Pigments of Pseudomonas Species. Part III.[1] The Synthesis of Demethylaeruginosin B and Aeruginosin B.†

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The structure of aeruginosin B is now defined as 2-amino-6-carboxy-10-methylphenazinium-8-sulphonate by the synthesis of the demethyl derivative. Aeruginosin B could not be synthesised by quaternisation of the demethyl compound or its derivatives, but has been made by the action of sulphite ion on aeruginosin A. Some limitations on the cyclisation of 2-aminodiphenylamines to phenazines in boiling nitrobenzene are described as well as a new phenazine synthesis.

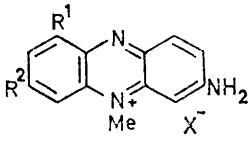
In part II of this series,[1] arguments were presented to show that aeruginosin B is an aminocarboxymethylphenazinium-sulphonate. On the basis of a reaction of the pigment in N-hydrochloric acid at 100° over an extended period (which was followed by changes in the electronic spectrum and by paper chromatography) and comparison with model compounds, the orientation of the substituents was deduced to be (I; $R^1 = CO_2H$, $R^2 = SO_3^-$, no X^-). This structure was consistent with the n.m.r. spectrum of the compound. This evidence was, to a large extent, circumstantial and more positive proof was sought in an unambiguous synthesis of the pigment or of its demethyl derivative.

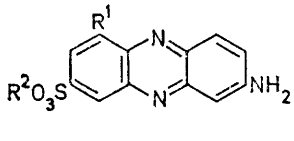
The synthesis of aeruginosin B presented a number of problems. The quaternisation of phenazines is not easy, particularly when electron-withdrawing substituents are present. In the synthesis[2] of aeruginosin A (I; $R^1 = CO_2^-$, $R^2 = H$, no X^-), the most effective reagent was methyl 2,4-dinitrobenzenesulphonate; the presence of the additional sulpho-group in demethylaeruginosin B was expected to make quaternisation even more difficult. Early experiments (with R. B. Herbert) showed that there were difficulties in the use of free sulphonic acids for the nitrobenzene cyclisation of 2-aminodiphenylamines to phenazines; again, during the quaternisation process, the acid groups need to be masked by easily removable groups. Sulphonic acid groups present difficulties in this respect; alkyl groups are too labile and the sensitivity of aeruginosin B to alkali and to prolonged acid hydrolysis[1] seemed to preclude the use of aryl esters or amides. Kenner and Murray,[3] however,

† A preliminary account of part of this work has been published: R. K. Bentley and F. G. Holliman, *Chem. Comm.*, 1966, 312.

[1] Part II, R. B. Herbert and F. G. Holliman, *J. Chem. Soc.* (*C*), 1969, 2517.
[2] Part I, F. G. Holliman, *J. Chem. Soc.* (*C*), 1969, 2514.
[3] G. W. Kenner and M. A. Murray, *J. Chem. Soc.*, 1949, S178.

have shown that aryl arenesulphonates are hydrogenolysed with Raney nickel as catalyst; on the other hand, they are stable to hydrogen in the presence of platinum or palladium, thus permitting ready reduction of nitro-groups during the synthesis of the 2-aminodiphenylamines required for the oxidative cyclisation in nitrobenzene to the phenazine system.[4] This differential catalytic effect was verified by the conversion of phenyl 3-nitrobenzenesulphonate into 3-aminobenzenesulphonic acid *via* phenyl 3-aminobenzenesulphonate.


(I)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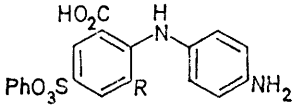
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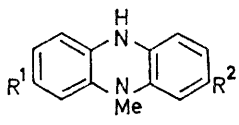
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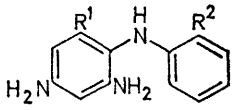
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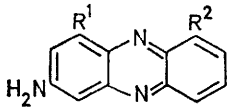

(VI)

A further model sequence, incorporating the cyclisation to a phenazine, was achieved in the synthesis of 8-aminophenazine-2-sulphonic acid (II; $R^1 = R^2 = H$). Although phenyl 4-aminobenzenesulphonate failed to react with 2,4-dinitrochlorobenzene or 2,4-dinitrophenyl toluene-*p*-sulphonate, the Ullmann reaction with 2,4-dinitrofluorobenzene gave phenyl 2′,4′-dinitrodiphenylamine-4-sulphonate. Hydrogenation with Adams catalyst gave the amino-compound without removal of the esterifying group and cyclisation in refluxing nitrobenzene then gave phenyl 8-aminophenazine-2-sulphonate (II; $R^1 = H$, $R^2 = Ph$). Disappointingly, the hydrogenolysis of the ester did not proceed easily: in absolute alcohol no change occurred, whilst in aqueous alcohol the reaction was only very slow and it seemed that the catalyst was being poisoned. On the other hand, alkaline hydrolysis to give 8-aminophenazine-2-sulphonic acid proceeded more readily than expected.

This series of reactions opened a route to demethylaeruginosin B, although failure to quaternise (II; $R^1 = H$, $R^2 = Ph$) with methyl 2,4-dinitrobenzenesulphonate and the unexpected difficulty with the hydrogenolysis of the sulphonate held little prospect for an extension to aeruginosin B itself. Phenyl 4-bromo-3-carboxy-5-nitrobenzenesulphonate was prepared from 4-bromo-3-carboxy-5-nitrobenzenesulphonyl chloride [5] by reaction with sodium phenoxide in aqueous solution, 1 molar equivalent only being used to avoid replacement of the activated bromine atom. An Ullmann reaction with *p*-phenylenediamine readily gave phenyl 4′-amino-2-carboxy-6-nitrodiphenylamine-4-sulphonate (III; $R = NO_2$) which was hydrogenated in aqueous ethanolic alkali to the diaminodiphenylamine (III; $R = NH_2$). Cyclisation in nitrobenzene was considerably faster than expected for a diphenylamine carrying two electron-attractive substituents. Brock and Holliman [6] had noted previously that the cyclisation of 2-aminodiphenylaminecarboxylic acids is faster than that of the corresponding esters and it seems therefore that the cyclisation can be acid catalysed. This has been borne out by other observations in this laboratory.[7] Alkaline hydrolysis of the resultant phenyl 8-amino-4-carboxyphenazine-2-sulphonate (II; $R^1 = CO_2H$, $R^2 = Ph$) gave the sulphonic acid (II; $R^1 = CO_2H$, $R^2 = H$). Comparison of this phenazine with demethylaeruginosin B by electrophoresis, paper chromatrography, and spectroscopy and by their behaviour when heated with dilute hydrochloric acid over an extended period (a criterion which had been shown to be sensitive to the relative position of sulphonamide and carboxy-groups in model 2-aminocarboxyphenazinesulphonamides [1]) indicated the identity of the two compounds. Thus the nature and orientation of the groups previously deduced [1] were confirmed.

The methyl ester (II; $R^1 = CO_2Me$, $R^2 = Ph$) was unstable in hot solvents, an additional impediment to its expected difficult quaternisation. Of the methylating agents tried, methyl 2,4-dinitrobenzenesulphonate was the most convenient, although only trace amounts of the metho-salt were obtained under the optimum conditions. Methyl trifluoromethanesulphonate, reported as a powerful methylating agent,[8] was equally disappointing. The trace quantities of phenazinium salt obtained by either method were subjected successively to hydrogenation with Raney nickel and acid hydrolysis to yield a product which moved at the same rate as aeruginosin B on paper chromatography and electrophoresis.

In seeking a more efficient synthesis of aeruginosin B, our attention was focused on a route in which quaternisation was to precede the introduction of the deactivating sulpho-group. McIlwain [9] has shown that quaternisation of phenazine activates the nucleus to attack by the sulphite ion giving a mono- and a di-sulphonic acid; dihydrophenazines are initially formed and can be oxidised, *via* a proposed radical-ion betaine, to sulphophenazinium betaines. Further, by analogy with similar attack by cyanide ion to give 2-cyano-10-methyl-5,10-dihydrophenazine (IV; $R^1 = CN$, $R^2 = H$), McIlwain assumed that the sulphonic acids were (IV; $R^1 = SO_3H$,

[4] G. Gaertner, A. Gray, and F. G. Holliman, *Tetrahedron*, 1962, **18**, 1105.
[5] R. B. Herbert and F. G. Holliman, *Tetrahedron*, 1965, **21**, 663.
[6] D. J. H. Brock and F. G. Holliman, unpublished work.
[7] D. S. Trickey and F. G. Holliman, unpublished work.
[8] J. Burdon and V. C. R. McLoughlin, *Tetrahedron*, 1965, **21**, 1.
[9] H. McIlwain, *J. Chem. Soc.*, 1937, 1704.

$R^2 = H$) and (IV; $R^1 = R^2 = SO_3H$) with the same orientation of the sulphonic acid groups with respect to the quaternary centre as required in aeruginosin B. These structures have now been shown to be correct. The dihydrodisulphonic acid was oxidised to the phenazyl betaine as described by McIlwain: an e.s.r. spectrum confirmed the radical-ion nature of the salt and the symmetry of the spectrum suggested a symmetrical dispostion of the sulpho-groups. The radical betaine, dissolved in aqueous ammonia, underwent extremely ready substitution of one of the sulpho-groups by an amino-group and oxidation to give 8-amino-10-methylphenazinium-2-sulphonate, identified by dequaternisation in alkali to a product identical with the 8-aminophenazine-2-sulphonic acid (II; $R^1 = R^2 = H$) described earlier. Unfortunately, this route could not be adapted to the synthesis of aeruginosin B as 5-methylphenazinium-1-carboxylate [10] reacted with sulphite to give a multiplicity of products.

It thus seemed likely that aeruginosin A would react with sulphite to give aeruginosin B. In surveying experiments, however, the product was apparently an aminophenazinium disulphonic acid betaine from its spectral and electrophoretic properties. Further, aeruginosin B reacted with sulphite to give a product apparently identical with that from aeruginosin A on electrophoretic and chromatographic criteria.

Several sulphite reactions are known to exhibit a marked pH dependence, the nature of the products being affected as well as the reaction rates.[11,12] The reaction between aeruginosin A and sulphite was surveyed in buffered solutions between pH 3 and 10. In a narrow range between pH 8 and 9, a monosulphonic acid betaine was obtained as well as the disulphonic acid betaine. The former proved to be identical with aeruginosin B in respect of electrophoresis, paper chromatography, and electronic and i.r. spectra. Although it was isolated in only 29% yield and the position of the sulphonic acid group, except by analogy, was equivocal, it is significant that the pH at which it was produced was the same as that of the culture when aeruginosin B is produced *in vivo*.[13] The possibility arises, therefore, that aeruginosin B may be an artefact arising through the biological reduction of sulphate to sulphite and its reaction with aeruginosin A.

Although the mechanisms of entry of the two sulphonic acid groups are by no means clear, it is apparent that the rate of entry of the second is comparable with that of the first. In an attempt to separate the two reactions, it was thought that a halogen atom in the 8-position of aeruginosin A (I; $R^1 = CO_2^-$, $R^2 = H$, no X^-) might undergo preferential nucleophilic displacement; on the other hand Bradley and Hannon [14] showed that benzenesulphinic acid reacted with 2-chlorophenazine (undoubtedly as the phenazinium salt since the sodium sulphinate did not react), to give, initially, a monosulphone in which the chlorine was retained, the halogen only being displaced in a second step to give a disulphone. 2-Amino-8-bromo-6-methoxycarbonyl-10-methylphenazinium 2,4-dinitrobenzenesulphonate [I; $R^1 = CO_2Me$, $R^2 = Br$, $X^- = 2,4\text{-}(NO_2)_2C_6H_3SO_3^-$] (synthesised by the usual route starting with 2,5-dibromo-3-nitrobenzoic acid and 4-aminoacetanilide), in fact, reacted with sulphite ion at all pH values to give a single product which was a bromine-free, disulphonic acid still containing the carboxy-function and apparently identical with the disulphonic acid obtained from aeruginosins A and B. The betaine, 8-bromoaeruginosin A (I; $R^1 = CO_2^-$, $R^2 = Br$, no X^-) behaved similarly.

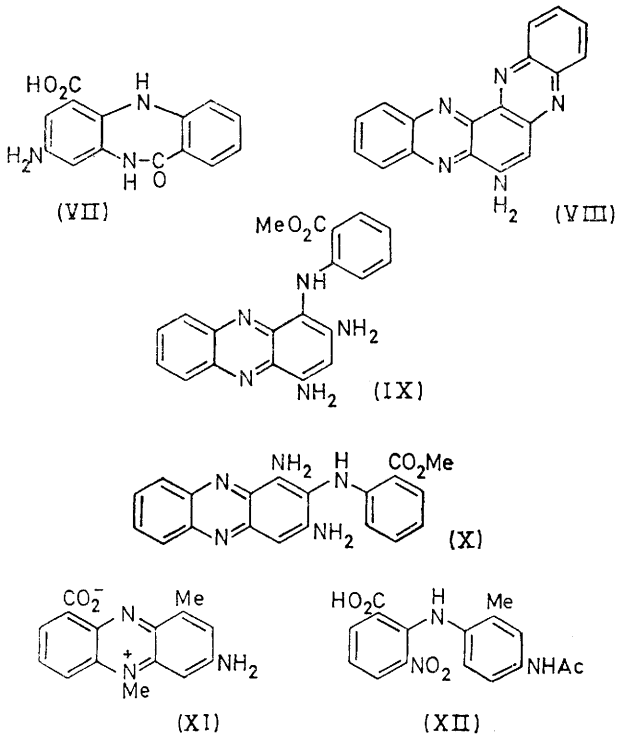
It seemed probable that the second sulphonic acid group was entering the aeruginosin B molecule (I; $R^1 = CO_2H$, $R^2 = SO_3^-$, no X^-) at the remaining unsubstituted, electron-deficient 4-position, both on theoretical grounds and on the basis of the n.m.r. spectrum of the disulphonic acid obtained from the 2-amino-8-bromo-6-methoxycarbonyl-10-methylphenazinium salt; this showed two singlets of equal intensity at τ 1·33 and 2·00 (in D_2O) and τ 1·63 and 2·67 (in alkaline D_2O). Attempts to synthesise a 2-amino-4-substituted-10-methylphenazinium-1-carboxylate (as I; $R^1 = CO_2^-$, $R^2 = H$, no X^-) were therefore made to see if the sulphite reaction would yield a monosulphonic acid only; if successful, a removable group could then be used to block this position in a synthesis of aeruginosin B. In the final outcome we were unsuccessful, but the synthetic sequences provided a number of interesting results.

The cyclisation of dimethyl 4,6-diaminodiphenylamine-2,2′-dicarboxylate (V; $R^1 = R^2 = CO_2Me$) gave only methyl 3-aminophenazine-1-carboxylate [15] (VI; $R^1 = CO_2Me$, $R^2 = H$), whilst the acid (V; $R^1 = R^2 = CO_2H$) gave the dibenzo[*b*,*e*][1,4]diazepinone (VII). Jourdan [16] has reported the analogous cyclisation of (V; $R^1 = H$, $R^2 = CO_2H$) on fusion or in boiling xylene; we find the dibenzodiazepinone is also the major product in boiling nitrobenzene, only a small yield of 7-aminophenazine-1-carboxylic acid (VI; $R^1 = H$, $R^2 = CO_2H$) being formed. An attempt to reduce diazepinone cyclisation in favour of phenazine formation by carrying out the nitrobenzene reaction on the sodium salts in the presence of sodium methoxide was successful only at the expense of concomitant decarboxylation, the product being 3-aminophenazine-1-carboxylic acid from (V; $R^1 = R^2 = CO_2H$) and 2-aminophenazine from (V; $R^1 = H$, $R^2 = CO_2H$).

In an attempted synthesis of methyl 7,9-diaminophenazine-1-carboxylate (VI; $R^1 = NH_2$, $R^2 = CO_2Me$) by the cyclisation of methyl 2′,4′,6′-triaminodiphenylamine-1-carboxylate (V; $R^1 = NH_2$, $R^2 = CO_2Me$), over an extended period the product was a mixture of an

[10] G. S. Hansford and F. G. Holliman, unpublished work.
[11] J. E. LuValle, *J. Amer. Chem. Soc.*, 1952, **74**, 2970.
[12] R. Sperling, *J. Chem. Soc.*, 1949, 1925.
[13] G. Kreft, Ph.D. Thesis, University of Cape Town, 1957.
[14] W. Bradley and J. D. Hannon, *J. Chem. Soc.*, 1962, 4438.
[15] D. J. H. Brock and F. G. Holliman, *Tetrahedron*, 1963, **19**, 1903.
[16] F. Jourdan, *Ber.*, 1885, **18**, 1444.

aminomethoxycarbonylquinoxalinophenazine (or phenazines) with the corresponding demethoxycarbonylated material. The latter was isolated and proved identical with a compound which had been obtained alongside 1,3-diaminophenazine by the nitrobenzene cyclisation of 2,4,6-triaminodiphenylamine [7] and was formulated as (VIII) in view of the reported instability of the alternative linear quinoxalino[2,3-*b*]phenazine system.[17] Al-



though (VIII) has been reported by Kehrmann and Poehl,[18] their data was insufficient to establish identity with our compound.* It is clear that (VIII) results from the participation of a molecule of nitrobenzene in a Wohl–Aue [19] type condensation either before or after the cyclisation of the diphenylamine system. Both processes are probable. When 1,3,5-triaminobenzene was heated under reflux in nitrobenzene, 1,3-diaminophenazine was initially formed in sufficiently good yield to prove the most direct preparation of this compound; [20] it was subsequently converted into the aminoquinoxalinophenazine (VIII). On the other hand, when the reaction time of (V; $R^1 = NH_2$, $R^2 = CO_2Me$) in nitrobenzene was shortened, a mixture of two diamino-(2′-methoxycarbonylphenyl)aminophenazines (IX) and (X) were produced together with some 1,3-diaminophenazine and traces of methyl 7,9-diaminophenazine-1-carboxylate (VI; $R^1 = NH_2$, $R^2 = CO_2Me$). The latter was quaternised and then treated with aqueous sodium sulphite; it was largely simply hydrolysed but a a small quantity of what appeared to be a monosulphonic acid was isolated.

Finally, 4-methylaeruginosin A (XI) was synthesised. 2-Bromo-3-nitrobenzoic acid condensed with 5-acetamido-2-aminotoluene to give 4′-acetamido-2′-methyl-2-nitrodiphenylamine-1-carboxylic acid (XII). Hydrolysis of the latter in aqueous alkali led to the surprising appearance of 7-amino-9-methylphenazine-1-carboxylic acid (VI; $R^1 = Me$, $R^2 = CO_2H$) as a minor product. The cyclisation of 2-nitrodiphenylamines by potassium hydroxide in high-boiling hydrocarbon solvents has been subsequently reported,[21] but our case is reminiscent of the cyclisation of 2-nitrodiphenylamine in alkaline conditions only in the presence of excess of aniline as reductant,[22] a function here fulfilled by the *p*-phenylenediamine system. The method was extended to 7-aminophenazine-1-carboxylic acid, 3-bromo-7-aminophenazine-1-carboxylic acid and demethylaeruginosin B, but in poor yield.† Simultaneous deacetylation and esterification of (XII) was achieved using dry methanolic hydrogen chloride and the product was successively reduced, cyclised, quaternised and hydrolysed to give 4-methylaeruginosin A. Surprisingly, this reacted with sulphite to give a major product which by electrophoresis and electronic spectra was very similar to the products from aeruginosins A and B and thus a disulphonic acid. A minor product had characteristics similar to aeruginosin B.

EXPERIMENTAL

Qualitative paper chromatography was performed on Whatman No. 1 paper by the descending method with the following solvents which are subsequently designated by their letters: (A) butanol and conc. hydrochloric acid (4 : 1) saturated with water; (B) butanol and acetic acid (4 : 1) saturated with water; (C) butanol, pyridine, and water (4 : 1 : 5), upper layer in the trough, lower layer in the tank. R_B Refers to the distance moved relative to aeruginosin B. Whatman No. 3MM paper was used when larger quantities were separated. Chromatography (charcoal) refers to the use of columns of a mixture of equal weights of Darco G60 charcoal and Celite 535 filter aid. Alumina used for column chromatography was either Peter Spence type O (referred to as ' alumina ') or the acidic grade of Woelm alumina which had been deactivated by successive treatments with water and methanol and then dried in air at room temperature (referred to as ' deactivated alumina '). Paper electrophoresis was performed on Whatman No. 1 paper in phosphate buffer at pH 7 at a potential gradient of 7·5 V/cm. in an apparatus similar to that described by Kunkel and Tiselius; [23] M_B refers to the distance moved relative to

* Kehrmann and Poehl's preparation has subsequently been repeated and the identity of their product with the aminoquinoxalinophenazine herein described has been demonstrated (with N. V. Ellerton).

† These yields have subsequently been raised to 60—80% by the inclusion of sodium borohydride (with S. R. Challand).

[17] G. A. Swan and D. G. Felton, ' Phenazines,' Interscience, New York, 1957, p. 509 and references there cited.

[18] F. Kehrmann and N. Poehl, *Helv. Chim. Acta*, 1926, **9**, 485.
[19] A. Wohl and W. Aue, *Ber.*, 1901, **34**, 2442.
[20] A. Albert and H. Duewell, *J. Soc. Chem. Ind.*, 1947, **66**, 11.
[21] B.P. 1,091,618 (*Chem. Abs.*, 1968, **69**, 43939).
[22] S. B. Serebryanyi, *Ukrain. Khim. Zhur.*, 1955, **21**, 350; I. Yoshioka and H. Otomasu, *Hoshi Yakka Daigaku Kiyo*, 1957, **6**, 44.
[23] H. Kunkel and A. Tiselius, *J. Gen. Physiol.*, 1951, **35**, 89.

aeruginosin B. Buffer solutions were prepared according to Conway.[24] U.v.–visible spectra were recorded on a Unicam SP 800 instrument. I.r. spectra were recorded on potassium chloride discs except where otherwise stated and with a Perkin-Elmer 125 grating spectrophotometer or a Unicam SP 200 instrument. N.m.r. spectra were measured on a Varian A60 instrument. M.p.s were measured on a hot stage and are uncorrected.

Phenyl Aniline-m-*sulphonate*.—A solution of phenyl *m*-nitrobenzenesulphonate [25] (1·0 g.) in absolute alcohol (75 ml.) was hydrogenated (2 atmos.; 100 mg. Adams catalyst) for 15 hr. The catalyst was removed and the colourless solution taken to dryness under reduced pressure. The brown oil crystallised from light petroleum (b.p. 60—80°; 75 ml.) and sufficient benzene for complete solution at the b.p. to give white needles of phenyl aniline-*m*-sulphonate (0·74 g., 83%), m.p. 69—70·5° (Found: C, 58·0; H, 4·7; N, 5·65. $C_{12}H_{11}NO_3S$ requires C, 57·8; H, 4·45; N, 5·6%).

Aniline-m-*sulphonic acid*.—A solution of phenyl aniline-*m*-sulphonate (250 mg.) in absolute alcohol (50 ml.) was hydrogenated (5 atmos.; Raney nickel) for 15 hr. The pale brown solution was filtered, and taken to dryness under reduced pressure. The residue was dissolved in water and passed through an ion-exchange column (Dowex 50W X8 100—200 mesh, H^+ form, 15 × 1 cm.). The eluate was taken to dryness under reduced pressure to give a brown semicrystalline residue of aniline-*m*-sulphonic acid, which could not be recrystallised; $\nu_{max.}$ (Nujol) 1025 cm^{-1} (S=O). It was converted into pyridinium 3-phthalimidobenzenesulphonate, m.p. 215—216·5° (lit.,[26] 220°), mixed m.p. 215—217°.

Phenyl 2′,4′-*Dinitrodiphenylamine*-4-*sulphonate*.—An intimate mixture of 2,4-dinitrofluorobenzene (11·1 g.), phenyl sulphanilate [27] (12·5 g.), anhydrous sodium carbonate (3·2 g.), potassium fluoride (1·8 g.), and freshly reduced copper (3·0 g.), was heated in an oil-bath at 160° for 1 hr. An ethyl acetate extract of the cooled mixture was taken to dryness and the residue was washed with warm hydrochloric acid (5N; 2 × 50 ml.) then water (2 × 50 ml.). Recrystallisation [ethanol–acetic acid (2 : 1)] gave yellow crystals of the *diphenylamine* (14·4 g., 69%), m.p. 133—137° (Found: C, 52·1; H, 3·1; N, 10·15; S, 7·9. $C_{18}H_{13}N_3O_7S$ requires C, 52·1; H, 3·2; N, 10·1; S, 7·7%).

Phenyl 2′,4′-*Diaminodiphenylamine*-4-*sulphonate*.—Phenyl 2′,4′-dinitrodiphenylamine-4-sulphonate (3·5 g.) in ethyl acetate (100 ml.) was hydrogenated (5 atmos.; 400 mg. Adams catalyst; 2 hr.). Removal of the solvent in nitrogen gave a pale purple residue which was recrystallised (absolute alcohol) to give *phenyl* 2′,4′-*diaminodiphenylamine*-4-*sulphonate* (2·20 g., 73%), as white needles, m.p. 168·5—169° (decomp.), slowly colouring in air (Found: C, 60·2; H, 4·9; N, 11·55. $C_{18}H_{17}N_3O_3S$ requires C, 60·8; H, 4·8; N, 11·8%).

Phenyl 8-*Aminophenazine*-2-*sulphonate*.—A solution of phenyl 2′,4′-diaminodiphenylamine-4-sulphonate (500 mg.) in nitrobenzene (50 ml.) was heated under reflux under nitrogen for 48 hr. Paper chromatography in butanol–hydrochloric acid showed a major orange-red spot at the solvent front and a minor red spot moving as 2-aminophenazine. Chromatography (alumina; 2% ethanol in ether) and recrystallisation (toluene) gave deep red needles of the *phenazinesulphonate* (189 mg., 38%), m.p. 187—187·5° (Found: C, 61·35; H, 4·1; N, 11·65. $C_{18}H_{13}N_3O_3S$ requires C, 61·5; H, 3·7; N, 11·9%).

8-*Aminophenazine*-2-*sulphonic Acid*.—A solution of phenyl 8-aminophenazine-2-sulphonate (150 mg.) in a mixture of absolute alcohol (50 ml.) and aqueous sodium hydroxide (N; 50 ml.) was heated under reflux for 30 min. The solution was adjusted to pH 4 with hydrochloric acid and introduced onto a charcoal column. After thorough washing of the column with water, the phenazine was eluted with 5% aqueous pyridine. The eluate was taken to dryness and the purple solid was recrystallised from water to give dark red plates of 8-*aminophenazine*-2-*sulphonic acid* (70 mg., 59%), m.p. >300° (Found: C, 52·05; H, 3·4; N, 15·0. $C_{12}H_9N_3O_3S$ requires C, 52·3; H, 3·3; N, 15·3%), R_B (A) = 1·4; M_B = 0·4; i.r. spectrum of the potassium salt: $\nu_{max.}$ 1025 cm^{-1} (S=O).

Phenyl 4-*Bromo*-3-*carboxy*-5-*nitrobenzenesulphonate*.—4-Bromo-3-carboxy-5-nitrobenzenesulphonyl chloride [5] (17·2 g.), phenol (4·7 g.), and sodium hydroxide (4·0 g.) in water (50 ml.) were shaken vigorously for 2 hr. at 0° and 2 hr. at room temperature. The solution was acidified, the precipitate was collected, washed with water, and recrystallised (20% aq. alcohol) to give white needles of the *ester* (11·0 g., 55%), m.p. 179—180° (Found: C, 39·1; H, 1·8; N, 3·55. $C_{13}H_8BrNO_7S$ requires C, 38·8; H, 2·0; N, 3·5%).

The use of excess of sodium phenoxide (17·4 g.) in boiling water gave *phenyl* 3-*carboxy*-4-*phenoxy*-5-*nitrobenzenesulphonate*, m.p. 158—160° (Found: C, 54·35; H, 3·05; N, 3·45; S, 7·55. $C_{19}H_{13}NO_8S$ requires C, 54·9; H, 3·1; N, 3·4; S, 7·7%); $\nu_{max.}$ ($CHCl_3$) 1260 $cm.^{-1}$ (C–O–C).

Phenyl 4′-*Amino*-2-*carboxy*-6-*nitrodiphenylamine*-4-*sulphonate*.—A solution of phenyl 4-bromo-3-carboxy-5-nitrobenzenesulphonate (4·0 g.), *p*-phenylenediamine (1·2 g.), and anhydrous sodium acetate (3·3 g.) in ethanol (50 ml.) was heated under reflux for 2·5 hr. The hot solution was filtered into warm dilute hydrochloric acid (0·1N; 100 ml.) and the mixture was left overnight at 0°; the brown precipitate was collected, washed with water, and dried. Recrystallisation (n-propanol) gave the *diphenylamine* (2·9 g., 68%), m.p. 208—209° (decomp.) (Found: C, 52·05; H, 3·8; N, 9·3; S, 7·05. $C_{19}H_{15}N_3O_7S,\frac{1}{2}H_2O$ requires C, 51·9; H, 3·7; N, 9·55; S, 7·3%).

Phenyl 4′,6-*Diamino*-2-*carboxydiphenylamine*-4-*sulphonate*.—Phenyl 4′-amino-2-carboxy-6-nitrodiphenylamine-4-sulphonate (1·0 g.) dissolved in aqueous alcoholic alkali (3 ml. 2N-sodium hydroxide, 50 ml. water, and 50 ml. absolute alcohol) was hydrogenated (5 atmos., 200 mg. Adams catalyst, 2 hr.). The solution was filtered under nitrogen into 0·06N-acetic acid (155 ml.). The pale purple precipitate was recrystallised (methanol under nitrogen) to give the *diaminodiphenylamine* (0·80 g., 86%) as needles, m.p. 234—237°, slowly colouring in air (Found: C, 57·05; H, 4·4; N, 10·25. $C_{19}H_{17}N_3O_5S$ requires C, 57·1; H, 4·3; N, 10·5%).

Phenyl 8-*Amino*-4-*carboxyphenazine*-2-*sulphonate*.—A solution of phenyl 4′,6-diamino-2-carboxydiphenylamine-4-sulphonate (500 mg.) in nitrobenzene (50 ml.) was heated under reflux under nitrogen for 12 hr. The solution was

[24] B. E. Conway, 'Electrochemical Data,' Elsevier, Amsterdam, 1952, pp. 206—214.
[25] H. H. Hodgson and J. H. Crook, *J. Chem. Soc.*, 1936, 1677.
[26] P. Chen and E. J. Cross, *J. Soc. Dyers and Colourists*, 1943, **59**, 144.
[27] S. I. Burmistov and A. D. Burylina, *Shornik. Statei. Obsch. Khim.*, 1953, **2**, 1065 (*Chem. Abs.*, 1955, **49**, 6861).

cooled, filtered, reduced to small volume *in vacuo* (<10 ml.) and set aside at 0° for 2 days. The dark purple precipitate of the *phenazine* (231 mg., 47%) was collected and washed with benzene and then ether. A further sample (67 mg.) of lower purity was obtained by addition of benzene (10 ml.) to the nitrobenzene mother liquors. Recrystallisation (nitrobenzene) gave dark purple crystals, m.p. >300° (Found: C, 57·8; H, 3·45; N, 10·9; S, 8·35. $C_{19}H_{13}N_3O_5S$ requires C, 57·7; H, 3·3; N, 10·6; S, 8·1%), $M_B = 0·4$.

The *methyl ester* was prepared with diazomethane in methanol–ether. It was dissolved in benzene containing a little nitrobenzene and chromatographed (alumina, 2% methanol in ether elution) to give bright red *needles* (65%), m.p. 94—98° (decomp.) (Found: C, 58·7; H, 4·05; N, 10·6; S, 8·1. $C_{20}H_{15}N_3O_5S$ requires C, 58·7; H, 3·7; N, 10·3; S, 7·7%), $M_B = 0$.

8-*Amino*-4-*carboxyphenazine*-2-*sulphonic Acid*.—A solution of the phenyl ester (100 mg.) in 50% aqueous alcoholic sodium hydroxide solution (0·5N; 100 ml.) was heated under reflux for 15 min.; it was then made just acidic with hydrochloric acid. The solution was chromatographed (charcoal, 2% aq. pyridine elution) to give an orange eluate which was taken to dryness *in vacuo*. The residue was recrystallised (water) to give dark purple needles of 8-amino-4-carboxyphenazine-2-sulphonic acid (35 mg., 43%), m.p. 300° (Found: C, 46·45; H, 3·1; N, 12·1; S, 9·55. $C_{12}H_9N_3O_5S,H_2O$ requires C, 46·3; H, 3·3; N, 12·45; S, 9·5%). This had identical properties to demethylaeruginosin B; R_B (A) = 1·1; R_B (B) = 1·4; M_B = 1·35: $\lambda_{max.}$ (0·5N-hydrochloric acid) 237, 293, 375, and 538 nm.; $\lambda_{max.}$ (0·5N-aqueous sodium hydroxide) 240, 282, 370, and 470 nm.; i.r. spectra of the potassium salts (prepared *via* an ion-exchange resin) were identical throughout. Equimolar solutions of the synthetic compound and of demethylaeruginosin B in 0·5N-hydrochloric acid were each heated at 100°, samples being removed at intervals up to 192 hr. and examined by paper chromatography and u.v.–visible spectroscopy; identical behaviour was observed.

Quaternisation of Phenyl 8-*Amino*-4-*methoxycarbonylphenazine*-2-*sulphonate and De-esterification of the Quaternary Salt*.—A solution of 2-amino-6-methoxycarbonyl-8-phenoxysulphonylphenazine (5 mg.) and methyl 2,4-dinitrobenzenesulphonate (5 mg.) in toluene (10 ml.; Na dried) in a stoppered flask was set aside at room temperature for 2 weeks during which the colour changed from orange to red and a little purple precipitate separated. The solution was decanted off, and the solid was washed with dry toluene. This solid, which had $M_B = -0·8$, R_F (C) = 0·7, was dissolved in 50% aq. alcohol (10 ml.) and hydrogenated (4 atmos.; Raney nickel; 1 hr.) to give a yellowish green solution. The catalyst was filtered off, the solution was taken to dryness under reduced pressure, and the residue was dissolved in dilute hydrochloric acid (N; 1 ml.). The solution, which on paper chromatography and electrophoresis showed a major red spot R_B (A) = 1·4; R_B (C) = 0·4; $M_B = 0$, was then heated in a sealed tube in a boiling water-bath for 1 hour. Examination by paper chromatography and electrophoresis showed a major red component whose behaviour was identical with that of aeruginosin B.

2-*Amino*-10-*methylphenazinium*-8-*sulphonate*.—10-Methylphenaziniumyl-2,8-disulphonate [9] (400 mg.) was dissolved in dilute ammonia solution (0·1N; 40 ml.). The colour quickly changed from green to purple. After being set aside overnight, the solution was adjusted to pH 4 with dilute hydrochloric acid, and chromatographed (charcoal). Elution with water removed inorganic salts and later gave an emerald green solution. Gradient-elution with 10% aqueous pyridine then gave a major red eluate which was taken to dryness under reduced pressure; the residue was recrystallised (water) to give purple needles of 2-*amino*-10-*methylphenazinium*-8-*sulphonate* (73 mg., 22%) (Found: C, 54·1; H, 4·15; N, 13·9, requires C, 54·0; H, 3·8; N, 14·5%).

The betaine (50 mg.) in aqueous sodium hydroxide solution (N; 100 ml.) was heated on a boiling water bath for 2 hr. to give an orange solution. After adjustment of the pH to 4 by addition of hydrochloric acid, the solution was chromatographed (charcoal). The column was washed thoroughly with water and was then gradient eluted with 7% aqueous pyridine. The orange eluate was taken to dryness under reduced pressure, and the solid was recrystallised from water to give 8-aminophenazine-2-sulphonic acid which had identical physical characteristics (u.v. and i.r. spectra, paper chromatography, and electrophoresis) with those reported previously.

Reactions of Aeruginosins A and B with Sulphite Ion.—An aqueous solution of aeruginosin A (10 mg. in 50 ml.) and sodium sulphite (400 mg.) was kept at room temperature for 2 days, acidified, and allowed to oxidise in the air overnight. The pH was adjusted to 5 and the solution was chromatographed (charcoal). A 1% aqueous pyridine eluate was evaporated to dryness, and the residue was dissolved in water and reprecipitated with absolute ethanol. It had R_B (A) = 1·05; R_B (B) = 0·50; M_B = 1·9; $\nu_{max.}$: 1717 (C=O), 1032 cm.$^{-1}$ (S=O); $\lambda_{max.}$ (0·5N-hydrochloric acid): 240, 300, 390, and 540 nm. A saturated aqueous solution of aeruginosin B (1 ml.) and sodium sulphite (1 mg.) left for 3 days and then acidified showed an apparently identical product when examined by paper chromatography and paper electrophoresis.

2-*Amino*-6-*carboxy*-10-*methylphenazinium*-8-*sulphonate* (*Aeruginosin B*).—Aeruginosin A (10·3 mg.) and anhydrous sodium sulphite (18 mg., 4 mole) were dissolved in an air-free borate buffer (pH 8·4; 100 ml.). The solution was set aside at room temperature under nitrogen for 4 days. Concentrated hydrochloric acid (5 ml.) was added and the solution was heated on a boiling water-bath for 15 min. in the presence of air. The solution was then stood at 0° for 24 hr. during which a purple precipitate separated. This was collected and washed with a little water. The mother liquors were adjusted to pH 4 and chromatographed (charcoal). After being washed with water, the column was eluted with 2% aqueous pyridine. The red eluate was taken to dryness and the residue, combined with the precipitate collected earlier, was dissolved in water and chromatographed (deactivated alumina). Water as eluant removed unchanged aeruginosin A and elution with 0·5% aqueous pyridine gave a major red fraction which was taken to dryness. The residue was recrystallised from water to give aeruginosin B (3·6 mg., 29%) identical with the natural product; R_B (A) = 1·0; R_B (B) = 1·0; M_B = 1·0; $\nu_{max.}$ 1725 cm.$^{-1}$ (C=O); 1044 cm.$^{-1}$ (S=O); $\lambda_{max.}$ (N-HCl): 239, 293, 376, 386sh, and 541 nm.

2,5-*Dibromobenzoic Acid*.*—A solution of silver nitrate (8·5 g.) in water (50 ml.) was added during 15 min. to a vigorously stirred solution of *o*-bromobenzoic acid (10 g.) in a mixture of acetic acid (150 ml.), nitric acid (35 ml.), water (25 ml.), and bromine (2·6 ml.). After a further 15 min. the reaction mixture was filtered, the filtrate was

* We are grateful to Dr. R. B. Herbert for these preparations.

reduced to half volume *in vacuo*, and diluted. The precipitated 2,5-dibromobenzoic acid recrystallised (aqueous ethanol) as white needles (11·2 g., 80%), m.p. 158—160° (lit.,[28] 153°) (Found: C, 29·9; H, 1·25. Calc. for $C_7H_4Br_2O_2$: C, 30·0; H, 1·45%).

2,5-*Dibromo*-3-*nitrobenzoic Acid*.*—(i) Nitric acid (*d* 1·5; 2·5 ml.) was added slowly to a solution of 2,5-dibromobenzoic acid in oleum (20%; 10 ml.). The reaction mixture was then heated at 100° for 4·5 hr., cooled, and added to ice. The precipitated *nitro-acid* recrystallised (acetic acid) as white needles, m.p. 222—227° (Found: C, 26·0; H, 1·1; Br, 48·7. $C_7H_3Br_2NO_4$ requires C, 25·8; H, 0·95; Br, 49·3%).

(ii) 2-Bromo-3-nitrobenzoic acid [29] (12·3 g.) was dissolved as far as possible in sulphuric acid (90%, 100 ml.). Silver sulphate (8·5 g.) was added and the mixture was heated and stirred at 100° whilst bromine (2·6 ml.) was added during 10 min. Heating was continued for 3 hr. after which the cooled mixture was poured onto ice. The dried precipitate was continuously extracted with chloroform (700 ml.); 2,5-dibromo-3-nitrobenzoic acid separated from the cool extract. The recrystallised acid (8·1 g., 50%), m.p. 230—233·5, had an i.r. spectrum identical to that described above.

4′-*Acetamido*-4-*bromo*-6-*nitrodiphenylamine*-2-*carboxylic Acid*.*—A solution of 2,5-dibromo-3-nitrobenzoic acid (5·85 g.), *p*-aminoacetanilide (3·0 g.), and sodium acetate (4·1 g.) in n-pentyl alcohol (60 ml.) was heated under reflux for 2 hr. The solvent was removed *in vacuo* and the residue was dissolved in water. Acidification (dilute hydrochloric acid) of the filtered solution precipitated the *diphenylamine* which was recrystallised (90% aqueous acetic acid): m.p. 258·5—260·5° (3·8 g., 53%) (Found: C, 45·5; H, 3·0; N, 10·4. $C_{15}H_{12}BrN_3O_5$ requires C, 45·7; H, 3·05; N, 10·7%).

4′-*Amino*-4-*bromo*-6-*nitrodiphenylamine*-2-*carboxylic Acid*.—A suspension of 4′-acetamido-4-bromo-6-nitrodiphenylamine-2-carboxylic acid (19·7 g.) in methanol (300 ml.) containing hydrochloric acid (5N; 10 ml.) was stirred and heated under reflux. Concentrated hydrochloric acid (2 ml.) was added to the mixture after each hour until complete dissolution was obtained. The mixture was heated for a further 2 hr. after which the solution was concentrated to 100 ml., and then cooled. Sodium hydroxide solution (N; 120 ml.) was added to it and the mixture was warmed to obtain complete dissolution. After adjustment of the pH to 5 (acetic acid) the mixture was set aside overnight; the brown precipitate was collected, washed with water, dried, and recrystallised (n-propanol) to give brown needles of the *diphenylamine* (16 g., 91%), m.p. 229·5—230·5° (Found: C, 44·15; H, 2·85; N, 12·15. $C_{13}H_{10}BrN_3O_4$ requires C, 44·3; H, 2·9; N, 11·9%).

Methyl 4′-*Amino*-4-*bromo*-6-*nitrodiphenylamine*-2-*carboxylate*.—A solution of the diphenylaminecarboxylic acid (7 gm.) in dry tetrahydrofuran (100 ml.) was treated with an excess of ethereal diazomethane. The solvent was distilled off, and the solid was recrystallised (dry methanol) to give almost black prisms of the *ester* (6·2 g., 86%), m.p. 169—169·5° (Found: C, 45·85; H, 3·6; Br, 21·4. $C_{14}H_{12}BrN_3O_4$ requires C, 45·9; H, 3·3; Br, 21·9%).

Methyl 4′,6-*Diamino*-4-*bromodiphenylamine*-2-*carboxylate*.—A solution of methyl 4′-amino-4-bromo-6-nitrodiphenylamine-2-carboxylate (2·0 g.) in methanol (150 ml.) was hydrogenated (5 atmos.; Raney nickel) until pale yellow (90 min.). The catalyst was filtered off and the solution was taken to dryness under reduced pressure of nitrogen. The solid was recrystallised from benzene–light petroleum (60—80°) (1 : 1) under nitrogen to give bright yellow needles of the *diaminodiphenylamine* (1·33 g., 73%), m.p. 151—152° (Found: C, 50·4; H, 4·25; Br, 24·1. $C_{14}H_{14}BrN_3O_2$ requires C, 50·0; H, 4·2; Br, 24·1%).

7-*Amino*-3-*bromophenazine*-1-*carboxylic Acid*.—4′-Amino-4-bromo-6-nitrodiphenylamine-2-carboxylic acid (500 mg.) in methanol (100 ml.) was hydrogenated (5 atmos.; Raney nickel) until pale yellow (90 min.). The catalyst was filtered off, and the solution was taken to dryness under reduced pressure of nitrogen.

The crude diamine dissolved in nitrobenzene (90 ml.) was heated under reflux under nitrogen for 15 hr.; it was then filtered whilst hot and set aside overnight. The purple precipitate of 7-amino-3-bromophenazine-1-carboxylic acid (252 mg.) was collected and washed with benzene and then ether. A further 69 mg. were obtained by chromatography (alumina) of the concentrated nitrobenzene mother liquors (total yield 75%). The product was recrystallised from nitrobenzene (Found: C, 49·35; H, 2·75; N, 12·95. $C_{13}H_8BrN_3O_2$ requires C, 49·1; H, 2·5; N, 13·2%).

Methyl 7-*Amino*-3-*bromophenazine*-1-*carboxylate*.—A solution of methyl 4′,6-diamino-4-bromodiphenylamine-2-carboxylate (2·0 g.) in nitrobenzene (500 ml.) was heated under reflux in air for 36 hr. After being taken to small volume *in vacuo*, chromatography (alumina) gave a major orange band with 1% methanol in ether. The eluate was taken to dryness and the residue was recrystallised (toluene) to give bright red needles of methyl 7-*amino*-3-*bromophenazine*-1-*carboxylate* (1·21 g., 61%), m.p. 225—227° (Found: C, 50·9; H, 3·25; N, 12·6. $C_{14}H_{10}BrN_3O_2$ requires C, 50·6; H, 3·0; N, 12·7%).

2-*Amino*-8-*bromo*-6-*methoxycarbonyl*-10-*methylphenazinium* 2,4-*dinitrobenzenesulphonate*.—(All apparatus was dried at 110°.) A solution of 7-amino-3-bromophenazine-1-carboxylate (664 mg.) in dry toluene (100 ml.) was heated under reflux in an oil-bath at 100°. A solution of methyl 2,4-dinitrobenzenesulphonate (890 mg.) in dry toluene (25 ml.) was filtered into the reaction flask. The temperature was maintained at 100° for 2·5 hr. during which purple crystals separated. The solution was cooled and the precipitate was collected and washed with toluene and then ether. The crude product was dissolved in hot water (1·5 l.) and the solution was cooled; it was then filtered and extracted with chloroform (2 × 500 ml.). The organic solution was extracted with water (500 ml.) and the aqueous extracts concentrated under reduced pressure to *ca.* 400 ml. This solution was set aside at 0° overnight to give bright red needles of the *phenazinium salt* (475 mg., 40%) m.p. 231—233°. Recrystallisation (water) raised the m.p. to 238—239° (Found: C, 43·2; H, 2·85; Br, 13·3; N, 12·05. $C_{21}H_{16}BrN_5O_9S$ requires C, 42·4; H, 2·7; Br, 13·4; N, 11·8%); $M_B = -1·1$.

Reaction of the 2-*Amino*-8-*bromo*-6-*methoxycarbonyl*-10-*methylphenazinium Ion with Sulphite Ion*.—A solution of 2-amino-8-bromo-6-methoxycarbonyl-10-methylphenazinium 2,4-dinitrobenzenesulphonate (100 mg.) and anhydrous sodium sulphite (2·0 g.) in water (500 ml.) was set aside at room temperature for 2 days. Concentrated hydro-

* We are grateful to Dr. R. B. Herbert for these preparations.

[28] A. Claus and A. Reh, *Annalen*, 1891, **266**, 207; H. Hubner, *Ber.*, 1877, **10**, 1705.

[29] P. J. Culhane, *Org. Synth.*, Coll. Vol. I, 1944, 125.

chloric acid (25 ml.) was added and the solution was heated on a boiling water-bath for 5 hr. The pH was adjusted to 4 (aqueous sodium hydroxide) and the solution was chromatographed (charcoal). The column was washed with water after which elution with 1% aqueous pyridine gave a purple eluate which was taken to dryness under reduced pressure. Addition of ethanol–ether (1 : 1) to a solution in the minimum of 80% aqueous alcohol gave a purple precipitate of an aminocarboxyphenazinedisulphonic acid (37 mg., 49%) [Found (after correction for residue): C, 37·3; H, 3·35; Br, nil; S, 14·5. $C_{14}H_{11}N_3O_8S_2,2H_2O$ requires C, 37·4; H, 3·3; S, 14·4%], R_B (A) = 1·05; R_B (B) = 0·5; M_B = 1·9; $\nu_{max.}$ 1717 (C=O) and 1032 cm.$^{-1}$ (S=O); τ 1·33, 2·0 (in D_2O); 1·63, 2·67, (equal intensities) (in D_2O–NaOD). It was apparently identical with the product obtained from aeruginosins A and B with unbuffered aqueous sodium sulphite.

2-Amino-8-bromo-10-methylphenazinium-6-carboxylate (8-Bromoaeruginosin A).—Crude 2-amino-8-bromo-6-methoxycarbonyl-10-methylphenazinium 2,4-dinitrobenzenesulphonate (from 664 mg. of phenazine) dissolved in dilute hydrochloric acid (N; 250 ml.) was heated at 100° for 1 hr.; it was then filtered whilst hot and the residue was washed with hot water. The pH of the combined filtrate and washings was adjusted to 3 (aqueous sodium hydroxide), and the solution was left overnight at 0° to give a red precipitate. The precipitate was collected and dissolved in water. The solution was passed through a deactivated alumina column to remove 7-amino-3-bromophenazine-1-carboxylic acid. The eluate was concentrated under reduced pressure to 50 ml., and was then boiled and filtered. Dark red needles of *8-bromoaeruginosin A* separated from the cool solution. A further quantity was obtained from the mother liquors by absorption on charcoal and gradient elution with 10% aqueous pyridine. The combined crops were recrystallised from water (217 mg., 30%) (Found: C, 45·75; H, 3·85; N, 11·5. $C_{14}H_{10}BrN_3O_2,2H_2O$ requires C, 45·7; H, 3·8; N, 11·4%).

Reaction of 8-Bromoaeruginosin A with Sulphite Ion.—A solution of 8-bromoaeruginosin A (2·0 mg.) and anhydrous sodium sulphite (7 mg.) in water (20 ml.) was set aside at room temperature for 3 days after which it was acidified and warmed on a water-bath for 10 min. Paper chromatography and electrophoresis showed the same major and minor components as described for the action of sulphite on the 2-amino-8-bromo-6-carbomethoxy-10-methylphenazinium ion. The same products were formed when the reaction mixture was buffered at various pH.

Dimethyl 4,6-Dinitrodiphenylamine-2,2′-dicarboxylate.—A solution of 4,6-dinitrodiphenylamine-2,2′-dicarboxylic acid[30] (1·0 g.) in dried methanol (50 ml.) was treated with ethereal diazomethane until an orange solid separated. The precipitate was collected and recrystallised (absolute methanol) to give bright yellow, fibrous needles of the *ester* (795 mg., 74%), m.p. 142—144° (Found: C, 51·35; H, 3·55; N, 11·55. $C_{16}H_{13}N_3O_8$ requires C, 51·2; H, 3·5; N, 11·2%).

Dimethyl 4,6-Diaminodiphenylamine-2,2′-dicarboxylate.—A solution of the foregoing ester (500 mg.) in dried methanol (150 ml.) was hydrogenated (5 atmos., 100 mg. Adams catalyst) until very pale yellow (2 hr.). The catalyst was filtered off and the filtrate was taken to dryness under reduced pressure of nitrogen. The residue was recrystallised [benzene–light petroleum (b.p. 60—80°) (1 : 4) sodium dried] under nitrogen to give cream-coloured needles of *dimethyl 4,6-diaminodiphenylamine-2,2′-dicarboxylate* (320 mg., 76%), m.p. 88—92° (Found: C, 58·0; H, 5·55; N, 12·6. $C_{16}H_{17}N_3O_4,H_2O$ requires C, 57·7; H, 5·7; N, 12·6%).

Nitrobenzene Cyclisation of Dimethyl 4,6-Diaminodiphenylamine-2,2′-dicarboxylate.—A solution of dimethyl 4,6-diaminodiphenylamine-2,2′-dicarboxylate (100 mg.) in nitrobenzene (25 ml.) was heated under reflux under nitrogen for 4 days; the solution was then taken to small volume *in vacuo* and chromatographed (alumina). Elution with methanol in ether gave many bands, the major one [R_B (A) = 1·85] giving a solid which was recrystallised from methanol (red needles) and identified as methyl 3-aminophenazine-1-carboxylate (m.p. 230—234°), by comparison with an authentic sample.[15]

4,6-Diaminodiphenylamine-2,2′-dicarboxylic Acid.—A solution of 4,6-dinitrodiphenylamine-2,2′-dicarboxylic acid[30] (2·0 g.) in methanol (50 ml.) was hydrogenated (5 atmos.; Raney nickel) for 24 hr. The light brown solution was concentrated under reduced pressure of nitrogen until crystallisation commenced. After several hours, the greyish precipitate of *4,6-diaminodiphenylamine-2,2′-dicarboxylic acid* (1·1 g., 67%) was collected, m.p. >300 (Found: C, 56·9; H, 4·6; N, 14·05. $C_{14}H_{13}N_3O_4,\frac{1}{2}H_2O$ requires C, 56·8; H, 4·8; N, 14·2%). Attempted recrystallisation of the diphenylamine from nitrobenzene gave a greenish precipitate, identified as *8-amino-6-carboxy-5,10-dihydro-11H-dibenzo[b,e][1,4]diazepin-11-one* (Found: C, 62·05; H, 3·9; N, 15·2. $C_{14}H_{11}N_3O_3$ requires C, 62·4; H, 4·1; N, 15·6%), $\lambda_{max.}$ (95% ethanol) 211, 236, 364 nm. (*cf.* 8-amino-5,10-dihydro-11*H*-dibenzo[*b*,*e*][1,4]diazepin-11-one[16]: $\lambda_{max.}$: 214, 228, and 344sh nm.

Nitrobenzene Cyclisation of 2′,4′-diaminodiphenylamine-2-carboxylic Acid.—A solution of 2′,4′-dinitrodiphenylamine 2-carboxylic acid[31] (1·0 g.) in methanol (100 ml.) was hydrogenated (5 atmos.; Raney nickel; 24 hr.). The catalyst was filtered off, and the greenish brown solution was concentrated under reduced pressure of nitrogen until crystallisation commenced. After a few hours the precipitate of 2′,4′-diaminodiphenylamine-2-carboxylic acid was collected and dissolved in nitrobenzene (100 ml.). The solution was heated under reflux under nitrogen for 15 hr. after which it was cooled, filtered, and extracted with aqueous sodium hydroxide (0·2N; 2 × 100 ml.). The alkaline solution was concentrated under reduced pressure and then neutralised (dilute hydrochloric acid) to give a purple precipitate of 7-aminophenazine-1-carboxylic acid[32] (100 mg., 14%). The nitrobenzene solution from the cyclisation was steam distilled. The greyish solid which remained was washed thoroughly with ethanol. The product (70 mg., 9%) was identified as 8-amino-5,10-dihydro-11*H*-dibenzo[b,e][1,4]diazepin-11-one by comparison with an authentic sample.[16]

Nitrobenzene Cyclisation of 4,6-Diaminodiphenylamine-2,2′-dicarboxylic Acid in the Presence of Base.—4,6-Diaminodiphenylamine-2,2′-dicarboxylic acid (296 mg.) and sodium methoxide (162 mg.) in nitrobenzene (150 ml.) were heated under reflux under nitrogen for 9 hr. A further 300 mg. of sodium methoxide was added immediately prior to the

[30] P. Cohn, *Monatsh.*, 1901, **22**, 396; A. Purgotti *et al.*, *Gazzetta*, 1903, **33** (II), 330.

[31] N. S. Drozdov and S. S. Drozdov, *Zhur. obshchei. Khim.*, 1934, **4**, 1 (*Chem. Abs.*, 1934, **28**, 5436).

[32] F. G. Holliman, B. A. Jeffery, and D. J. H. Brock, *Tetrahedron*, 1963, **19**, 1841.

solution being cooled. The reaction mixture was left at 0° overnight causing the separation of a black precipitate. The solid was collected, washed thoroughly with benzene, dried, and dissolved in water. The solution was filtered and then neutralised with dilute hydrochloric acid to give a purple precipitate of 3-aminophenazine-1-carboxylic acid; this was identified by esterification and comparison with an authentic sample of methyl 3-amino-phenazine-1-carboxylate.[15]

Methyl 2′,4′,6′-Trinitrodiphenylamine-2-carboxylate.—Methyl anthranilate (3·0 g.) picryl chloride (7·5 g.) and anhydrous sodium carbonate (5 g.) in absolute alcohol (20 ml.) were stirred and heated under reflux for 45 min. The solution was cooled and the orange precipitate was collected and washed thoroughly with water. Recrystallisation (absolute ethanol) gave yellow needles of the *diphenylamine* (4·5 g., 62%), m.p. 178—181° (Found: C, 46·6; H, 3·0; N, 15·45. $C_{14}H_{10}N_4O_8$ requires C, 46·4; H, 2·8; N, 15·5%).

The Cyclisation of Methyl 2′,4′,6′-Triaminodiphenylamine-2-carboxylate in Nitrobenzene.—(i) A solution of methyl 2′,4′,6′-trinitrodiphenylamine-2-carboxylate (500 mg.) in methanol (25 ml.) was hydrogenated (5 atmos.; 100 mg. Adams catalyst) until colourless (1 hr.). The solution was filtered into nitrobenzene (100 ml.) and the methanol was distilled off in an atmosphere of nitrogen. The nitrobenzene solution was heated under reflux under nitrogen for 24 hr., reduced to small volume *in vacuo* and chromatographed (alumina). Gradient elution with 4% methanol in ether gave a major orange eluate which was taken to dryness; the residue recrystallised (toluene) as reddish brown needles, $\lambda_{max.}$ (95% ethanol) 209, 232, 268, 287sh, 354sh, 366, and 430 nm; $\nu_{max.}$ 1720 cm^{-1} (C=O). It was apparently a mixture of an aminoquinoxalinophenazine-carboxylic acid methyl ester with an aminoquinoxalinophenazine [Found: C, 68·3; H, 3·95; N, 21·3%; M^+, 355 (weak), 297 (strong). $C_{20}H_{13}N_5O_2$ requires C, 67·6; H, 3·7; N, 19·7%; M^+, 355. $C_{18}H_{11}N_5$ requires C, 72·7; H, 3·75; N, 23·45%; M^+, 297].

The mixture (40 mg.) was dissolved in dilute hydrochloric acid (N; 50 ml.) and the solution was heated under reflux for 1 hr.; it was then made alkaline with sodium hydroxide solution. The reaction mixture was extracted with benzene (3 × 50 ml.) and the organic solution was dried ($MgSO_4$) and then chromatographed (alumina). A major yellow band was eluted with 1% methanol in ether. The residue from this eluate was recrystallised (toluene) to give reddish brown needles of 6-aminoquinoxalino[2,3-*a*]phenazine, m.p. 350° (Found: C, 72·45; H, 3·85; N, 23·7%; M^+, 297. $C_{18}H_{11}N_5$ requires C, 72·7; H, 3·75; N, 23·45%; *M*, 297); $\lambda_{max.}$ (95% alcohol): 207, 233, 267, 285sh, 351sh, 364, and 428 nm.

(ii) The above experiment was repeated but with only an 8 hr. period under reflux in nitrobenzene. Chromatography of the product (alumina) with gradient elution with 2% methanol in ether gave several minor yellow bands, a major lilac band (A), a minor orange band of the quinoxalinophenazines described above, and two purple bands (B and C). The eluate of band A was taken to dryness and the residue was recrystallised (toluene) to give red needles of a mixture of isomeric diamino(2′-methoxycarbonylanilino)-phenazines (Found: C, 67·4; H, 5·2; N, 19·35%; M^+, 359. $C_{20}H_{17}N_5O_2$ requires C, 66·8; H, 4·8; N, 19·5%; M^+, 359); $\nu_{max.}$: 1687 cm^{-1} (C=O). This mixture was rechromatographed (alumina) with 0·8% methanol in ether as eluant. A partial separation into red and purple bands was obtained. The first eluate (red band) was taken to dryness and the solid was recrystallised (toluene) to give dark red needles, m.p. 211—215° (t.l.c. showed slight contamination with the other isomer), $\nu_{max.}$ 1680 cm^{-1} (C=O); $\lambda_{max.}$ (95% alcohol): 241, 297, 336sh, 404, and 525 nm. The second eluate (purple band) was treated similarly to give dull, pinkish red needles uncontaminated by the other isomer, m.p. 251—254°,* $\nu_{max.}$ 1680 cm^{-1} (C=O); $\lambda_{max.}$ (95% alcohol): 240, 302, 350, 391, and 527 nm. The eluate of band B was taken to dryness and the solid was recrystallised (toluene) to give 1,3-diaminophenazine, m.p. 284—285°, (lit.,[20] 284—285°) identical with an authentic sample. Band C, similarly treated gave red needles of methyl 7,9-diaminophenazine-1-carboxylate (18 mg., 5%) m.p. 258—260° (Found: C, 62·8; H, 4·7; N, 20·8. $C_{14}H_{12}N_4O_2$ requires C, 62·7; H, 4·5; N, 20·9%), R_B (A) = 1·9; $\lambda_{max.}$ (95% alcohol): 220, 241sh, 305, 406, and 527 nm; $\nu_{max.}$ 1725 cm^{-1} (C=O).

1,3-*Diaminophenazine.*—A solution of 1,3,5-trinitrobenzene (250 mg.) in methanol (20 ml.) was hydrogenated (5 atmos.; 100 mg. Adams catalyst) for 5 hr. The colourless solution was filtered into nitrobenzene (50 ml.) and the methanol was distilled off in an atmosphere of nitrogen. The nitrobenzene solution was heated under reflux under nitrogen for 40 hr. after which it was taken to small volume *in vacuo* and chromatographed (alumina); 1·5% methanol in ether eluted a major purple band. This eluate was taken to dryness and the solid was recrystallised (benzene) to give 1,3-diaminophenazine (86 mg., 35%), m.p. 284—285°.

6-*Aminoquinoxalino*[2,3-a]*phenazine.*—The above experiment was repeated but with the reflux period in nitrobenzene extended to 6 days. The nitrobenzene was distilled off *in vacuo* and the solid was washed with a little benzene and recrystallised (toluene) to give reddish brown needles of 6-aminoquinoxalino[2,3-*a*]phenazine (219 mg., 63%), identical with the product described above.

2,4-*Diamino*-6-*methoxycarbonyl*-10-*methylphenazinium* 2,4-*Dinitrobenzenesulphonate.*—A solution of methyl 7,9-diaminophenazine-1-carboxylate (54 mg.) and methyl 2,4-dinitrobenzenesulphonate (80 mg.) in dry toluene (50 ml.) was heated in an oil-bath at 100° for 3 hr. A colour change from red to green was observed and a green solid separated. The solution was cooled and the green precipitate was collected, washed with toluene then ether, and dissolved in water (100 ml.). The aqueous solution was extracted with chloroform (2 × 50 ml.), concentrated under reduced pressure to *ca.* 20 ml., boiled, filtered, and left overnight at 0°. The green precipitate of the 2,4-diamino-6-methoxycarbonyl-10-methylphenazinium salt was collected and dried at 100° *in vacuo* over P_2O_5 (29 mg., 27%), m.p. 120—123° (Found: C, 45·4; H, 3·95; N, 15·25. $C_{21}H_{18}N_6O_9S,-1\frac{1}{2}H_2O$ requires C, 45·2; H, 3·8; N, 15·1%).

Reaction of the 2,4-Diamino-6-methoxycarbonyl-10-methylphenazinium Ion with Sulphite Ion.—A solution of 2,4-diamino-6-methoxycarbonyl-10-methylphenazinium 2,4-dinitrobenzenesulphonate (30 mg.) and anhydrous sodium sulphite (500 mg.) in water (100 ml.) was set aside at room temperature for 3 days. Concentrated hydrochloric acid (1·5 ml.) was added and the solution was set aside overnight. After being brought to pH 4 (aqueous sodium hydroxide), the solution was chromatographed (charcoal). The column was washed thoroughly with water, and then gradient eluted with 10% aqueous pyridine. A minor emerald-green

* Synthesis has shewn the purple compound to be (IX) (with N. V. Ellerton).

fraction followed by a major dark green fraction were eluted. The eluate of the minor product was taken to dryness under reduced pressure, and the solid was recrystallised from water to give a brownish green precipitate with the properties expected for a 2,4-diamino-6-carboxy-10-methylphenazinium-sulphonate; $M_B = 0.95$; $\nu_{max.}$ 1710 cm.$^{-1}$ (C=O); 1018 cm.$^{-1}$ (S=O). The eluate of the major product was similarly treated to give a product chromatographically and electrophoretically identical to that obtained by the acid hydrolysis of the 2,4-diamino-6-methoxycarbonyl-10-methylphenazinium salt, and presumably 4-aminoaeruginosin A; R_B (A), 1·15; R_B (B), 2·0; M_B, −0·2.

4-Amino-3-methylacetanilide.—A solution of 3-methyl-4-nitroacetanilide [33] (7·5 g.) in methanol (150 ml.) was hydrogenated (2 atmos.; 1 g. Adams catalyst, 2 hr.). The catalyst was filtered off and the solution was taken to dryness under reduced pressure. The residual oil crystallised (benzene) as white needles of *4-amino-3-methylacetanilide*, m.p. 101—103°, in almost quantitative yield (Found: C, 65·75; H, 7·1; N, 17·0. $C_9H_{12}N_2O$ requires: C, 65·8; H, 7·4; N, 17·1%).

4′-Acetamido-2′-methyl-6-nitrodiphenylamine-2-carboxylic Acid.—An intimate mixture of 2-bromo-3-nitrobenzoic acid [29] (7·4 g.), 4-amino-3-methylacetanilide (4·9 g.) and anhydrous sodium acetate (5·0 g.) was fused at 180° (on an oil-bath) for 2 hr. After being cooled, the brown solid was dissolved in a mixture of absolute alcohol (15 ml.) and aqueous sodium hydroxide (2N; 45 ml.). The filtered solution was acidified (hydrochloric acid). The red tar which separated was washed repeatedly with water until it solidified. Crystallisation (50% aqueous alcohol) gave thick, brown needles of the *diphenylamine* (6·25 g., 62%) m.p. 233—234° (Found: C, 58·2; H, 4·35; N, 13·1. $C_{16}H_{15}N_3O_5$ requires: C, 58·3; H, 4·6; N, 12·8%).

Methyl 4′-Amino-2′-methyl-6-nitrodiphenylamine-2-carboxylate.—(*a*) A solution of the above diphenylamine (2·0 g.) in dry methanol (100 ml.) was heated under reflux overnight whilst dry hydrogen chloride was passed through it. The solution was concentrated under reduced pressure until separation of the amine hydrochloride commenced. The solution was diluted with an equal volume of water after which it was made faintly alkaline with ammonia. The reddish brown precipitate was collected, washed with dilute sodium carbonate solution and then water, and then finally dried. Recrystallisation (dry methanol) gave red plates of *methyl 4′-amino-2′-methyl-6-nitrodiphenylamine-2-carboxylate* (1·08 g., 59%), m.p. 194—197° (Found: C, 59·8; H, 5·2; N, 14·2. $C_{15}H_{15}N_3O_4$ requires C, 59·8; H, 5·0; N, 13·9%).

(*b*) A solution of 4′-acetamido-2′-methyl-6-nitrodiphenylamine-2-carboxylic acid (5·0 g.) in aqueous sodium hydroxide solution (2N, 100 ml.) was heated under reflux for 90 min. The cooled solution was filtered and acidified with glacial acetic acid. The reddish brown precipitate (4·1 g.) was collected, washed with water, and dried *in vacuo* over sodium hydroxide. Paper chromatography showed that the major, yellow product [R_B (A) = 1·8] was contaminated by a minor, red material [R_B (A) = 1·4]. After esterification of the crude mixture with methanol and hydrogen chloride, chromatography (alumina; elution by 0·5% methanol in ether) gave a major brown fraction from which methyl 4′-amino-2′-methyl-6-nitrodiphenylamine-2-carboxylate, identical with that described above, was isolated. The other component was isolated from a slower-running orange fraction and identified as *methyl 7-amino-9-methylphenazine-1-carboxylate*, identical with the material described below.

Methyl 7-Amino-9-methylphenazine-1-carboxylate.—A solution of methyl 4′-amino-2′-methyl-6-nitrodiphenylamine-2-carboxylate (750 mg.) in methanol (50 ml.) was hydrogenated (5 atmos.; 150 mg. Adams catalyst) until colourless. The solution was filtered into nitrobenzene (200 ml.), and the methanol was distilled off in an atmosphere of nitrogen. The nitrobenzene solution was heated under reflux under nitrogen for 48 hr. and then taken to small volume *in vacuo* and chromatographed (alumina). Elution with 2% methanol in ether gave a major orange band. Evaporation of the eluate and recrystallisation of the residue (toluene) gave bright red needles of methyl 7-amino-9-methylphenazine-1-carboxylic acid (222 mg., 33%), m.p. 240—244° (Found: C, 67·25; H, 5·1; N, 16·0. $C_{15}H_{13}N_3O_2$ requires C, 67·4; H, 4·9; N, 15·7%).

2-Amino-4,10-dimethylphenazinium-6-carboxylate (4-methylaeruginosin A).—A solution of methyl 7-amino-9-methylphenazine-1-carboxylate (160 mg.) and methyl 2,4-dinitrobenzenesulphonate (260 mg.) in dry toluene (40 ml.) was heated under reflux at 100° (on an oil-bath) for 90 min., during which dark red crystals separated. The solution was cooled and the precipitate was collected and washed with dry toluene and then ether. A solution of the crude phenazinium salt in hydrochloric acid (N; 75 ml.) was heated under reflux on a boiling water-bath for 30 min. Reddish brown crystals of 2-amino-6-carboxy-4,10-dimethylphenazinium chloride were deposited from the cool solution. The mixture was set aside overnight after which the precipitate was collected and dissolved in hot water (30 ml.). The solution was filtered, cooled, and carefully neutralised with sodium hydroxide solution to give a red precipitate. Recrystallisation of this from water gave dark red needles of *4-methylaeruginosin A* (107 mg., 59%), m.p. >300° (Found: C, 59·2; H, 5·6; N, 13·7. $C_{15}H_{13}N_3O_2,2H_2O$ requires C, 59·4; H, 5·65; N, 13·9%).

Reaction of 4-Methylaeruginosin A with Sulphite Ion.—A solution of 4-methylaeruginosin A (1·0 mg.) and anhydrous sodium sulphite (15 mg.) in water (5 ml.) was set aside at room temperature for 24 hr. during which time the colour slowly became yellow. Acidification gave a reddish brown colour which became purple only when the mixture was exposed to the air for several hours. The solution was applied as a band to Whatman No. 3MM paper and chromatographed (A). The paper was dried and the two purple bands were irrigated with water. Examination showed the bluish purple product to be a disulphonic acid, [R_B (A) = 1·1; R_B (B) = 0·55; M_B 1·9; $\lambda_{max.}$ (aqueous buffer pH 7·0) 243, 301, 393sh, 405, and 553 nm.; $\lambda_{max.}$ (2N-hydrochloric acid) 243, 304, 389, and 566 nm.] similar to the product from aeruginosin B, and the minor purple product to be a monosulphonic acid [R_B (A) = 1·05; R_B (B) = 1·7; M_B = 1·0].

Cyclisation of 4′-Amino-6-nitrodiphenylamine-2-carboxylic Acids to Phenazines in Aqueous Alkali.—(a) *7-Aminophenazine-1-carboxylic acid*. A solution of 4′-amino-6-nitrodiphenylamine-2-carboxylic acid (273 mg.) in sodium hydroxide solution (2N; 5 ml.) was heated under reflux for 10 hr. The cooled solution was filtered and acidified with glacial acetic acid to give a brown precipitate which was collected, washed with water, and dried at 100°. Recrystallisation (nitrobenzene) gave dark red needles of 7-aminophenazine-

[33] E. Noelting and L. Stoeckling, *Ber.*, 1891, **24**, 564.

1-carboxylic acid (33 mg., 14%), identical with an authentic specimen.[32]

(b) 7-*Amino*-9-*methylphenazine*-1-*carboxylic acid* was prepared by a similar method and in similar yield from either 4′-amino-2′-methyl-6-nitro-diphenylamine-1-carboxylic acid or its 4′-acetyl derivative; m.p. >300° (Found: C, 66·1; H, 4·5; N, 16·45. $C_{14}H_{11}N_3O_2$ requires C, 66·4; H, 4·4; N, 16·6%).

(c) 7-*Amino*-3-*bromophenazine*-1-*carboxylic acid*. When a solution of 4′-amino-4-bromo-6-nitrodiphenylamine-2-carboxylic acid (1·76 g.) in aqueous sodium hydroxide (2N; 25 ml.) was stirred and heated under reflux, a brown precipitate formed. After 2·5 hr. the mixture was cooled and the precipitate was collected, washed with brine and dissolved in hot water; the solution was then filtered. Acidification (acetic acid) gave a purple precipitate which crystallised from nitrobenzene to give purple crystals of 7-amino-3-bromophenazine-1-carboxylic acid (585 mg., 37%) identical with that described above.

(d) 8-*Amino*-4-*carboxyphenazine*-2-*sulphonic acid* (*demethylaeruginosin B*). Sodium 4-bromo-3-carboxy-5-nitrobenzenesulphonate [5] (1·04 g.) and *p*-phenylenediamine (324 mg.) in sodium hydroxide solution (2N; 15 ml.) were heated under reflux for 1·5 hr. The solution was cooled, filtered, and concentrated hydrochloric acid was added to give a pH of 4. The mixture was set aside overnight at 0° after which the precipitate was collected and washed with water. Recrystallisation from water gave 8-amino-4-carboxyphenazine-2-sulphonic acid (35 mg.). A further 64 mg. were obtained from the mother liquors by chromatography (charcoal) as previously described, total yield 99 mg. (10%) identical with the product described above.

[0/628 *Received, April 22nd,* 1970]