505. *Actinomycin. Part II.* Studies on the Chromophoric Grouping.*

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The molecular weight of actinomycin B has been revised on the basis of quantitative hydrogenation to 1240 ± 20 corresponding to an approximate formula $C_{61}H_{88}O_{16}N_{12}$. Hydrolysis with hot aqueous barium hydroxide leads to an insoluble purple barium salt which on acidification yields the red peptide-free quinone, actinomycinol B, $C_{16}H_{13}O_5N$, in which the original chromophoric system of actinomycin has been modified. The formulation of this compound as a substituted anthraquinone has been shown to be untenable and other structures containing a heterocyclic nitrogen atom are discussed.

In our previous paper (Dalgliesh, Johnson, Todd, and Vining, *J.*, 1950, 2946) we described the acid hydrolysis of the red quinonoid antibiotic actinomycin (Waksman and Woodruff, *Proc. Soc. Expt. Biol. N.Y.*, 1940, **45**, 609) and the identification of the five amino-acids which were obtained together with ammonia and other unidentified products in the hydrolysate. The amino-acids comprised sarcosine, L-threonine, methyl-L-valine, L-proline, and D-valine and it was of interest that this was the first report of the occurrence of sarcosine in peptide combination although since that time it has been reported in groundnut protein (Haworth, MacGillivray, and Peacock, *Nature*, 1951, **167**, 1068) and in the antibiotic vinactin C (Townley, Mull, and Scholz, Abstr., XIIth Intern. Congr. Chem., New York, 1951, p. 284).

The actinomycin B used for these studies was prepared by Lehr and Berger (*Arch. Biochem.*, 1949, **23**, 503) and it was shown (Dalgliesh *et al.*, *loc. cit.*) that the amino-acids obtained from the acid hydrolysis were identical with those obtained similarly from the actinomycin A of Waksman and Tishler (*J. Biol. Chem.*, 1942, **142**, 519). At the same time as we began our degradative investigations (Dalgliesh and Todd, *Nature*, 1949, **164**, 830), Brockmann and Grubhofer (*Naturwiss.*, 1949, **36**, 376) commenced chemical studies on actinomycin C which was very similar to actinomycins A and B except that it contained D-*allo*isoleucine in place of D-valine (*idem, ibid.*, 1950, **37**, 494). More recently Brockmann, Grubhofer, Kass, and Kalbe (*Ber.*, 1951, **84**, 260) have described a quantitative estimation of the amino-acids in their actinomycin C acid hydrolysate; the results—*N*-methyl-L-valine 42, L-proline 30, D-*allo*isoleucine 24, L-threonine 4·3, sarcosine 1·6, and D-valine 1%—led them to suggest that their starting material was not homogeneous and this was confirmed by counter-current distribution. It seems therefore that there is a group of actinomycins which differ slightly in the peptide portion of the molecule although there may be further differences revealed when the complete structure of the coloured fragment is fully known. Full details of the amino-acid content of another recently described actinomycin are not yet available although valine seems to be present and not *iso*leucine (Sarlet, *Enzymologia*, 1950, **14**, 49; *Nature*, 1951, **168**, 469), and nothing is known of the nature of the peptide in actinomycin J [Hirata and Nakanishi, *J. Penicillin (Japan)*, 1949, **2**, 180].

The molecular formula of actinomycin B was previously given as $C_{41}H_{58}O_{11}N_8$ on the basis of analyses and molecular weight determinations. Brockmann and Grubhofer (*loc. cit.*) originally formulated actinomycin C as $C_{40}H_{57}O_{11}N_7$ but a detailed study of the quantitative hydrogenation, on the assumption that the molecule contains only one quinone and no other group capable of reversible reduction, consistently gave a value of 1200 ± 25 for the molecular weight. In view of these results and the variable figures we have obtained for the molecular weight of actinomycin B by cryoscopic or ebullioscopic methods, we also have carried out quantitative hydrogenations and have obtained a value of 1240 ± 20 which necessitates a revision of the molecular formula of actinomycin B to

* Part I, *J.*, 1950, 2946.

$C_{61}H_{88}O_{16}N_{12}$. In consequence there are probably nine moles of amino-acid produced from each mole of actinomycin after acid hydrolysis rather than five as reported previously. A quantitative estimation of each of the five different amino-acids has not yet been carried out but the action of D-amino-acid oxidase (Dalgliesh *et al.*, *loc. cit.*) indicated that there was probably more than one D-valine unit.

Although Brockmann *et al.* (*Naturwiss.*, 1949, **36**, 376; *Ber.*, 1951, **84**, 260) reported that they were unable to prepare an acetyl derivative from actinomycin C and only a diacetyl derivative of the leuco-compound, we have obtained a homogeneous though non-crystalline orange tetra-acetyl derivative from actinomycin B (calculated on the basis of the revised molecular formula) and a crystalline yellow hexa-acetyl dihydro-derivative. Acetyl derivatives of actinomycin A were described by Waksman and Tishler (*loc. cit.*). Like the German workers we have been unable to obtain crystalline methyl derivatives from actinomycin, and no reaction has been observed with fluoro-2 : 4-dinitrobenzene.

All previous workers have agreed that the red actinomycins are quinonoid in nature on account of their reversible reduction and the ready formation of reduced acetyl derivatives. The vigorous acid hydrolysis which is required to liberate the individual amino-acids converts the quinonoid portion of the molecule into a black insoluble material and consequently, in order to study the nature of the chromophoric grouping, we have investigated the action of alkalis on actinomycin B. The action of 10% aqueous sodium hydroxide or barium hydroxide at room temperature caused slow evolution of ammonia (one mole) and formation of a red amphoteric amorphous product, the analysis of which approximated to $C_{61}H_{87}O_{17}N_{11},2H_2O$. The general properties of this compound still closely resembled those of actinomycin, *viz.*, the absorption in the visible and the ultra-violet region, the quinonoid nature, and the charring on treatment with hot concentrated hydrochloric acid, but it has not been investigated further as yet. The action of hot 2N-barium hydroxide on actinomycin B caused evolution of ammonia and rapid formation of a purple precipitate together with the five free amino-acids. This precipitate was a barium salt and on acidification yielded a red crystalline compound which was no longer a peptide. While this work was in progress Brockmann and Grubhofer (*loc. cit.*, 1950) issued a preliminary announcement of a similar degradation of their actinomycin C but as full experimental details of their work are not yet available it is not possible to make a detailed comparison of the two products, which are undoubtedly very similar. They named their compound despeptidoactinomycin C but this name is not altogether satisfactory as it implies that the compound contains the same chromophoric system as actinomycin itself. This is not the case, for, whereas actinomycin is readily charred by hot concentrated hydrochloric acid, this derivative is unchanged after treatment with 6N-hydrochloric acid at 100° for 24 hours, even in the presence of the free amino-acids liberated from the hydrolysis of actinomycin itself. Furthermore a comparison of the absorption spectra (Fig. 1) shows that a deep-seated change in the chromophoric system has occurred. We therefore propose to introduce the term actinomycinol B for this product from the hydrolysis of actinomycin B with barium hydroxide.

It was found that the best yields of actinomycinol B (about 27%) were obtained after heating actinomycin B with 2N-barium hydroxide for eight hours under reflux. During the hydrolysis one mol. of ammonia was evolved rapidly and a second very slowly, but no carbon dioxide evolution was observed. Actinomycinol B was purified by chromotography on silica, by sublimation, and by crystallisation from nitrobenzene, being then obtained as red needles which gave analytical values corresponding to a formula $C_{16}H_{13}O_5N$ and contained two *C*-methyl but no *O*-methyl or *N*-methyl groups. Molecular-weight determinations by quantitative micro-hydrogenation confirmed this formula. The compound appeared to be quinonoid because of its ready reversible reduction and to contain acidic hydroxyl groups because of its solubility in alkalis including sodium hydrogen carbonate. In all of these properties, as well as in the decomposition above 300° without melting, in the colours given with concentrated sulphuric acid (brown), sodium hydroxide (red), and sodium hydrogen carbonate (reddish brown), and in the fact that it was not reduced with stannous chloride, there was complete agreement with the properties described by Brockmann and Grubhofer (*loc. cit.*) for their despeptidoactinomycin C.

However, whereas the latter product was stated to be basic in that it could be extracted out of ether into 5% hydrochloric acid, the reverse was true of our compound and its basic properties were very weak indeed. Differences were also observed in the properties of the acetyl derivatives. Actinomycinol B formed a triacetyl derivative, m. p. 210° (from toluene), and a penta-acetyl leuco-derivative, m. p. 237—238°, but the triacetyl derivative of despeptidoactinomycin C was reported to have m. p. 164° when crystallised from toluene and 210° from methanol, and the penta-acetyl leuco-compound was reported as melting at 269—271°.

The reversible reduction of actinomycinol B together with the analysis of the acetyl compounds indicates the presence of a quinone but no other easily reducible group, and of at least two phenolic hydroxyl groups, and also that either the other oxygen atom or the nitrogen atom carries an acetylatable hydrogen atom. The two *C*-methyl groups being taken into account, actinomycinol B thus becomes $C_{12}H_5ON(2CO)(2CH_3)(2OH)$ and it would therefore appear that the molecule is essentially aromatic and polycyclic with no large aliphatic fragments. It may also be noted that, if the molecule is assumed to be carbocyclic and to contain three fused six-membered rings, then all the carbon atoms can be accounted for. In view of the wide natural distribution of hydroxyanthraquinone

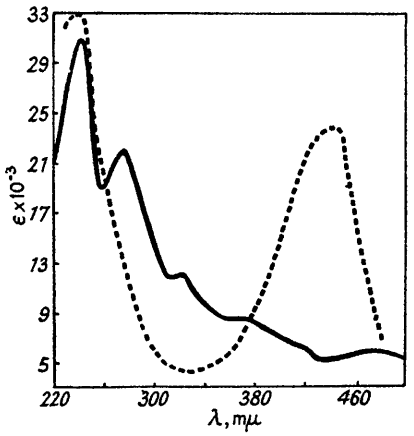


FIG. 1 *Ultra-violet absorption spectra of actinomycin B and actinomycinol B (in methanol).*

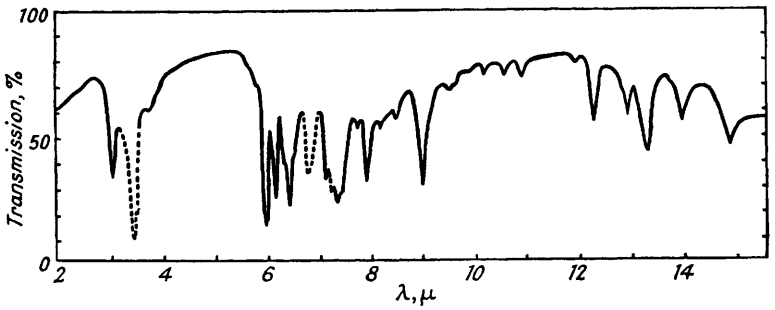
– – – – – *Actinomycin B.*
——— *Actinomycinol B.*

derivatives, we have examined very carefully the possibility that actinomycinol B might be an aminotrihydroxydimethylanthraquinone. At first sight there is much in favour of such a hypothesis; the colour and absorption spectrum and the facts that it sublimes without melting and gives a bright red solution in sodium hydroxide are all compatible with a hydroxyanthraquinone structure. The solubility in sodium hydrogen carbonate solution and aqueous ammonia would favour a β-hydroxyanthraquinone structure, whereas the brown coloration with ferric chloride is reminiscent of α-hydroxy- or α-amino-anthraquinones. The ready formation of lakes with magnesium or aluminium salts observed with actinomycinol B is also a property of anthraquinones containing hydroxy- and/or amino-groups in the *o*-positions to one another. The formation of only a triacetyl derivative from an aminotrihydroxy-compound might possibly be explained on steric grounds.

In the first place we sought to establish the function of the nitrogen atom in actinomycinol B and in particular to examine the possibility of a primary amino-group being present. The very weak basic properties coupled with the failure to obtain a condensation product with benzaldehyde made the presence of a β-aminoanthraquinone grouping very doubtful. On the other hand the characteristic colour reaction for α-aminoanthraquinones with sulphuric acid and paraformaldehyde (cf. I.G. Farbenind., G.P. 455,822) was not observed, and although one mol. of ammonia was liberated on fusion with potassium hydroxide this is by no means diagnostic of a primary amino-group. All attempts to remove the hypothetical primary amino-group by diazotisation followed by reduction

($NH_2 \longrightarrow H$) or hydrolysis ($NH_2 \longrightarrow OH$) have failed and we have been unable to obtain a nitrogen-free compound in this way although a wide variation in conditions of diazotisation was investigated. In parallel studies on authentic amino-hydroxy-anthraquinones, 4-aminoalizarin could be converted without difficulty either into alizarin ($NH_2 \longrightarrow H$) or purpurin ($NH_2 \longrightarrow OH$); 3-aminoalizarin gave alizarin by all methods (cf. Schultz and Erber, *J. pr. Chem.*, 1906, [ii], **74**, 275) and 1-amino-4 : 5 : 8-trihydroxyanthraquinone gave 1 : 4 : 5 : 8-tetrahydroxyanthraquinone by all methods. By diazotising actinomycinol B in nitrosylsulphuric acid and then heating the product with water at 100°, or by heating an ethanolic solution of the product under reflux or treating the product with cuprous oxide in cold ethanol (Hodgson and Turner, *J.*, 1943, 86), or by diazotising in ethanol with amyl nitrite and concentrated sulphuric acid and heating the solution (Knoevenagel, *Ber.*, 1890, **23**, 2995), products were obtained which still contained nitrogen and resembled the original material, although the analysis and properties of the acetylated products indicated that some changes had occurred.

FIG. 2. *Infra-red spectrum of actinomycinol B (Nujol mull).*



Further evidence against the presence of a primary amino-group has come from an examination of the infra-red spectrum of actinomycinol B (Fig. 2). The infra-red spectra of a number of aminoanthraquinones have been recorded by Flett (*J.*, 1948, 1441) who has shown that, whereas the normal carbonyl absorption band of anthraquinone is at 1676 cm.$^{-1}$, the presence of an amino-group in the molecule causes the appearance of a second absorption band at 1612 cm.$^{-1}$ for α-aminoanthraquinone and at 1625 cm.$^{-1}$ for β-aminoanthraquinone. All primary aminoanthraquinones in solution and most of them in the solid state show absorption bands characteristic of the NH_2-group, *e.g.*, at 3300 and 3420 cm.$^{-1}$ in 1-aminoanthraquinone and at 3220, 3330, and 3470 cm.$^{-1}$ in 2-aminoanthraquinone. In the case of actinomycinol B, however, the spectrum is complicated by the presence of hydroxyl groups. Flett (*loc. cit.*) showed that β-hydroxyanthraquinones, in which no hydrogen bonding with the quinonoid carbonyl groups is possible, show normal hydroxyl and carbonyl frequencies near 3350 and 1675 cm.$^{-1}$ respectively. The presence of an α-hydroxyl group, however, invariably caused the appearance of a low carbonyl frequency in the range 1590—1640 cm.$^{-1}$ while the usual hydroxyl absorption band did not appear. Similar effects have been noted with other hydroxy-polycyclic quinones (Johnson, Quayle, Robinson, Sheppard, and Todd, *J.*, 1951, 2633).

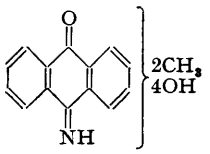
We have therefore examined the infra-red spectra of certain amino-hydroxyanthraquinones of known structure and have found that the above generalisations of Flett appear to hold good in this series also (Table).

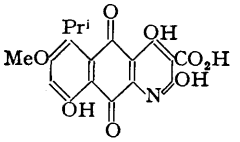
The infra-red spectrum of actinomycinol B indicates that there is one bonded and one non-bonded carbonyl group in the molecule and since there is only a single band in the amino-hydroxyl region of the spectrum it is unlikely that actinomycinol B can be an anthraquinone bearing a primary amino-group although the spectroscopic evidence does not exclude a possible secondary amino-group. Thus the combined evidence of chemical properties and infra-red spectrum eliminates a primary amino-anthraquinone structure for actinomycinol B. It has already been pointed out that a dimethylanthraquinone nucleus would account for all the carbon atoms of actinomycinol B, and hence secondary or

Carbonyl, amino-, and hydroxyl frequencies of amino-hydroxyanthraquinones.

	Frequency (cm.$^{-1}$)		
	C=O		NH_2 or OH
	(*a*)	(*b*)	
Anthraquinone	1676	—	—
1 : 2-Dihydroxy-3-amino-	1669	1631	3205, 3356, 3425, 3472
1 : 2-Dihydroxy-4-amino-	—	1613, 1587	3226, 3367
1 : 4 : 5-Trihydroxy-8-amino-	—	1592	3300, 3472
Actinomycinol B	1667	1616	3278

tertiary amino-groups could not be present as substituents; if actinomycinol B does not contain a primary amino-group it cannot be a derivative of anthraquinone. The possibility of an iminoanthraquinone formula (I) is eliminated (cf. Scholl and Parthey, *Ber.*, 1906, **39**, 1201) because of the stability of actinomycinol B towards strong acids and the results of acetylation.

(I) 



(II)

Another point of difference between actinomycinol B and the authentic amino-hydroxyanthraquinones was that a gradual shift in the position and intensity of the ultra-violet absorption bands occurred when actinomycinol B was examined in ethanol whereas the substituted anthraquinones were quite stable under these conditions. The acetyl derivatives of actinomycinol B were also unstable in alcohols, unlike the acetylated anthraquinone derivatives.

A decision on the precise nature of the nucleus in actinomycinol B must await the elucidation of the structure of the product from zinc dust fusion or, alternatively, the structures of the oxidative degradation products. Brockmann and Grubhofer (*Naturwiss.*, 1950, **37**, 494) isolated a colourless compound, m. p. 80°, from the zinc dust distillation of their despeptidoactinomycin C, and we have carried out some preliminary experiments with actinomycinol B which suffice to confirm that the latter compound is not a substituted anthraquinone. Hydroxy-methylanthraquinones are known to be converted into methylanthracenes by distillation with zinc (*e.g.*, Flumiani, *Monatsh.*, 1924, **45**, 43) although cases of the removal or migration of methyl groups have been reported (*e.g.*, Fairbourne and Foster, *J.*, 1930, 1275) and we have obtained mixtures of methylanthracenes from 1 : 3- and 1 : 4-dimethylanthraquinones. Anthracene was isolated without difficulty from 3- and 4-aminoalizarins and from 1-amino-4 : 5 : 8-trihydroxyanthraquinone by the action of zinc in a zinc chloride–sodium chloride melt (Clar, *Ber.*, 1939, **72**, 1645), but with actinomycinol B a similar process gave a very small yield of a neutral colourless crystalline substance which has not yet been obtained sufficiently pure or in sufficient quantity (<1 mg.) to carry out carbon, hydrogen, and nitrogen analyses. It had m. p. 117—121° but the position of the absorption bands in its ultra-violet spectrum (maxima at 2270, 2720, and 2800 Å) which was unchanged on acidification did not correspond to those of the alkylanthracenes (Clar, *Ber.*, 1932, **65**, 506) and the chromatographic properties of the compound were also at variance with those of authentic methylanthracenes. Further work on this compound is in progress and a discussion on its structure must be deferred until more information is available. However, it is evident that any structure for actinomycinol B must account for the following facts: (*a*) it is virtually non-basic and gives rise to a colourless, neutral compound on zinc dust fusion; (*b*) its infra-red spectrum shows the presence of no more than one hydrogen atom directly attached to nitrogen; (*c*) it yields triacetyl and penta-acetyl dihydro-derivatives in which at least one of the acetyl groups is apparently labile. It may be significant that if the molecule of actinomycinol B contained a cyclic amide structure, $-N{=}C(OH)- \rightleftharpoons -NH-CO-$, this would account

for the lack of basicity, the potential >NH grouping, and the easily hydrolysable acetyl derivative (*e.g.*, Goutarel, Janot, Prelog, and Taylor, *Helv. Chim. Acta*, 1951, **34**, 1139). Cyclic amide structures are fairly common in Nature, and Kögl and his co-workers (*Rec. Trav. chim.*, 1940, **59**, 1180; 1944, **63**, 251; 1945, **64**, 23) have advocated the quinone structure (II) for the fungus pigment phomazarin although no positive evidence was obtained for the presence of the pyridone ring in so far as no pyridine derivatives were isolated either from oxidations or zinc dust fusions.

Experimental

Actinomycin B.—Anhydrous actinomycin B (92·9 mg.) in purified dioxan (10 c.c.) was hydrogenated in the presence of Adams's platinum catalyst. The absorption of hydrogen (1·87 c.c. at 25·5°/755 mm.) did not change on continuation of the experiment for a further hour, and the molecular weight found was 1258 (Calc. for $C_{61}H_{88}O_{16}N_{12}$: *M*, 1245). Another experiment gave a value of 1217. A sample of actinomycin B dried at 120°/0·1 mm. over phosphoric oxide was analysed [Found: C, 58·7; H, 7·1; N, 13·4; *C*-Me, 6·8; *N*-Me, 6·3; active H (Zerewitinoff), 0·39. Calc. for $C_{61}H_{88}O_{16}N_{12}$: C, 58·8; H, 7·1; N, 13·5; 3*C*-Me, 6·5; 3*N*-Me, 7·0; 5 active H, 0·40%).

Tetra-acetylactinomycin B.—(*a*) Actinomycin B (200 mg.) was dissolved in dry pyridine (10 c.c.), and acetyl chloride (3 c.c.) added dropwise to the stirred and well-cooled solution. After 16 hours, the mixture was diluted with water (25 c.c.), and the precipitate (100 mg.) collected. The product separated from aqueous ethanol as an amorphous orange-red solid, m. p. 227—229°, and could be chromatographed as a single band on neutral alumina (Found, in a sample dried at 90°/10^{-4} mm. over phosphoric oxide: C, 58·9; H, 6·4; N, 12·2; *O*-Ac, 11·8. $C_{69}H_{96}O_{20}N_{12}$ requires C, 58·7; H, 6·8; N, 11·9; 4*O*-Ac, 12·2%). The *acetyl* derivative was insoluble in light petroleum, water, and sodium hydroxide solution, but soluble in benzene, acetone, ethanol, ethyl acetate, and chloroform. It also formed a deep red solution in 10% hydrochloric acid.

(*b*) Actinomycin B (100 mg.) was suspended in acetic anhydride (2 c.c.), and 70% perchloric acid (1 drop) was added. After 12 hours, the solution was poured on ice. The precipitated acetyl derivative was identical with that obtained in the previous experiment. The ultra-violet absorption showed maxima at 4480 (ε, 21,700) and 4340 Å (ε, 21,400) and an inflection at 2180—2210 Å (ε, 38,400).

Hexa-acetyldihydroactinomycin B.—Actinomycin B (250 mg.) was suspended in acetic anhydride (1 c.c.) with zinc dust (1 g.), and 70% perchloric acid (1 drop) was added. The bright red solution slowly changed to pale green and after 12 hours became greenish-yellow. It was then poured on ice, the precipitate dissolved in chloroform, and the extract washed and dried. Concentration of the chloroform solution (to 3 c.c.) followed by dilution with carbon disulphide (12 c.c.) gave the *product* as yellow prisms (160 mg.), m. p. 262°, which was raised to m. p. 265—266° by recrystallisation from toluene (Found, in an air-dried sample: C, 56·4; H, 6·9; N, 11·3; *O*-Ac, 14·6; loss of weight on drying, 3·7. $C_{73}H_{108}O_{25}N_{12},3H_2O$ requires C, 56·4; H, 6·95; N, 10·9; 6*O*-Ac, 16·6; $3H_2O$, 3·5%). The product was insoluble in light petroleum or water, slightly soluble in ether, and soluble in benzene, acetone, ethanol (solution unstable on warming), and chloroform. It was insoluble in alkalis but readily dissolved in concentrated hydrochloric acid. The ultra-violet absorption showed a single maximum at 2830 Å (ε, 3570).

Hydrolysis of Actinomycin B with Cold Barium Hydroxide.—Actinomycin B (50 mg.) was suspended in 0·4N-barium hydroxide and kept at room temperature for 14 hours whereafter it had completely dissolved to a reddish-brown solution; ammonia was evolved during this time. The *product* could not be extracted from the alkaline solution with chloroform but after neutralisation it was extracted by this solvent and precipitated from it, by addition of ethyl acetate–acetone, as a red amorphous solid, m. p. 195—197°. It was insoluble in ether and benzene but soluble in chloroform, ethyl acetate, water, ethanol, and acetone. It gave no ferric reaction but was reversibly reduced with sodium dithionite [Found, in an air-dried sample: C, 57·4; H, 7·1; N, 12·1; active H (Zerewitinoff), 0·9; loss of weight on drying, 2·5. $C_{61}H_{91}O_{19}N_{11}$ requires C, 57·1; H, 7·15; N, 12·0; 12 active H, 0·94; $2H_2O$, 2·8%]. Light absorption: Maxima at 4460 (ε, 19,260) and 4300 Å (ε, 18,500) and an inflection at 2300—2340 Å (ε, 39,090).

Actinomycinol B.—(i) Actinomycin B (500 mg.) was heated with 2N-barium hydroxide (40 c.c.) under reflux for 8 hours. The actinomycin rapidly dissolved to form a brownish-purple solution and eventually a purple precipitate was deposited. Considerable frothing occurred during the heating and ammonia was evolved. The purple precipitate of the barium

salt was separated, washed, and treated with excess of dilute hydrochloric acid and then thoroughly extracted with chloroform; all the solid material was transferred to the organic phase. The chloroform extract was washed and the solvent removed, leaving an orange-red solid (69 mg.) which was extracted with ethyl acetate at room temperature. The residue from the extraction (24 mg.) was recrystallised from nitrobenzene and gave red needles with a bronze metallic reflex. They darkened above 300° without melting (Found, in a sample dried at 80° and 10^{-2} mm. for 12 hours over phosphoric oxide: C, 63·9; H, 4·4; N, 5·0; *C*-Me, 10·1; *O*-Me, 0·0; *N*-Me, 0·0. $C_{16}H_{13}O_5N$ requires C, 64·2; H, 4·4; N, 4·7; 2*C*-Me, 10·05%; *M*, 299). Light absorption in chloroform: Maxima at 2900 (ε, 23,400), 3000 (ε, 20,400), and 4920 Å (ε, 5540); inflexions at 2790—2820 (ε, 21,900) and 3220—3300 Å (ε, 4790) respectively. The product, *actinomycinol B* (23·6 mg.), in purified dioxan absorbed 2·28 c.c. of hydrogen when hydrogenated over Adams's platinum catalyst at 25·5°/758 mm., corresponding to a molecular weight of 266. Actinomycinol B was insoluble in water and petroleum, slightly soluble in chloroform, ether, ethyl acetate, and methanol, and soluble in acetone, acetic acid, pyridine, and dioxan. It was slightly soluble in concentrated hydrochloric acid, to an orange-red solution but was completely extracted therefrom by chloroform or ether. It was soluble in aqueous sodium hydrogen carbonate or aqueous ammonia to a red-brown solution but the deep red solution in sodium hydroxide was unstable and deposited a brown precipitate on being kept. Actinomycinol B could not be extracted into organic solvents from aqueous alkaline solutions. Its brown solution in sulphuric acid was not fluorescent, nor was its solution in acetic acid, and ethanolic solutions changed to dark brown with ferric chloride and violet-red with stannous chloride. Hydrogenation or zinc and acetic acid caused reversible reduction.

(ii) In similar hydrolysis the ammonia evolved was determined by titration as the hydrolysis proceeded. 0·84 Mol. was liberated after 1 hour, 1·7 mol. after 9 hours, and 2·1 mol. after 18 hours.

Triacetylactinomycinol B.—Actinomycinol B (41 mg.) was mixed with acetic anhydride (2 c.c.) and 70% perchloric acid (1 drop), and after 2 hours the product was poured on ice and the precipitate (40 mg.) recrystallised from toluene. *Triacetylactinomycinol B* was thus obtained as yellow needles, m. p. 209·5—210° (Found, in a sample dried at 80°/0·1 mm. over phosphoric oxide: C, 61·8; H, 4·4. $C_{22}H_{19}O_8N$ requires C, 62·1; H, 4·5%). The acetyl derivative was insoluble in hydrochloric acid and sodium hydrogen carbonate solution but dissolved in aqueous sodium hydroxide to a deep violet-brown solution. Light absorption in ether: Maxima at 4260 (ε, 5090), 2910 (ε, 19,500), 2770 (ε, 20,000), 2710 (ε, 20,400), and 2350 Å (ε, 49,680).

Penta-acetyldihydroactinomycinol B.—Actinomycinol B (22 mg.) and zinc dust (1 g.) were suspended in acetic anhydride (1 c.c.), and 70% perchloric acid (1 drop) added. After 48 hours excess of zinc was removed and the solution poured on ice. The precipitate was extracted with toluene and washed, and the extract, which had an intense blue fluorescence, was washed, dried, and concentrated until the product crystallised on cooling as pale yellow rods (19 mg.), m. p. 237—238° (Found, in a sample dried at 80°/0·1 mm. for 24 hours over phosphoric oxide: C, 61·5; H, 5·1; *O*-Ac, 41·1. $C_{26}H_{25}O_{10}N$ requires C, 61·1; H, 4·9; 5*O*-Ac, 43·6%). Light absorption in ethanol: Maxima at 3910 (ε, 4270), 3150 (ε, 7620), and 2650 Å (ε, 9320) and an inflexion at 2240—2380 Å (ε, 21,630).

Fusion of Actinomycinol B with Potassium Hydroxide.—Actinomycinol B (10 mg.) was heated with molten potassium hydroxide (300 mg.) during 45 minutes and the liberated ammonia (1·1 mol.) was determined by titration.

Diazotisation Experiments.—(i) *Cuprous oxide method.* 1-Amino-4 : 5 : 8-trihydroxyanthraquinone (25 mg.) was dissolved in sulphuric acid (0·25 c.c.) containing sodium nitrite (35·5 mg.), and the solution added slowly to glacial acetic acid (1 c.c.) cooled in ice. The resulting mixture was added to a suspension of cuprous oxide (0·17 g.) in ethanol (1 c.c.). After 3 hours, water (2 c.c.) was added and the precipitate separated, dried, and extracted with ether. The ethereal solution was evaporated and the residue recrystallised from aqueous ethanol, giving brown needles with a green metallic reflex whose colour reactions (sulphuric acid, sodium hydroxide, acetic acid, pyridine) were indistinguishable from those of authentic 1 : 4 : 5 : 8-tetrahydroxyanthraquinone. The product darkened and sublimed above 300° but did not melt. The acetyl derivative crystallised from toluene as yellow needles, m. p. 281·5—282·5° (Allen, Frame, and Wilson, *J. Org. Chem.*, 1941, **6**, 732, give m. p. 281—282° for 1 : 4 : 5 : 8-tetra-acetoxyanthraquinone). A mixture of 1-acetamido-4 : 5 : 8-triacetoxyanthraquinone, m. p. 274°, and 1 : 4 : 5 : 8-tetra-acetoxyanthraquinone formed two pale yellow bands when applied as a solution in benzene (500 parts)–ethanol (1 part) to a column of silica.

A similar experiment with actinomycinol B gave a product which still contained nitrogen

and, although very similar to the starting material, the acetylated product could be separated from triacetylactinomycinol B by chromatography.

(ii) *Hypophosphorous acid.* 4-Aminoalizarin (20 mg.) was dissolved in cooled sulphuric acid (0·25 c.c.) containing sodium nitrite (100 mg.). After 3 hours hypophosphorous acid (1 c.c. of 40%) was added dropwise to the cooled solution (ice). After 20 hours at 0°, the precipitate (9 mg.) was separated and recrystallised from aqueous ethanol, to give alizarin as orange needles, m. p. 289—291° alone or mixed with an authentic specimen.

(iii) *Amyl nitrite method.* 1-Amino-4 : 5 : 8-trihydroxyanthraquinone (37 mg.), amyl nitrite (100 mg.), and concentrated sulphuric acid (0·2 c.c.) were dissolved in ethanol (200 c.c.). After 10 minutes, the solution was heated under reflux for 1 hour, concentrated (to 10 c.c.), and diluted with water (20 c.c.). The precipitate (35 mg.) was recrystallised from pyridine, to give brown needles (16 mg.) of 1 : 4 : 5 : 8-tetrahydroxyanthraquinone identical with the product from (i) above.

A similar experiment with actinomycinol B gave a red crystalline product which still contained nitrogen and closely resembled the material obtained by method (i).

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