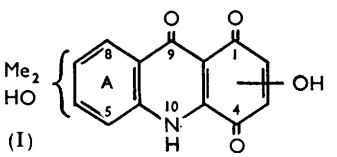
87. *Actinomycin. Part VI.* The Structure and Synthesis of Actinomycinol.*

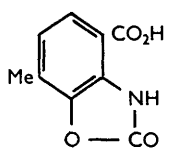
By W. G. HANGER, W. C. HOWELL, and A. W. JOHNSON.

Actinomycinol, a degradation product of actinomycin, has been shown to be 2 : 5-dihydroxy-3 : 6-dimethylacridone-1 : 4-quinone. The structure has been confirmed by synthesis.

THE action of hot aqueous barium hydroxide on actinomycin produces a crystalline peptide-free hydroxy-quinone, depeptidoactinomycin [1] or actinomycinol.[2] This compound has been obtained from all of the actinomycins examined,[3] suggesting that the chromophore is a common feature in the actinomycin group, although the absorption spectrum of actinomycinol, which is quite different from that of the parent actinomycin, clearly indicates that extensive rearrangement has occurred during the reaction.[2] Actinomycinol is a dihydroxy-quinone, $C_{15}H_{11}O_5N$, containing two *C*-methyl groups. A variety of *N*-and *O*-methyl, acetyl, and benzoyl derivatives of both actinomycinol and dihydroactinomycinol has been prepared by Brockmann and his colleagues.[3,4] The nature of the nucleus of actinomycinol has been the subject of several communications [1,2,5] but the correct partial structure (I), based on acridone-1 : 4-quinone, was suggested by Brockmann and Muxfeldt [6] in 1954. Although actinomycinol did not contain a carboxyl group it was soluble in aqueous sodium hydrogen carbonate, and this was interpreted as indicative of a hydroxyl substituent in the quinone ring (a vinylogous carboxyl system), which was confirmed by formation of a phenazine with *o*-phenylenediamine.



(I)



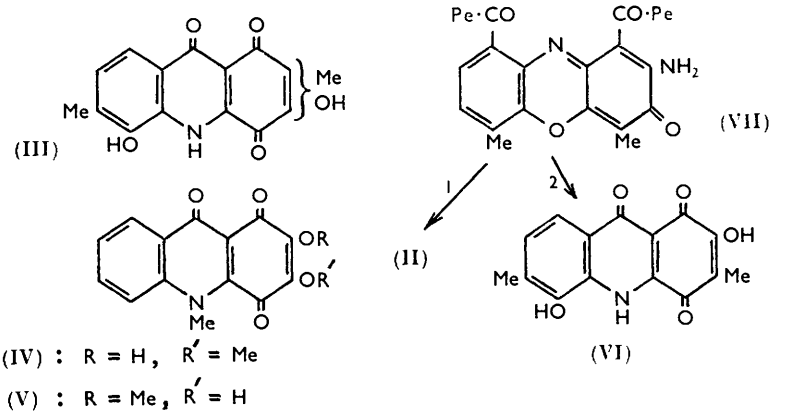
(II)

The infrared spectrum of actinomycinol [2] gives some support to the partial structure (I). In the carbonyl region, it shows strong bands at 1664 cm.$^{-1}$ with an inflection at 1656 (quinone-carbonyl groups) and 1616 cm.$^{-1}$ (" extended " amide carbonyl [7]). The spectrum also shows a sharp band at 3278 cm.$^{-1}$ (NH) corresponding to a band in the infrared spectrum of acridone at 3270 cm.$^{-1}$. The band at 810 cm.$^{-1}$ in the actinomycinol spectrum can probably be correlated with the presence of two adjacent $\geq$CH groups in the aromatic ring A.

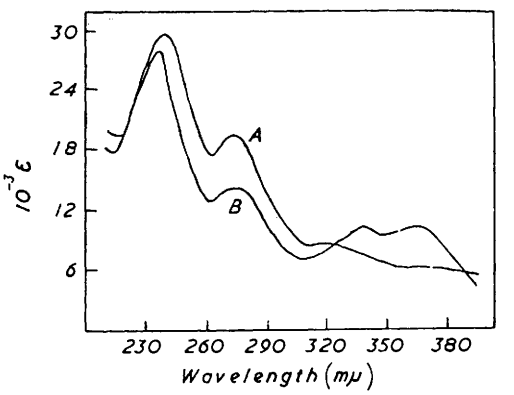
* Part V, *J.*, 1957, 3280.

[1] Brockmann and Grubhofer, *Naturwiss.*, 1950, **37**, 494; *Chem. Ber.*, 1953, **86**, 1407.
[2] Johnson, Todd, and Vining, *J.*, 1952, 2672.
[3] Brockmann and Vohwinkel, *Naturwiss.*, 1954, **41**, 257; *Chem. Ber.*, 1956, **89**, 1373.
[4] Brockmann and Muxfeldt, *Chem. Ber.*, 1956, **89**, 1379.
[5] Brockmann and Budde, *Naturwiss.*, 1953, **40**, 529.
[6] Brockmann and Muxfeldt, *Naturwiss.*, 1954, **41**, 500.
[7] Witkop and Goodwin, *J. Amer. Chem. Soc.*, 1953, **75**, 3371.

In Part IV [8] of this series, we described the degradation of actinomycin B to 7-methylbenzoxazolone-4-carboxylic acid (II). It is evident that the aromatic ring in compound (II) corresponds to ring A of actinomycinol, thus permitting an expansion of the structural formula to (III). The orientation of the remaining hydroxyl and methyl groups was determined by a comparison of the properties of actinomycinol with those of 2-hydroxy-3-methoxy-10-methylacridone-1 : 4-quinone (IV) and the 3-hydroxy-2-methoxy-isomer (V),



the structures of which have been proved by Crow and Price [9] in their structural work on melicopicine and related acridone alkaloids. Thus, whereas the quinone (IV) is red, both in the solid state and in solution, the isomer (V) is a greenish-yellow unstable compound giving green solutions in non-polar solvents. The absorption spectrum of the former



Ultraviolet absorption spectra of (1) *actinomycinol and* (2) *3-hydroxy-2-methoxy-10-methylacridone-1 : 4-quinone.*

closely resembles that of actinomycinol (see Figure), and the quinone-carbonyl band in the infrared spectrum is at 1668 cm.$^{-1}$ (actinomycinol, 1664 cm.$^{-1}$) whereas the corresponding band in the spectrum of the isomer (V) is displaced to 1635 cm.$^{-1}$. These observations, which are paralleled by the stabilities of isomers among the safranine dyes,[10] have led us to formulate actinomycinol as 2 : 5-dihydroxy-3 : 6-dimethylacridone-1 : 4-quinone (VI), and the relation between actinomycin [11] (VII) and its degradation products (II) and (VI) is illustrated.

The formation of actinomycinol involves several stages. On the phenoxazin-3-one

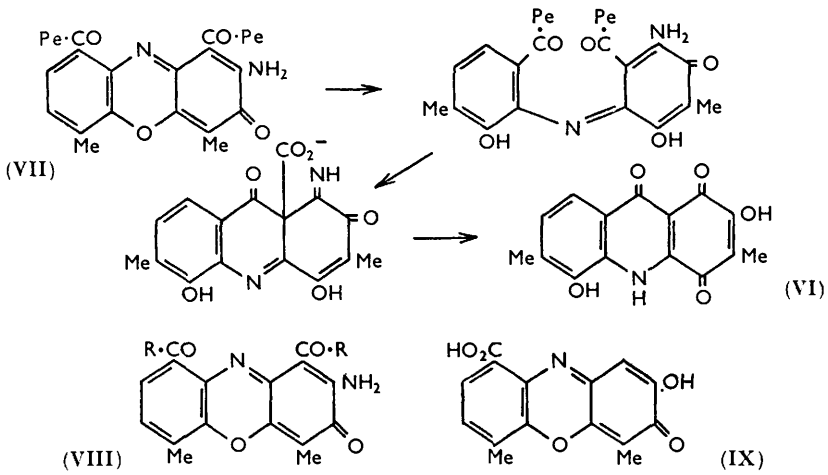
[8] Bullock and Johnson, *J.*, 1957, 1602.
[9] Crow and Price, *Austral. J. Sci. Res.*, 1949, **2**, 282.
[10] Balls, Hewitt, and Newman, *J.*, 1912, **101**, 1840.
[11] Brockmann, Bohnsack, Franck, Gröne, Muxfeldt, and Süling, *Angew. Chem.*, 1956, **68**, 70.

formulation (VII) for actinomycin, the reaction may be regarded as follows, although the precise order of the various stages has not been established and it is not suggested that the postulated intermediates are necessarily capable of separate existence: (1) hydrolysis of the ether (vinylogous ester) bridge of the heterocyclic ring; this is known [12] to occur with alkali under very mild conditions; (2) Dieckmann-type cyclisation, possibly with prior hydrolysis of the amide linkages, in order to relieve the strain in the 2 : 2′-disubstituted quinone anil; [12] (3) decarboxylation after hydrolysis of the peptide chains; (4) hydrolysis of the quinone-amino-group (amide character), and final rearrangement to actinomycinol.

Slight modifications of the actinomycin molecule seriously affect this sequence of events and preclude actinomycinol formation. For example, " deaminoactinomycin " (OH for NH_2 in VII) gives no actinomycinol on treatment with barium hydroxide [13] and likewise we have been unable to obtain actinomycinol from actinocin dimethyl ester [12,13] (VIII; R = OMe) or actinocyldi(glycine methyl ester) [13] (VIII; R = $NH{\cdot}CH_2{\cdot}CO_2Me$), now prepared from methyl 3-hydroxy-4-methyl-2-nitrobenzoate by a modified route.

The reaction of barium hydroxide with actinocin dimethyl ester (VIII; R = OMe) gave 2 : 5-dihydroxy-3-methylbenzoquinone, which also has been isolated by Brockmann and Muxfeldt [14] after reaction of actinomycin C with warm 20% hydrochloric acid. We have isolated this hydroxy-quinone, together with actinocinin [14,15] (IX) from the acid-degradation products of actinocin dimethyl ester.

A preliminary communication [16] of the deduction of structure (VI) was submitted for publication on the same day as one from Brockmann and Muxfeldt [17] who had reached a similar conclusion by careful analysis of the absorption spectrum of dihydroactinomycinol tetramethyl ether on the basis of their " displacement rule." [18]



Structure (VI) for actinomycinol has been confirmed by synthesis. In considering possible approaches, it was decided to build up the acridone system by cyclisation of the appropriate 2-carboxydiphenylamine, which itself could be obtained by condensation of an *o*-chlorobenzoic acid with 2 : 4 : 5-trimethoxy-3-methylaniline. This intermediate, however, proved to be rather inaccessible and in an independent synthesis of actinomycinol [19]

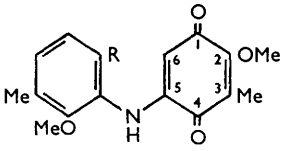
[12] Angyal, Bullock, Hanger, Howell, and Johnson, *J.*, 1957, 1592.
[13] Brockmann and Muxfeldt, *Angew. Chem.*, 1956, **68**, 69.
[14] *Idem, ibid.*, p. 67.
[15] Brockmann and Gröne, *ibid.*, p. 66.
[16] Angyal, Bullock, Hanger, and Johnson, *Chem. and Ind.*, 1955, 1295.
[17] Brockmann and Muxfeldt, *Angew. Chem.*, 1955, **67**, 617.
[18] Brockmann, Muxfeldt, and Haese, *Chem. Ber.*, 1956, **89**, 2174.
[19] Brockmann and Muxfeldt, *Angew. Chem.*, 1955, **67**, 618; *Chem. Ber.*, 1956, **89**, 1397.

the German workers substituted 4 : 5-dimethoxy-3-methyl-2-nitroaniline as the second component.

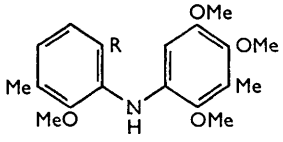
The difficulty of preparing these intermediates led us to investigate the formation of the arylaminoquinones by addition of suitably substituted anilines to *p*-benzoquinones. In the first instance it was proposed to introduce the carboxyl group (R = CO_2H in X) required for acridone formation by a separate process after the addition, but later the anthranilic acid was added directly to the quinone. These methods have the advantage that the requisite quinone, 2-methoxy-3-methyl-*p*-benzoquinone,[20] was obtainable from the intermediate amine, 2-methoxy-*m*-toluidine, by oxidation with chromic acid, an observation which suggested that actinomycin might be formed biogenetically by dimerisation of a benzenoid intermediate, as proved to be the case. Although the normal addition of arylamines to simple quinones affords both mono- and di-adducts,[21] only monoaddition occurs with 2-methoxy-3-methyl-*p*-benzoquinone, and the initial aminoquinol is oxidised immediately by the original quinone to give the adduct (X; R = H). Demethylation and/or demethoxylation has been reported in certain cases during the addition of amines to quinones under forcing conditions [22] but no such reaction was observed in the present instance.

In predicting the point of addition of the amine to 2-methoxy-3-methyl-*p*-benzoquinone it was expected that the effect of the methoxyl group was to neutralise partly the electron-withdrawing properties of the quinonoid 4-carbonyl group (vinylogous ester system) and the overriding directional influence for nucleophilic addition was therefore exerted by the 1-carbonyl group, causing the amine to add at $C_{(5)}$ as shown in (X; R = H). This was verified by hydrolysis of the adduct with sulphuric acid, 2 : 5-dihydroxy-3-methylbenzoquinone being obtained (dimethyl ether,[23] m. p. 104—105°).

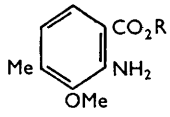
The quinone (X; R = H) was methylated reductively before attempts were made to introduce the carbonyl bridge, and the product, 2 : 4 : 5 : 2′-tetramethoxy-3 : 3′-dimethyldiphenylamine (XI; R = H) was treated with oxalyl chloride in the presence of aluminium chloride,[24] but yielded a complex mixture from which we were unable to isolate any of the desired products. In another attempt carbonyl chloride was substituted for oxalyl chloride but to no avail.



(X)



(XI)



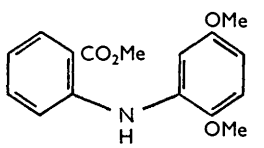
(XII)

The synthesis was therefore modified in such a way that the potential carbonyl group of the acridonequinone was included in the original amine before addition to the quinone, *i.e.*, the substituted anthranilic acid or its ester (XII; R = H or Me) was used in the addition reaction. The addition of anthranilic acids to benzoquinones is a well-known reaction, and mono- and di-adducts can be formed. In a recent study,[25] Acheson and Sansom reported only the diaddition product from anthranilic acid and *p*-benzoquinone but we have found that the monoaddition product [26] is obtainable if addition is carried out in aqueous acetic acid at room temperature and that it can be reductively methylated to 2 : 5-dimethoxy-2′-methoxycarbonyldiphenylamine (XIII).

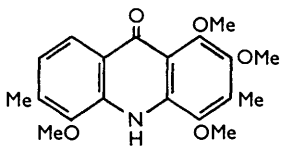
The 3-methoxy-4-methylanthranilic acid (XII; R = H) required for the synthesis of

[20] Majima and Okazaki, *Ber.*, 1916, **49**, 1482.
[21] Suida and Suida, *Annalen*, 1918, **416**, 113.
[22] Anslow and Raistrick, *J.*, 1939, 1446.
[23] Anslow, Ashley, and Raistrick, *J.*, 1938, 439.
[24] Stollé, *Ber.*, 1913, **46**, 3915; *J. prakt. Chem.*, 1922, **105**, 137.
[25] Acheson and Sansom, *J.*, 1955, 4440.
[26] Astre, *Bull. Soc. chim. France*, 1896, **15**, 1025.

actinomycinol was obtained from 7-methoxy-6-methylisatin,[8] itself prepared from 2-methoxy-*m*-toluidine, by oxidation with alkaline hydrogen peroxide according to the method of Baker *et al.*[27] Although the addition of unsubstituted anthranilic acid or its methyl ester to *p*-benzoquinone is complete after one hour at room temperature, addition of the acid or ester (XII; R = H or Me) to 2-methoxy-3-methylbenzoquinone was much slower and even at 60° in aqueous ethanol required several days. The product (X; R = CO_2H) was reductively methylated to yield the ester (XI; R = CO_2Me) which was hydrolysed to the acid (XI; R = CO_2H). This acid was cyclised by phosphorus oxychloride, and the 9-chloroacridine derivative (not isolated) was hydrolysed with dilute acid to a product, m. p. 158—159°, presumably (XIV), dihydroactinomycinol tetramethyl ether, or an isomer.



(XIII)



(XIV)

Reductive methylation[4] of actinomycinol (from actinomycin) gives a compound, m. p. 183—184°, also claimed to be dihydroactinomycinol tetramethyl ether, but the structural difference between this and the synthetic product is still under investigation. The synthetic acridone of m. p. 158—159° was demethylated completely by hydrobromic acid or pyridine hydrobromide and, after aerial oxidation of a weakly alkaline solution of the product, the actinomycinol so obtained was identical with that[2] derived from actinomycin in infrared, ultraviolet, and visible absorption spectra.

Experimental

95% Ethanol was used as solvent in determinations of absorption spectra, and Nujol mulls were used for infrared spectra, except where otherwise stated.

Actinomycinol B.—Prepared from actinomycin B as described in Part II,[2] it formed red needles after sublimation at 160°/10^{-4} mm. (Found: C, 63·0; H, 4·0; N, 5·0. Calc. for $C_{15}H_{11}O_5N$: C, 63·2; H, 3·9; N, 4·9%). The infrared spectrum showed max. at 3279, 2660, 2330, 1664, 1616, 1548, 1357, 1285, 1259, 1220, 1174, 1109, 1049, 1015, 980, 943, 915, 837, 812, 773, 750, 714, and 670 cm.$^{-1}$.

2-Hydroxy-3-methoxy-10-methylacridone-1 : 4-quinone[9] formed red needles, m. p. 236—238°, $\lambda_{max.}$ 235, 276, 336, and 365—368 mμ (log ε 4·44, 4·14, 4·00, and 4·01 respectively), $\nu_{max.}$ at 1668, 1600, 1580, 1520, 1410, 1310, 1280, 1260, 1205, 1165, 1135, 1095, 1060, 1040, 1020, 955, 930, 865, 820, 790, 770, 755, and 745 cm.$^{-1}$.

3-Hydroxy-2-methoxy-10-methylacridone-1 : 4-quinone[9] had infrared max. at 3250, 1635, 1575, 1540, 1475, 1300, 1255, 1220, 1190, 1165, 1130, 1100, 1040, 980, 945, 920, 860, 805, 780, 770, 765, 725, and 708 cm.$^{-1}$.

3-Benzyloxy-4-methyl-2-nitrobenzoic Acid.—A solution of potassium hydroxide (1·8 g.) in methanol (20 c.c.) was added to methyl 3-hydroxy-4-methyl-2-nitrobenzoate[12] (5·67 g.) and benzyl chloride (3·8 g.) in methanol (15 c.c.), and the mixture heated under reflux for 19 hr. The cooled mixture was diluted with water (200 c.c.) and extracted with ether (3 × 100 c.c.) and the combined ethereal extracts were washed and dried. Acidification of the aqueous layer and washings gave the unchanged hydroxy-ester (1·02 g.). After removal of the solvent from the ethereal solution, the oily residue so obtained was hydrolysed directly by boiling 20% aqueous sodium hydroxide (50 c.c.) for 5 min. After cooling, the product was diluted with water (150 c.c.) and then extracted with ether to remove neutral products. Acidification of the aqueous layer gave an almost quantitative (6·30 g.) yield, allowing for unchanged starting material, of the *product* which crystallised from methanol in colourless prisms, m. p. 176—177° (Found, on a sample sublimed at 160°/0·05 mm.: C, 62·7; H, 4·35; N, 5·1. $C_{15}H_{13}O_5N$ requires C, 62·7; H, 4·55; N, 4·9%).

N-(*3-Benzyloxy-4-methyl-2-nitrobenzoyl glycine Methyl Ester.*—The foregoing acid (6·32 g.) was

[27] Baker, Schaub, Joseph, McEvoy, and Williams, *J. Org. Chem.*, 1952, **17**, 141.

suspended in benzene and heated under gentle reflux with thionyl chloride (21 g.) for 1 hr. The resulting solution was concentrated under reduced pressure to remove the excess of thionyl chloride, and the residual acid chloride, which solidified on cooling, was redissolved in dry benzene (50 c.c.). Finely powdered glycine methyl ester hydrochloride (2·8 g.) was added and the mixture heated under reflux for 20 hr., then any undissolved material was separated from the hot solution. The filtrate was cooled and the crystals which separated were removed and washed with benzene and ether. By gradual concentration of the mother-liquors a further quantity of the product was obtained and the combined product (5·55 g., 70%) was recrystallised from aqueous ethanol, to give the *ester* as colourless feathery needles, m. p. 123—123·5° (Found: C, 60·5; H, 5·2; N, 7·9. $C_{18}H_{18}O_6N_2$ requires C, 60·3; H, 5·1; N, 7·8%).

N-(3-*Hydroxy*-4-*methylanthraniloyl*)*glycine Methyl Ester Hydrochloride*.—The nitro-ester (2·47 g.) from the preceding experiment was suspended in ethanol (25 c.c.) and hydrogenated over Raney nickel (1 g.) at atmospheric pressure and room temperature. After the absorption of hydrogen had ceased, the catalyst was separated and the filtrate diluted with ether (100 c.c.) and saturated with dry hydrogen chloride. The *amine hydrochloride* (1·58 g., 83%) slowly separated after gradual dilution of the solution with ether. Recrystallised from ethanol–ether it had m. p. 174—174·5° (decomp.) (Found: C, 47·9; H, 5·5; Cl, 12·85. $C_{11}H_{15}O_4N_2Cl$ requires C, 48·1; H, 5·5; Cl, 12·9%).

Actinocyldi(*glycine Methyl Ester*).—*N*-(3-Hydroxy-4-methylanthraniloyl)glycine methyl ester (0·438 g.) was dissolved in phosphate buffer (500 c.c.; pH 7·1) and kept at 40° while a solution of potassium ferricyanide (1·05 g.) in water (40 c.c.) was added, dropwise with stirring. After cooling, the product which had separated as a bright orange flocculent solid was collected, washed, and dried. It crystallised from chloroform (hot extraction from a Soxhlet thimble), to give *actinocyldi*(*glycine methyl ester*) (0·29 g., 78%) as fine orange needles, decomp. at 283—285° (Found: C, 56·5; H, 4·9; N, 11·8. $C_{22}H_{22}O_8N_4$ requires C, 56·2; H, 4·7; N, 11·9%), $\lambda_{max.}$ in MeOH, 237, 423, and 443 mμ (log ε 4·61, 4·45, and 4·48 respectively), in dioxan 239, 425, and 445 mμ (log ε 4·59, 4·44, and 4·46 respectively).

Action of Barium Hydroxide on Actinocin Dimethyl Ester.—A suspension of actinocin dimethyl ester [12, 13] (107 mg.) in 2N-barium hydroxide (20 c.c.) was heated under reflux for 4·5 hr. The ester slowly dissolved and purple-brown solid was precipitated. This was separated, washed with water, and dissolved in 2N-hydrochloric acid (30 c.c.) to give a brownish-orange solution, which was extracted with chloroform (3 × 50 c.c.). Removal of the solvent from the washed and dried extract gave a brown solid (19·5 mg.) which, unlike actinomycinol, was completely soluble in cold ethyl acetate. This material crystallised from nitrobenzene in copper-bronze plates which were further purified by sublimation at 90°/0·05 mm. The orange-red prisms of 2 : 5-dihydroxy-3-methylbenzoquinone thus obtained had m. p. 175—177° (partial decomp.) and formed a violet solution in dilute alkali (Found: C, 54·8; H, 4·1. Calc. for $C_7H_6O_4$: C, 54·55; H, 3·9%), $\lambda_{max.}$ 288 and 416 mμ (log ε 4·35 and 2·46), in chloroform 289 and 423 mμ (log ε 4·36 and 2·43).

Action of Hydrochloric Acid on Actinocin Dimethyl Ester.—The ester (313 mg.) was heated in 20% hydrochloric acid (60 c.c.) under reflux for 1 hr. On cooling, the deep red solution deposited very dark green crystals (232 mg.) which were separated, washed, and dried. The filtrate and washings were extracted with chloroform (4 × 100 c.c.), and the combined chloroform extracts washed, dried, and evaporated, to yield a crimson solid (20 mg.). This was sublimed and gave 2 : 5-dihydroxy-3-methyl-*p*-benzoquinone (8 mg.) as brownish-orange crystals, m. p. 174—177° (decomp.). The dark precipitate obtained as above was dissolved in saturated sodium hydrogen carbonate solution (50 c.c.) and then extracted with chloroform and ether. The aqueous solution was made slightly acid by addition of dilute hydrochloric acid, a dark red solid separating. It was extracted into chloroform (3 × 75 c.c.) and, by gradual concentration of the washed and dried chloroform solution, several crops of crimson needles of *actinocinin* [14, 15] were obtained which decomposed at 260° (Found, in a sample dried at 70° *in vacuo*: C, 62·9; H, 3·9; N, 5·0. $C_{15}H_{11}O_5N$ requires C, 63·2; H, 3·9; N, 4·9%). Light absorption max. were at 236 and 415 mμ (log ε 4·39 and 4·25).

2-*Methoxy*-3-*methyl*-p-*benzoquinone*.—A solution of 2-methoxy-*m*-toluidine (40 g.) in 10N-sulphuric acid (1 l.) was cooled to 0° and stirred vigorously while a solution of sodium dichromate (40 g.) in water (225 c.c.) was added during 1 hr. After an additional 5 hours' stirring the mixture was kept at <10° for 12 hr. It was then treated with a second portion of sodium dichromate (80 g.) in water (400 c.c.), as before. Stirring was discontinued after 3 hr. and the

black viscous mixture was extracted with ether (5 × 400 c.c.). The ethereal extract was washed and dried and the solvent distilled off through a small Vigreux column. Distillation of the residue under reduced pressure gave 2-methoxy-3-methyl-*p*-benzoquinone (14·7 g., 33%) as an orange-yellow oil, b. p. 104—105°/12 mm., which rapidly solidified to a yellow crystalline mass, m. p. 19—20° (Found: C, 63·4; H, 5·4. Calc. for $C_8H_8O_3$: C, 63·2; H, 5·3%).

2-*Methoxy*-5-(2-*methoxy*-m-*toluidino*)-3-*methyl*-p-*benzoquinone*.—To a suspension of 2-methoxy-3-methyl-*p*-benzoquinone (2·2 g.) in water (220 c.c.) was added a solution of 2-methoxy-*m*-toluidine (1 g.) in acetic acid (0·5 c.c.) and water (5 c.c.). Darkening was observed immediately and, within a short time, a purple precipitate had been formed. After the mixture had been shaken at room temperature for 5 hr. the precipitate was separated, washed with water, and dried (1·87 g.). In view of the ready polymerisation of the product it was used without further purification in the next stage (below). It had absorption max. at 520, 327, and 257 mμ (log ε 3·49, 3·88, and 4·14 respectively) and inflections at 333 and 276 mμ (log ε 3·87 and 4·03).

2 : 4 : 5 : 2′-*Tetramethoxy*-3 : 3′-*dimethyldiphenylamine*.—An ethanolic solution (75 c.c.) of the foregoing anilino-quinone (1 g.) was hydrogenated in the presence of Adams platinum catalyst (0·15 g.) for 2 hr., during which the colour changed from intense purple to pale green. Dimethyl sulphate (2 c.c.) and a suspension of finely ground anhydrous potassium carbonate (5 g.) and further catalyst (0·1 g.) in ethanol (50 c.c.) were then added to the hydrogenation product while still in an atmosphere of hydrogen. Hydrogenation was continued for a further 24 hr. After filtration through " Supercel " the ethanolic solution was almost colourless. As inorganic material was present after removal of the solvent, the dried residue was extracted with chloroform (4 × 50 c.c.), and the solvent removed from the combined extracts, to give a pale yellow oil (1·1 g.) which slowly solidified. Repeated recrystallisation from methanol gave colourless prisms of the *diphenylamine*, m. p. 107° (Found: C, 68·4; H, 7·4; N, 4·5. $C_{18}H_{23}O_4N$ requires C, 68·1; H, 7·3; N, 4·4%), $\lambda_{max.}$ 302 and 277 mμ (log ε 3·87 and 4·10), $\nu_{max.}$ 3440, 1605(s), 1587, 1510, 1488, 1455, 1420, 1385, 1370, 1360, 1310(s), 1250, 1225(s), 1205(s), 1162(s), 1130, 1090(s), 1060(s), 1005(s), 994(s), 852, 830, 808, 783(s), 750, and 712 cm.$^{-1}$.

2 : 5-*Dihydroxy*-3-*methyl*-p-*benzoquinone*.—2-Methoxy-5-(2-methoxy-*m*-toluidino)-3-methyl-*p*-benzoquinone (0·34 g.) was heated under reflux with 10N-sulphuric acid (7 c.c.) for 4 min. Initially the amino-quinone floated on the sulphuric acid as a purple oil but after 2—3 min. it dissolved and an orange sublimate collected in the condenser. The mixture was extracted with chloroform (5 × 25 c.c.), and the extracts were washed and evaporated *in vacuo*. Sublimation (95°/2 mm.) of the brown residue gave 2 : 5-*dihydroxy*-3-*methyl*-p-*benzoquinone* as orange prisms (0·1 g.), m. p. and mixed m. p. 173—175° (with sublimation) (Found: C, 54·7; H, 3·9. $C_7H_6O_4$ requires C, 54·6; H, 3·9%). This quinone gave a deep red ferric reaction and a violet colour with dilute alkali.

2 : 5-*Dimethoxy*-3-*methyl*-p-*benzoquinone*.—2 : 5-Dihydroxy-3-methyl-*p*-benzoquinone (34 mg.) in ethanol (15 c.c.) was shaken with dimethyl sulphate (0·2 c.c.) and anhydrous potassium carbonate (0·5 g.) at room temperature for 3 days. The solid was separated, the filtrate evaporated to dryness, and the residue treated with dilute hydrochloric acid (2 c.c.) and extracted with chloroform (3 × 10 c.c.). From the extract a yellow residue (3 mg.) was obtained. After two sublimations (95°/2 mm.), 2 : 5-dimethoxy-3-methyl-*p*-benzoquinone was obtained as golden-yellow needles (2 mg.), m. p. 104—105°. The brown solid separated as above was acidified and extracted with chloroform. After working up as described above, unchanged 2 : 5-dihydroxy-3-methylbenzoquinone was obtained as orange prisms (18 mg.), m. p. and mixed m. p. 173—174°.

2-2′-*Carboxyanilino*-1 : 4-*benzoquinone*.—To a suspension of *p*-benzoquinone (2·36 g.) in water (240 c.c.) was added anthranilic acid (1·0 g.) in acetic acid (2 c.c.). On addition of the acid an immediate purple colour developed and a brown solid separated. The reaction was complete after 4 hours' shaking at room temperature. Then the dark brown solid (1·8 g.) was separated and dried (P_2O_5) and used without further purification.

2 : 5-*Dimethoxy*-2′-*methoxycarbonyldiphenylamine*.—The foregoing quinone addition compound (1·8 g.) was dissolved in ethanol and hydrogenated in the presence of Adams platinum catalyst (0·2 g.). When hydrogenation was complete, dimethyl sulphate (4 c.c.) and a suspension of finely powdered anhydrous potassium carbonate (9 g.) and platinum catalyst (0·2 g.) in ethanol (90 c.c.) were added, the atmosphere of hydrogen being maintained. Hydrogenation was then continued for a further 60 hr. On exposure to air the resulting solution remained colourless and the residue obtained from filtration was washed with ethanol and then water, and the combined filtrates were evaporated *in vacuo* until no alcohol remained and oily green

drops appeared in the aqueous solution. Extraction with chloroform (5 × 25 c.c.) gave, after removal of the solvent and sublimation at 170°/1 mm., a pale green oil (2·2 g.) which slowly crystallised. Several recrystallisations from methanol gave the *diphenylamine ester* (1·7 g.) as colourless plates m. p. 65—67° (Found: C, 66·3; H, 5·7; N, 4·8. $C_{16}H_{17}O_4N$ requires C, 66·9; H, 5·95; N, 4·9%), $\nu_{max.}$ 1683(s), 1600(s), 1577(s), 1522(s), 1492, 1330, 1310, 1282, 1259(s), 1230, 1215, 1198, 1162, 1128, 1083, 1055, 1043, 1020, 961, 951, 914, 892, 852(s), 824, 803(s), 755, and 723 cm.$^{-1}$. A determination in hexachlorobutadiene revealed additional bands at 3300, 2941, 2845, 1451, 1432, and 1418 cm.$^{-1}$.

3-*Methoxy*-4-*methylanthranilic Acid*.—To a stirred aqueous solution of 7-methoxy-6-methylisatin [8] (8 g.) in 5% aqueous sodium hydroxide (75 c.c.) was added, during 20 min., a 30% solution of hydrogen peroxide (17 c.c.), the temperature rising to a maximum of 51° and the colour diminishing from the initial orange to pale yellow. After a further 20 min. the solution was acidified to pH 4 with dilute hydrochloric acid. The pale fawn solid which had been precipitated was separated, washed with a little water (10 c.c.), and dried. Crystallisation from toluene (after removal of insoluble material) afforded the acid as brown needles (5·4 g., 70%). Sublimation at 120°/1 mm. gave the pure *acid* as pale yellow needles, m. p. 162° (Found: C, 59·7; H, 6·0; N, 7·8. $C_9H_{11}O_3N$ requires C, 59·7; H, 6·1; N, 7·7%), $\nu_{max.}$ 3420, 3330, 2560, 2490, 1650(s), 1612, 1588(s), 1540, 1450(s), 1410, 1300(s), 1250, 1235(s), 1216, 1167, 1130, 1060, 1010(s), 933, 868, 825, 728(s), 718(s), and 705 cm.$^{-1}$.

To a solution of the acid (0·1 g.) in ether (25 c.c.) was added excess of ethereal diazomethane, and the mixture kept at room temperature for 1 hr. Removal of the solvent gave a white solid (0·11 g.) which on sublimation at 80°/1 mm. gave *methyl* 3-*methoxy*-4-*methylanthranilate* (0·1 g.) as white prisms, m. p. 88—89° (Found: C, 61·7; H, 6·8; N, 7·2. $C_{10}H_{13}O_3N$ requires C, 61·5; H, 6·7; N, 7·2%), $\nu_{max.}$ (i) 3465, 3360, 1690(s), 1620(s), 1600, 1550, 1305, 1235, 1180(s) 1165, 1090, 1060(s), 995, 975, 945, 860, 795, 772(s), 752(s), and 720 cm.$^{-1}$, (ii) in hexachlorobutadiene, additionally at 2936, 2860, 1450, and 1430 cm.$^{-1}$.

2-*Carboxy*-2′ : 4′ : 5′ : 6-*tetramethoxy*-3′ : 5-*dimethyldiphenylamine*.—A solution of 4-methyl-3-methoxyanthranilic acid (0·60 g.) and 2-methoxy-3-methyl-*p*-benzoquinone (1·1 g.) in methanol (25 c.c.) was heated under reflux for 4 days. After being kept at room temperature for 1 day, the deep red solution was concentrated to 10 c.c. and taken up in benzene (100 c.c.). After several washings with water the cherry-red extract was cautiously evaporated to dryness *in vacuo*, to give a dark red viscous oil. This material was hydrogenated in absolute ethanol (90 c.c.) at room temperature and pressure over Adams platinum catalyst (0·15 g.). After 30 min., anhydrous potassium carbonate (9 g.), dimethyl sulphate (4 c.c.), and additional catalyst (0·15 g.) were quickly added and hydrogenation continued for 3 days. Additional portions of potassium carbonate (9 g.), dimethyl sulphate (4 c.c.), and catalyst (0·1 g.) were added and hydrogenation continued for 24 hr. The catalyst and other insoluble material were removed and the ethanol was evaporated from the filtrate. The aqueous residue was extracted with chloroform (5 × 20 c.c.). Evaporation of the solvent from the combined extracts gave a red oil. Acidification and chloroform-extraction of the aqueous layer gave *ca.* 40 mg. of red acidic material. The above red oil in chloroform (25 c.c.) was extracted with concentrated hydrochloric acid (4 × 15 c.c.). The combined acid extracts were diluted with water (100 c.c.), and the aqueous solution re-extracted with chloroform (5 × 15 c.c.). After removal of the chloroform, brown crystals (0·15 g.) were obtained which recrystallised from methanol to give colourless plates of methyl 3-methoxy-4-methylanthranilate, m. p. and mixed m. p. 88—89°. The chloroform solution, after extraction with concentrated acid, was washed with water and evaporated, whereupon the crude diphenylamine ester was obtained as a dark oil. This oil was heated under reflux with ethanol (25 c.c.) and 2N-sodium hydroxide (15 c.c.) for 8 hr., the ethanol evaporated, and non-acidic material removed in chloroform. The alkaline solution was diluted with 2N-hydrochloric acid (15 c.c.) and extracted with chloroform. Removal of the chloroform *in vacuo* gave a brown acidic oil which was subjected to a counter-current distribution (48 transfers) using the solvent system, ethyl acetate–phosphate buffer solution (pH 6·9; M/15-solution). To each of the resultant fractions, 3N-sulphuric acid (2 c.c.) was added and, after being shaken, the organic layers were separated, washed, and evaporated. Tubes 37—47 contained crystalline residues which were combined (0·61 g.) and crystallised from ether–methanol to furnish the *carboxydiphenylamine* as colourless prisms, m. p. 168—169° (decomp.) (Found: C, 63·3; H, 6·1; N, 4·1. $C_{19}H_{23}O_6N$ requires C, 63·15; H, 6·4; N, 3·9%), main infrared bands at 3200, 1675, 1605, 1568, 1498, 1218, 1192, and 848 cm.$^{-1}$.

1 : 2 : 4 : 5-*Tetramethoxy*-3 : 6-*dimethylacridone*.—The above acid (68 mg.) was heated under reflux with phosphorus oxychloride (0·5 c.c.) for 1 hr. The deep red solution so obtained was evaporated to dryness *in vacuo* and gave a red residue, to which 0·5N-hydrochloric acid (5 c.c.) was added and the mixture was heated to 100° for 30 min. Chloroform extraction (5 × 10 c.c.) of the cooled solution gave an orange-red extract which was washed with sodium carbonate (2 × 10 c.c.) and then water (3 × 5 c.c.), and the solution (yellow, with green fluorescence) was evaporated to dryness, an orange solid (60 mg.) being obtained. Although this product crystallised from ether the product still contained a small amount of a red impurity. Chromatography on magnesium trisilicate with benzene–ethanol (98 : 2) as solvent gave a yellow eluate (150 c.c.) having a pronounced fluorescence, and evaporation of the solvent furnished a yellow crystalline *acridone* (55 mg.), m. p. 158—159° after crystallisation from methanol–light petroleum (b. p. 60—80°) (Found: C, 67·0; H, 6·3; N, 4·4; MeO, 36·5. $C_{19}H_{21}O_5N$ requires C, 66·5; H, 6·2; N, 4·1; 4MeO, 36·1%), $\nu_{max.}$ 263, 314, and 390 mμ (log ε 4·78, 3·46, and 3·85 respectively).

2 : 5-*Dihydroxy*-3 : 6-*dimethylacridone*-1 : 4-*quinone* (*Actinomycinol*).—The above tetramethoxyacridone (75 mg.) was heated under reflux with concentrated hydrobromic acid (2 c.c.) for 1 hr. After cooling, the mixture was poured into 0·1N-sodium hydroxide (100 c.c.). A brownish suspension was formed and the solution was rendered alkaline by adding 1N-aqueous sodium hydroxide (20 c.c.), whereupon the solid dissolved to give a port-wine-coloured solution which was aerated vigorously for 1 hr. The resulting solution was made weakly acid and exhaustively extracted with chloroform. The combined extracts were dried (Na_2SO_4) and evaporated to dryness to give a dark solid (29 mg.). Crystallisation from 50% aqueous acetic acid (20 c.c.) gave *actinomycinol* as red needles (17 mg.) which were purified further by sublimation at 160°/10^{-4} mm. (Found: C, 62·9; H, 4·0; N, 4·6. $C_{15}H_{11}O_5N$ requires C, 63·15; H, 3·9; N, 4·9%), $\lambda_{max.}$ 245, 276, 322, 370, and 476 mμ (log ε 4·47, 4·28, 3·86, 3·72, and 3·59 respectively). The infrared spectrum of the synthetic specimen showed max. at 3280, 2660, 2330, 1660, 1614, 1545, 1355, 1290, 1255, 1175, 1110, 1047, 1015, 982, 944, 912, 810, 770, 750, and 715 cm.$^{-1}$. Actinomycinol from actinomycin showed ultraviolet max. at 244, 277, 322, 370, and 475 mμ (log ε 4·49, 4·28, 3·91, 3·75, and 3·60), and infrared max. at 3279, 2660, 2330, 1664, 1616, 1548, 1357, 1285, 1259, 1174, 1110, 1049, 1015, 980, 943, 915, 812, 773, 750, and 714 cm.$^{-1}$.

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