data are published, we propose that such substances remain unclassified; the present practice of giving a designation to these poorly defined fractions has resulted in a rash of meaningless letters and numbers which contribute nothing but confusion to the actinomycin nomenclature.

The new biosynthetic actinomycins, designated E and F by Schmidt-Kastner,[37] and similar preparations we have obtained in our own laboratory may represent a unique group of actinomycins which have been produced through special conditions of nutrition. Whether they should be given new designations, e.g., E and F, or represent members of an actinomycin type normally produced in trace amounts, e.g., with sarcosine *S. antibioticus* produces an A complex containing up to 60 per cent actinomycin $A_{II}$ and $A_{III}$, remains to be decided on the basis of more complete chemical and biological characterization. This is brought out in Table 4.

*Conclusions.*—On the basis of the facts presented above, we therefore propose the following:

1. The term "actinomycin" should be restricted in use to chemically pure substances. Where a product is known to consist of a mixture, it should be referred to as an "actinomycin complex" or "actinomycin mixture," to indicate chemical inhomogeneity.

2. The present system of classification based on the combination of components present in varying amounts, e.g., A, B, X, C, etc., be abandoned. Also the use of letters, subscripts, and other confusing symbols be done away with. Since identical actinomycins can be found in different complexes, the use of any symbol indicating the complex of origin should be avoided. The pure actinomycin components should be renamed, but, in order to avoid superimposing a completely new set of symbols, only the Roman subscripts already employed shall be used:

| | |
|---|---|
| $A_I$, $B_I$, $X_{0\beta}$ | = Actinomycin I, |
| $A_{II}$, $B_{II}$ | = Actinomycin II, |
| $A_{III}$, $B_{III}$ | = Actinomycin III, |
| $A_{IV}$, $B_{IV}$, $D_{IV}$, $C_1$, $I_1$, $X_1$ | = Actinomycin IV, |
| $A_V$, $B_V$, $X_2$ | = Actinomycin V, |
| $C_2$ | = Actinomycin VI, |
| $C_3$ | = Actinomycin VII. |

Other minor components of different complexes and the new biosynthetic actino-

mycins might all be given a similar distinguishing symbol as soon as their homogeneity and difference from already named types is established.

3. The naturally produced actinomycin complexes will thus be made up of the various actinomycins in different proportions. What was known as "actinomycin A" will simply be an "actinomycin mixture" consisting of actinomycins I–V, and "actinomycin C" will consist of actinomycins IV, VI and VII.

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‡ Personal communication.

[1] S. A. Waksman and H. B. Woodruff, *Proc. Soc. Exptl. Biol. Med.*, **45**, 609, 1940.

[2] S. A. Waksman, H. Robinson, H. J. Metzger, and H. B. Woodruff, *Proc. Soc. Exptl. Biol. Med.*, **47**, 261, 1941.

[3] S. A. Waksman and M. Tishler, *J. Biol. Chem.*, **142**, 519, 1942.

[4] S. A. Waksman, W. B. Geiger, and D. M. Reynolds, these PROCEEDINGS, **32**, 117, 1946.

[5] M. Welsch, *Comp. rend. soc. biol.*, **140**, 1157, 1946.

[6] P. C. Trussell and E. M. Richardson, *Can. J. Research*, **26c**, 27, 1948.

[7] H. Umezawa, S. Hayano, T. Takeuchi, and Y. Mizuhara, *Japan Med. J.*, **1**, 100, 1948.

[8] W. Kocholaty, W. R. Junowicz-Kocholaty, and A. Kelner, *Arch. Biochem.*, **17**, 181, 1948.

[9] H. Lehr and J. Berger, *Arch. Biochem.*, **23**, 503, 1949.

[10] C. E. Dalgliesh and A. R. Todd, *Intern. Congr. Biochem. Abstr. Comm. 1st Congr., Cambridge, England*, p. 246, 1949.

[11] C. E. Dalgliesh and A. R. Todd, *Nature*, **164**, 830, 1949.

[12] C. E. Dalgliesh, A. W. Johnson, A. R. Todd, and L. C. Vining, *J. Chem. Soc.*, p. 2946, 1950.

[13] H. Brockmann and N. Grubhofer, *Naturwissenschaften*, **36**, 376, 1949.

[14] *Ibid.*, **37**, 494, 1950.

[15] H. Brockmann and N. Pfennig, *Naturwissenschaften*, **39**, 429, 1952.

[16] H. Brockmann, H. Linge, and H. Gröne, *Naturwissenschaften*, **40**, 224, 1953.

[17] H. Brockmann, *Angew. Chem.*, **66**, 1, 1954.

[18] H. Brockmann and H. Gröne, *Naturwissenschaften*, **41**, 65, 1954.

[19] R. A. Manaker, F. J. Gregory, L. C. Vining, and S. A. Waksman, *Antibiotics Ann.*, p. 853, 1954/1955.

[20] H. Brockmann, N. Grubhofer, W. Kass, and H. Kalbe, *Chem. Ber.*, **84**, 260, 1951.

[21] H. Brockmann and H. Gröne, *Naturwissenschaften*, **40**, 222, 1953.

[22] H. Brockmann and H. Gröne, *Chem. Ber.*, **87**, 1036, 1954.

[23] H. Brockmann and G. Pampus, *Angew. Chem.*, **67**, 519, 1955.

[24] L. C. Vining and S. A. Waksman, *Science*, **120**, 389, 1954.

[25] F. J. Gregory, L. C. Vining, and S. A. Waksman, *Antibiotics and Chemotherapy*, **5**, 409, 1955.

[26] G. G. Roussos and L. C. Vining, *J. Chem. Soc.*, p. 2469, 1956.

[27] L. H. Pugh, E. Katz, and S. A. Waksman, *J. Bacteriol.*, **72**, 660, 1956.

[28] W. A. Goss, E. Katz, and S. A. Waksman, these PROCEEDINGS, **42**, 10, 1956.

[29] W. A. Goss and E. Katz, *Appl. Microbiol.*, **5**, 95, 1957.

[30] R. Corbaz, L. Ettlinger, W. Keller-Schierlein, and K. Zähner, *Arch. Mikrobiol.*, **26**, 182, 1957.

[31] H. Brockmann, *Giorn. ital. chemioterap.*, **3**, 491, 1956.

[32] H. Sarlet, *Enzymologia*, **14**, 49, 1950.

[31] H. Sarlet, *Biochim. et Biophys. Acta*, **13**, 143, 1954.

[34] V. Monesi, V. Veronesi, and R. Craveri, Boll. Soc. ital. Ematol., **2**, 243, 1954.

[35] H. Umezawa, K. Maeda, and Y. Okami, *J. Antibiotics* (Japan), **4**, 335, 1951.

[36] A. Nishibori, *J. Antibiotics*, Ser. A, **9**, 31, 1956.

[37] G. Schmidt-Kastner, *Naturwissenschaften*, **43**, 131, 1956.