Synthesis of Some Methoxy- and Hydroxy-phenazine-1-carboxylic Acids †

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The synthesis of the naturally occurring 6- and 9-hydroxyphenazine-1-carboxylic acids, (3) and (4), is described. Methyl 6-methoxyphenazine-1-carboxylate (12) is identified as a metabolite from *Streptomyces luteoreticuli*, and a metabolite of *Pseudomonas aureofaciens* is identified as 2-hydroxyphenazine-1-carboxylic acid (2) by comparison with synthetic material.

THE elaboration of phenazine metabolites by micro-organisms often involves hydroxylation of one or more of the aromatic rings. This reaction may involve simple hydroxylation as in the transformation of phenazine-1-carboxylic acid (1) into 2-hydroxyphenazine-1-carboxylic acid (2) in *Pseudomonas aureofaciens* cultures.[1] Alternatively a more complex process may be involved, as in the conversion of (1) into pyocyanin (7) by *Ps. aeruginosa*[1] or of 6-hydroxyphenazine-1-carboxylic acid (3) into iodinin (8) by *Brevibacterium iodinum*;[2] in these cases the carboxy-function is replaced by a hydroxy-group. In the course of our studies on phenazine biosynthesis we have synthesised both the hydroxy-acids (2) and (3) as well as 9-hydroxyphenazine-1-carboxylic acid (4) which is also a microbial metabolite.[3]

We recently developed a useful method for the synthesis of substituted phenazines which is particularly well suited to the preparation of 1-carboxyphenazine derivatives.[4] The method involves the reductive cyclisation of appropriately substituted 2-nitrodiphenylamines with sodium borohydride in ethanolic sodium ethoxide, and was applied to the synthesis of 6-methoxyphenazine-1-carboxylic acid (5) and 9-methoxyphenazine-1-carboxylic acid (6) from compounds (9) and (10), respectively. As expected the cyclisation of (9) gave 8-methoxyphenazine-1-carboxylic acid (11) along with the required 6-methoxy-isomer (5), but the facility with which (9) could be prepared from 2-bromo-3-nitrobenzoic acid[5] and *m*-anisidine outweighed the advantage of an unambiguous route to (5) which would have required a much less accessible 2-nitrodiphenylamine. The two isomeric phenazines were readily separated and were distinguished by their n.m.r. spectra.

In addition to the methoxy-acids small amounts of the corresponding ethoxy-compounds were also isolated, presumably as a result of direct nucleophilic displacement of the methoxy-group in the phenazine by ethoxide ion. If the cyclisation of (9) was carried out in t-butyl alcohol containing sodium t-butoxide the only phenazines isolated were (5) and (11). Cyclisation of (10) in ethanolic sodium ethoxide additionally gave small amounts of phenazine-1-carboxylic acid (1), formed as a result of cyclisation on to the carbon atom bearing the methoxy-group in (10).

In the n.m.r. spectrum of the 9-methoxy-acid (6) the signal at highest field (δ 7.80) can be assigned to the proton at C-8 on the basis of substituent effects; the absence of this signal in the n.m.r. spectrum of 6,8-dideuterio-9-methoxyphenazine-1-carboxylic acid[6] confirmed this assignment. However this signal does not show the more usual splitting into a doublet or double doublet [*cf.* the spectrum of (5), where the high field (δ 7.72) doublet is apparent with J 7.5 Hz]. Instead the spectrum is ' deceptively simple ' in that this signal (X) appears as a triplet with a coupling constant (J 4.5 Hz) which is expected[7] of an ABX system where, as here, $\delta_{AB} \longrightarrow 0$; the coupling conforms to the expected value of $(J_{AX} + J_{BX})/2$. The corresponding signal in the spectrum of the methyl ester of (6) is more complex in showing four strong transitions in which the outer two lines are separated by the expected value of $J_{AX} + J_{BX}$. This is also expected of an ABX system in which δ_{AB} is small.[8]

Attempted demethylation of (6) with boron tribromide[9] and boron trichloride[10] proved unsatisfactory but success was achieved with aluminium chloride.[11] 9-Hydroxyphenazine-1-carboxylic acid (4) was obtained,

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[10] F. M. Dean, J. Goodchild, L. E. Houghton, J. A. Martin, R. B. Morton, B. Parton, A. W. Price, and S. Somvchien, *Tetrahedron Letters*, 1966, 4153; W. Gerrard and M. F. Lappert, *Chem. Rev.*, 1958, **58**, 1081.

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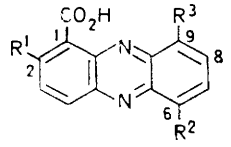
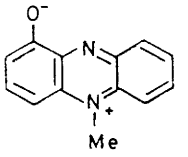
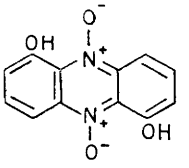
with properties similar to those reported for material isolated from microbial culture [3] and also synthesised,[3,12] by a less convenient route.



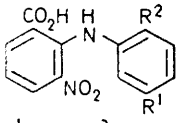
(1) $R^1=R^2=R^3=H$
(2) $R^1=OH, R^2=R^3=H$
(3) $R^1=R^3=H, R^2=OH$
(4) $R^1=R^2=H, R^3=OH$
(5) $R^1=R^3=H, R^2=OMe$
(6) $R^1=R^2=H, R^3=OMe$



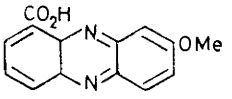
(7)



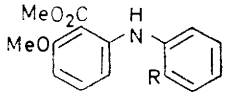
(8)



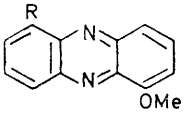
(9) $R^1=OMe, R^2=H$
(10) $R^1=H, R^2=OMe$



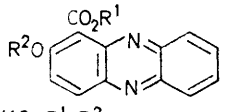
(11)



(14) $R=NH_2$
(15) $R=NO_2$



(12) $R=CO_2Me$
(13) $R=OMe$



(16) $R^1=R^2=Me$
(17) $R^1=Me, R^2=H$
(18) $R^1=H, R^2=Me$

Aluminium chloride was also successfully employed for the demethylation of 6-methoxyphenazine-1-carboxylic acid (5) [to (3)]. The hydroxy-acid (3) was subsequently shown to be an important intermediate in the biosynthesis of iodinin (8).[2,13]

Several methoxy-phenazine derivatives have recently been isolated from *Streptomyces luteoreticuli* cultures.[14] One of these was identified [15] as the 6-methoxy-derivative (12). The quoted physical data [14] for this compound closely correspond to those obtained for the methoxy-ester (12) prepared from the corresponding acid (5), and the structure for the metabolite is accordingly confirmed.

1,6-Dimethoxyphenazine (13) is, like (12), a metabolite of *S. luteoreticuli* and, by analogy with the biosynthesis of iodinin (8),[2] may reasonably be thought of as arising from either 6-methoxy- or 6-hydroxy-phenazine-1-carboxylic acid.

Cultures of *Ps. aureofaciens* produce an abundance of phenazine-1-carboxylic acid (1) and two minor metabolites.[16] The structure (2), assigned to one of these mainly on the basis of spectroscopic evidence,[17,18] was substantiated by showing *inter alia* that a degradation product was identical with 2-methoxy-1-methylphenazine,[18] and was confirmed by *X*-ray analysis of the metabolite itself.[19] We have independently confirmed the correctness of the structural conclusion in a synthesis of (2) by application of the earlier and more general, but less convenient route to substituted phenazines, namely the oxidative cyclisation of 2-aminodiphenylamines in boiling nitrobenzene. In this case the diphenylamine was (14), readily obtained by reduction of (15), itself prepared by condensation of methyl 2-amino-6-methoxybenzoate and *o*-iodonitrobenzene. Cyclisation of (14) gave methyl 2-methoxyphenazine-1-carboxylate (16), identical with a sample of the dimethyl derivative of naturally occurring (2).

Demethylation of synthetic (16) with tin(II) chloride and hydrochloric acid [18] gave, in our experience, low yields of methyl 2-hydroxyphenazine-1-carboxylate (17). Moreover treatment of this ester with aqueous alkali resulted in decarboxylation as well as hydrolysis (*cf.* ref. 18). On the other hand demethylation with boron trichloride [10] afforded 2-methoxyphenazine-1-carboxylic acid (18). No further reaction occurred even in the presence of a large excess of boron trichloride, but isolation of this product and further treatment with boron trichloride gave, in good yield, 2-hydroxyphenazine-1-carboxylic acid (2), identical with the natural product. Two steps are apparently necessary for the complete demethylation of (16); the complex formed on ester demethylation must be broken before cleavage at the aryl methoxy-group can be effected.

EXPERIMENTAL

N.m.r. spectra were recorded at 90 MHz. M.p.s were determined with a Kofler hot-stage apparatus. Chromatography refers to column chromatography on Kieselgel G (nach Stahl).[20] Where preparative t.l.c. was used instead, this is stated.

N-(3-*Methoxyphenyl*)-3-*nitroanthranilic Acid* (9).—A solution of 2-bromo-3-nitrobenzoic acid [5] (2.47 g), *m*-anisidine (1.48 g), and anhydrous sodium carbonate (1.5 g) in pentyl

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[13] R. B. Herbert, F. G. Holliman, and P. N. Ibberson, *Tetrahedron Letters*, 1974, 151.
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[15] S. Yamanaka, *Chiba Igakkai Zasshi*, 1972, **48**, 63 (*Chem. Abs.*, 1972, **77**, 162, 986).
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alcohol (50 ml) was refluxed for 4 h. Steam distillation left an oil which was taken into dichloromethane. The solution was washed with dilute mineral acid and dried. Evaporation, and recrystallisation of the residue from ethyl acetate–hexane gave yellow prisms of the *anthranilic acid* (9) (1.6 g, 56%), m.p. 189—191° (Found: C, 57.9; H, 4.15; N, 9.8. $C_{14}H_{12}N_2O_5$ requires C, 58.4; H, 4.15; N, 9.7%).

6-*Methoxyphenazine*-1-*carboxylic Acid* (5).—A solution of the anthranilic acid (9) (0.60 g), sodium borohydride (0.15 g), and sodium ethoxide (2M) in ethanol (100 ml) was refluxed for 20 h. The precipitate was collected and dissolved in water. The solution was filtered, acidified, and extracted with chloroform. The extract was dried and evaporated. The yellow solid thus obtained was chromatographed (benzene containing 40% ethyl acetate). Four compounds were eluted in the following order: 8-*ethoxyphenazine*-1-*carboxylic acid* (0.028 g, 5%), m.p. 237.5—239° (from aq. EtOH), $\nu_{max.}$ (KCl) 1 735 cm^{-1}; $\lambda_{max.}$ (EtOH) 265 (log ε 4.49), 368 (3.74), and 415 nm (3.66); *m/e* 268 (M^+), 224 ($M^+ - 44$), and 196; δ ($CF_3{\cdot}CO_2H$) 9.20 (1 H, dd, *J* 1.5 and 7.5 Hz, C-2 H), 9.00 (1 H, dd, *J* 1.5 and 8.5 Hz, C-4 H), 8.73 (1 H, d, *J* 10.5 Hz, C-6 H), 8.28 (1 H, dd, *J* 8.5 and 7.5 Hz, C-3 H), 7.97 (1 H, dd, *J* 2.5 and 10.5 Hz, C-7 H), 7.92 (1 H, d, *J* 2.5 Hz, C-9 H), 4.62 (2 H, q, *J* 7.0 Hz), and 1.70 (3 H, t, *J* 7.0 Hz) (Found: C, 66.9; H, 4.35; N, 10.25. $C_{15}H_{12}N_2O_3$ requires C, 67.1; H, 4.5; N, 10.45%); 8-*methoxyphenazine*-1-*carboxylic acid* (11) (0.143 g, 27%), m.p. 292—293° (from benzene–chloroform); $\nu_{max.}$ (Nujol) 1 730 cm^{-1}; $\lambda_{max.}$ (EtOH) 258 (log ε 4.57), 365 (3.49), and 400 nm (3.38); *m/e* 256, 254 (M^+), 210 ($M^+ - 44$), and 167; δ ($CF_3{\cdot}CO_2D$) 9.27 (1 H, d, *J* 7.5 Hz, C-2 H), 9.03 (1 H, d, *J* 8.5 Hz, C-4 H), 8.63 (1 H, d, *J* 10.5 Hz, C-6 H), 8.33 (1 H, dd, *J* 8.5 and 7.5 Hz, C-3 H), 8.1—7.9 (2 H, m, C-7 and -9 H), and 4.37 (3 H, s) (Found: C, 66.2; H, 4.05; N, 10.75. $C_{14}H_{10}N_2O_3$ requires C, 66.2; H, 3.95; N, 11.0%); 6-*ethoxyphenazine*-1-*carboxylic acid* (0.035 g, 6%), m.p. 238.5—239.5° (from aq. EtOH); $\nu_{max.}$ (KCl) 1 730 cm^{-1}; $\lambda_{max.}$ (EtOH) 264 (log ε 4.54), 370 (3.94), and 425 nm (3.48); *m/e* 270, 268 (M^+), and 224 ($M^+ - 44$); δ ($CF_3{\cdot}CO_2H$) 9.31 (1 H, dd, *J* 1.5 and 7.5 Hz, C-2 H), 9.02 (1 H, dd, *J* 1.5 and 9.0 Hz, C-4 H), 8.53 (1 H, dd, *J* 7.5 and 9.0 Hz, C-3 H), 8.41 (1 H, dd, *J* 7.5 and 8.5 Hz, C-8 H), 8.19 (1 H, dd, *J* 1.0 and 8.5 Hz, C-9 H), 7.64 (1 H, dd, *J* 1.0 and 7.5 Hz, C-7 H), 4.64 (2 H, q, *J* 7.0 Hz), and 1.72 (3 H, t, *J* 7.0 Hz) (Found: C, 67.3; H, 4.65; N, 10.2%); 6-*methoxyphenazine*-1-*carboxylic acid* (5) (0.182 g, 34%), m.p. 292.5—293° (from aq. EtOH); $\nu_{max.}$ (KCl) 1 720 cm^{-1}; $\lambda_{max.}$ (EtOH) 264 (log ε 4.67), 365 (3.94), and 428 nm (3.40); *m/e* 254 (M^+), 210 ($M^+ - 44$, *m** 174), and 181 [($M^+ - 44$) −29, *m** 159]; δ ($CF_3{\cdot}CO_2D$) 9.39 (1 H, d, *J* 7.5 Hz, C-2 H), 9.11 (1 H, d, *J* 9.0 Hz, C-4 H), 8.7—8.35 (2 H, m, C-3 and -8 H), 8.28 (1 H, d, *J* 9.0 Hz, C-9 H), 7.72 (1 H, d, *J* 7.5 Hz, C-7 H), and 4.43 (3 H, s) (Found: C, 65.85; H, 3.85; N, 10.65%).

A mixture of 6-methoxy- (12%) and 8-methoxy-phenazine-1-carboxylic acid (13%) was obtained by reductive cyclisation of (9) (0.60 g) with sodium borohydride (0.15 g) and sodium t-butoxide (2M) in refluxing t-butyl alcohol for 18 h (experiment with T. Etherington).

Methyl 8-*Methoxyphenazine*-1-*carboxylate*.—The acid (11) was esterified with an excess of ethereal diazomethane in methanol–dimethylformamide. Preparative t.l.c. (2% MeOH in $CHCl_3$) gave *methyl* 8-*methoxyphenazine*-1-*carboxylate* (66%), m.p. 100—101° (from aq. EtOH); $\nu_{max.}$ (Nujol) 1 730 cm^{-1}; $\lambda_{max.}$ (EtOH) 254 (log ε 4.84), 356 (3.95), and 397 nm (3.90); *m/e* 268 (M^+); δ ($CDCl_3$) 8.34 (1 H, dd, *J* 1.5 and 8.5 Hz, C-2 H), 8.22 (1 H, dd, *J* 1.5 and 7.0 Hz, C-4 H), 8.08 (1 H, d, *J* 10.0 Hz, C-6 H), 7.71 (1 H, dd, *J* 7.0 and 8.5 Hz, C-3 H), 7.55 (1 H, dd, *J* 2.5 and 10.0 Hz, C-7 H), 7.47 (1 H, d, *J* 2.5 Hz, C-9 H), 4.07 (3 H, s), and 4.01 (3 H, s) (Found: C, 66.9; H, 4.7; N, 10.15. $C_{15}H_{12}N_2O_3$ requires C, 67.1; H, 4.5; N, 10.45%).

Methyl 6-*Methoxyphenazine*-1-*carboxylate* (12).—Esterification of (5) with diazomethane as above gave *methyl* 6-*methoxyphenazine*-1-*carboxylate* (69%), m.p. 139—141° (from aq. EtOH); $\nu_{max.}$ (KCl) 1 725 cm^{-1}; $\lambda_{max.}$ (EtOH) 264 (log ε 4.69), 348 (3.74), 366 (3.93), and 415 nm (3.42); *m/e* 270 ($M^+ + 2$), 268 (M^+), 267 ($M^+ - 1$), 254, 253 ($M^+ - 15$), 239, 223, 210, 209, 199, 184, and 156; δ ($CDCl_3$) 8.55 (1 H, dd, *J* 1.5 and 8.5 Hz, C-2 H), 8.25 (1 H, dd, *J* 1.5 and 7.0 Hz, C-4 H), 8.0—7.4 (3 H, m, C-3, -8, and -9 H), 7.09 (1 H, dd, *J* 1.5 and 8.0 Hz, C-7 H), 4.17 (3 H, s), and 4.09 (3 H, s) (Found: C, 66.85; H, 4.55; N, 10.5%).

6-*Hydroxyphenazine*-1-*carboxylic Acid* (3).—A mixture of 6-methoxyphenazine-1-carboxylic acid (0.048 g), anhydrous aluminium chloride (0.20 g), and dry benzene (10 ml) was refluxed for 7 h. The residue obtained after removal of the benzene was treated with ice–water, aqueous sodium hydroxide (2M; 5 ml) was added, and the mixture was heated at 100 °C for 1 h. The solution was acidified and extracted with chloroform. This extract was dried. Evaporation gave 6-*hydroxyphenazine*-1-*carboxylic acid* (0.029 g, 64%), m.p. 250—251° (from aq. EtOH); $\nu_{max.}$ (KCl) 1 695 cm^{-1}; $\lambda_{max.}$ (EtOH) 269 (log ε 4.47), 366 (3.76), 373 (3.79), and 456nm (3.21); *m/e* 240 (M^+), 196 ($M^+ - 44$), and 168 ($M^+ - 44 - 28$); δ ($CF_3{\cdot}CO_2H$), 9.34 (1 H, dd, *J* 1.0 and 7.0 Hz, C-2 H), 9.00 (1 H, dd, *J* 1.0 and 8.5 Hz, C-4 H), 8.53 (1 H, dd, *J* 8.0 and 9.0 Hz, C-8 H), 8.40 (1 H, dd, *J* 7.0 and 8.5 Hz, C-3 H), 8.18 (1 H, dd, *J* 9.0 and 1.0 Hz, C-9 H), 7.76 (1 H, dd, *J* 1.0 and 8.0 Hz, C-7 H) (Found: C, 65.0; H, 3.45; N, 11.45. $C_{13}H_8N_2O_3$ requires C, 65.0; H, 3.35; N, 11.7%).

N-(2-*Methoxyphenyl*)-3-*nitroanthranilic Acid* (10).—A mixture of 2-bromo-3-nitrobenzoic acid [5] (3.04 g), *o*-anisidine (2.78 g), and sodium carbonate (2.2 g) was heated in refluxing pentyl alcohol (60 ml) for 18 h. The aqueous residue obtained after steam distillation was acidified. The orange precipitate which formed was collected, washed with dichloromethane, and recrystallised (aq. EtOH) to give red prisms of the *anthranilic acid* (10) (1.48 g, 42%), m.p. 229—231° (Found: C, 58.4; H, 4.25; N, 9.8. $C_{14}H_{12}N_2O_5$ requires C, 58.4; H, 4.15; N, 9.7%).

9-*Methoxyphenazine*-1-*carboxylic Acid* (6).—The anthranilic acid (10) (0.587 g) and sodium borohydride (0.087 g) were heated with sodium ethoxide (2M) in refluxing ethanol (60 ml) with stirring for 26 h. Water was added to dissolve the precipitate which formed; the solution was filtered and the ethanol removed under reduced pressure. Acidification gave a precipitate which was collected. Chromatography ($CHCl_3$ containing 10% MeOH) gave 9-*methoxyphenazine*-1-*carboxylic acid* as the major product, m.p. 262.5—263° (from EtOH) (0.266 g, 52%); $\nu_{max.}$ (KCl) 1 715 cm^{-1}; $\lambda_{max.}$ (EtOH) 263 (log ε 4.52), 366 (3.86), and 420sh nm (3.42); *m/e* 254 (M^+), 253, 235 [($M^+ - 1$) −18, *m** 219], 225 [($M^+ - 1$) −28], and 210 ($M^+ - 44$); δ ($CF_3{\cdot}CO_2H$) 9.29 (1 H, dd, *J* 2.0 and 7.0 Hz, C-2 H), 9.00 (1 H, dd, *J* 2.0 and 9.0 Hz, C-4 H), 8.40 (1 H, dd, *J* 7.0 and 9.0 Hz, C-3 H), 8.32 (2 H, d, line separation 4.5 Hz, C-6 and -7 H), 7.80 (1 H, t, line separation 4.5 Hz, C-8 H), and 4.40 (3 H, s) (Found:

C, 66.6; H, 4.1; N, 10.95. $C_{14}H_{10}N_2O_3$ requires C, 66.2; H, 3.95; N, 11.0%).

Phenazine-1-carboxylic acid (isolated as its methyl ester by chromatography after methylation of the crude cyclisation mixture) and *9-ethoxyphenazine-1-carboxylic acid* (0.062 g, 11%) were isolated as minor products. The latter had m.p. 230—231.5°; $\nu_{max.}$ (Nujol) 1 730 cm^{-1}; $\lambda_{max.}$ (EtOH) 265 and 370 nm; *m/e* 268 (M^+), 253 ($M^+ - 15$, *m**239), 235 [($M^+ - 15$) − 18, *m** 219], and 224 ($M^+ - 44$); δ ($CF_3{\cdot}CO_2H$; 60 MHz) 9.29 (1 H, dd, *J* 1.0 and 7.0 Hz, C-2 H), 9.00 (1 H, dd, *J* 1.0 and 9.0 Hz, C-4 H), 8.8—8.2 (3 H, m, C-3, -6, and -7 H), 7.76 (1 H, t, line separation 4.5 Hz, C-8 H), 4.7 (2 H, q, *J* 6.5 Hz), and 1.8 (3 H, t, *J* 6.5 Hz) (Found: C, 67.25; H, 4.45; N, 10.5. $C_{15}H_{12}N_2O_3$ requires C, 67.1; H, 4.5; N, 10.45%).

Methyl 9-Methoxyphenazine-1-carboxylate.—9-Methoxyphenazine-1-carboxylic acid was esterified with methanol and concentrated sulphuric acid (2 h under reflux). Non-acidic material was chromatographed (preparative t.l.c.; 5% MeOH in $CHCl_3$). *Methyl 9-methoxyphenazine-1-carboxylate* was isolated as the major product (68%), m.p. 114—116° (from aq. EtOH); $\nu_{max.}$ (KCl) 1 728 cm^{-1}; $\lambda_{max.}$ (EtOH) 263 (log ε 4.82), 346 (3.95), 364 (4.09), and 410 nm (3.73); *m/e* 268 (M^+), 267, 253, 239, 235, 222, 208, 179, and 176; δ ($CF_3{\cdot}CO_2H$), 9.23 (1 H, dd, *J* 1.0 and 7.0 Hz, C-2 H), 9.09 (1 H, dd, *J* 1.0 and 9.0 Hz, C-4 H), 8.5—8.2 (3 H, m, C-3, -6, and -7 H), 7.84 (1 H, four lines, the outer ones being separated by 8.5 Hz, C-8 H), 4.43 (3 H, s), and 4.32 (3 H, s) (Found: C, 67.45; H, 4.6; N, 10.5. $C_{15}H_{12}N_2O_3$ requires C, 67.1; H, 4.5; N, 10.45%).

9-Hydroxyphenazine-1-carboxylic Acid (4).—9-Methoxyphenazine-1-carboxylic acid (0.047 g) was suspended in dry benzene (10 ml). The mixture was refluxed with anhydrous aluminium chloride (0.10 g) for 5 h. The benzene was evaporated off and the residue was treated with ice–water and extracted with chloroform to remove unchanged starting material. The aqueous solution was basified (10 ml of 2M-NaOH) and heated at 100 °C for 30 min. Acidification precipitated the *9-hydroxyphenazine-1-carboxylic acid* (0.021 g, 47%), m.p. 290° (from aq. EtOH); $\nu_{max.}$ (KCl) 1 720 cm^{-1}; $\lambda_{max.}$ (EtOH) 270.5 and 372 nm; *m/e* 240.053 40 ($C_{13}H_8N_2O_3$ requires *M*, 240.052 96), 222 ($M^+ - 18$, *m** 205), 195 [($M^+ - 18$) − 28, *m** 169], and 196 ($M^+ - 44$) (Found: C, 60.4; H, 4.2; N, 10.4. $C_{13}H_8N_2O_3,H_2O$ requires C, 60.5; H, 3.9; N, 10.9%).

Methyl 6-Methoxy-N-(2-nitrophenyl)anthranilate (15).—Methyl 2-amino-6-methoxybenzoate [prepared from 2-methoxy-6-nitrobenzoic acid [21] by esterification [22] followed by hydrogenation in ethanol over platinum] (0.10 g), *o*-iodonitrobenzene (0.217 g), potassium carbonate (0.10 g), and copper powder (0.001 g) were thoroughly mixed and heated at 200 °C (bath temp.) for 15 min. The product was extracted into chloroform and purified by chromatography (benzene). *The anthranilate* (15) (0.055 g, 33%) was obtained as orange plates, m.p. 130—131° (from ethanol); $\nu_{max.}$ (KCl) 1 712 cm^{-1} (Found: C, 59.2; H, 5.00; N, 9.6. $C_{15}H_{14}N_2O_5$ requires C, 59.6; H, 4.65; N, 9.3%).

Methyl 2-Methoxyphenazine-1-carboxylate (16).—The anthranilate (15) (2.3 g) was hydrogenated in ethanol over platinum oxide (0.2 g). The amine (14) obtained after evaporation of solvent was used without purification for the next step.

A solution of this amine in nitrobenzene was refluxed under nitrogen for 162 h. Removal of the nitrobenzene left the crude product, which was chromatographed (benzene and 1 : 1 benzene–ether). The *methyl 2-methoxyphenazine-1-carboxylate* obtained was recrystallised from light petroleum; yield 0.464 g (23%); m.p. 129—130°; $\nu_{max.}$ (KCl) 1 737 cm^{-1}; $\lambda_{max.}$ (EtOH) 215 (log ε 4.47), 257 (4.87), 363 (3.94), and 398 nm (3.75); δ ($CDCl_3$) 8.31 (1 H, d, *J* 9 Hz, C-4 H), 8.3—8.1 (2 H, m), 7.9—7.8 (2 H, m), 7.83 (1 H, d, *J* 9 Hz, C-3 H), 4.13 (3 H, s), and 4.10 (3 H, s) (Found: C, 67.05; H, 4.45; N, 10.65. $C_{15}H_{12}N_2O_3$ requires C, 67.1; H, 4.5; N, 10.45%), identical (t.l.c., i.r., u.v., and ^1H n.m.r. spectra, and m.p. and mixed m.p.) with that obtained by methylation (ethereal diazomethane in tetrahydrofuran) of 2-hydroxyphenazine-1-carboxylic acid obtained from *Pseudomonas aureofaciens* cultures.

2-Hydroxyphenazine-1-carboxylic Acid (2).—To methyl 2-methoxyphenazine-1-carboxylate (5 mg) in dichloromethane at 0 °C was added a cooled (−70 °C) solution of boron trichloride (0.2 ml) in dichloromethane (1 ml). The reaction was kept at 0 °C for 10 min, then aqueous sodium acetate was added followed by aqueous sodium hydroxide. The solution was acidified and extracted with chloroform. The extracts were dried and evaporated leaving a residue which had properties consistent with 2-methoxyphenazine-1-carboxylic acid [18] [m.p. 193—195°; $\nu_{max.}$ ($CHCl_3$) 1 715 cm^{-1}].

Treatment of this material with boron trichloride as above gave *2-hydroxyphenazine-1-carboxylic acid* (overall 56%) which was recrystallised from benzene after preparative t.l.c. (10% MeOH in $CHCl_3$). This material was identical (t.l.c., i.r. and u.v. spectra, and melting behaviour) with that obtained from *Ps. aureofaciens* cultures (Found: C, 64.85; H, 3.35; N, 11.95. $C_{13}H_8N_2O_3$ requires C, 65.0; H, 3.35; N, 11.7%).

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