2-Pyruvoylaminobenzamide, a Metabolite of *Penicillium chrysogenum* and *P. notatum*

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A new metabolite of *Penicillium chrysogenum* and *P. notatum* is shown to be 2-pyruvoylaminobenzamide.

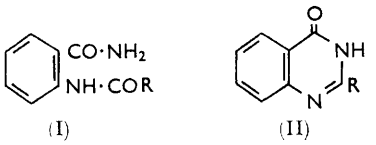
We have isolated from fermentations of *Penicillium chrysogenum* and of *P. notatum* a new metabolite, present in the culture filtrate, to which we assign the structure 2-pyruvoylaminobenzamide (Ia).

The (neutral) metabolite, $C_{10}H_{10}N_2O_3$, is optically inactive and shows bands in the i.r. characteristic of amide groups (1691, 1661, 1592, and 1523 cm.$^{-1}$) as well as a carbonyl band at 1730 cm.$^{-1}$ and two bands attributable to hydroxy- or imino-groups. The n.m.r. spectrum shows signals due to four aromatic protons, an acetyl or allylic methyl group, and three exchangeable protons at τ −2·7, 1·7, and 2·3. Acid hydrolysis of the metabolite gives anthranilic acid, accounting for the four aromatic protons.

On treatment with alkali, the metabolite loses the elements of water to give the quinazolinone (IIa), not previously described, which shows n.m.r. signals due to four aromatic protons, a methyl group, and an exchangeable proton (very broad signal at about τ −3·0). Clearly, no C*H* has been lost in the reaction. The quinazolinone

(IIa) has also been isolated in low yield from fermentations of *P. chrysogenum* where it presumably occurs as an artefact; the metabolite is slowly converted into the quinazolinone on standing at room temperature in buffer at pH 6 (but not at pH 4).

That the methyl group present in the metabolite and in its dehydration product is part of a *C*-acetyl group is

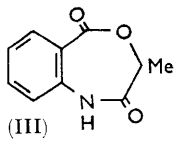


(I)

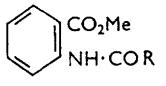
a; R = Ac
b; R = Me CH·OH
c; R = Me CH·OAc

(II)

a; R = Ac
b; R = Me CH·OH



(III)



(IV)

a; R = Me CH·OH
b; R = Ac

shown by reduction of the metabolite, with simultaneous loss of the elements of water, with sodium borohydride to give the secondary alcohol (IIb) (quartet at τ 4·9, doublet at 8·4) which had been synthesised [1] by treatment of the benzoxazepine (III) with methanolic ammonia. Repetition of this synthesis gave material identical with the naturally derived alcohol. The alcohol (IIb) can be oxidised with chromic acid to the product (IIa) obtained by treatment of the metabolite with base.

The keto-group in the metabolite can be reduced catalytically without loss of water to give the alcohol (Ib) which forms a crystalline acetate (Ic).

The above facts suggested structure (Ia) for the metabolite and this was confirmed by its synthesis from anthranilamide and pyruvoyl chloride, and by synthesis of the acetate (Ic) from anthranilamide and α-acetoxypropionyl chloride.

The major product from treatment of the benzoxazepine (III) with methanolic ammonia is the methyl ester (IVa) which has not previously been described. If aqueous ammonia is used the quinazolinone (IIb) is obtained in high yield. The ester (IVa) can be oxidised with chromic acid to the keto-ester (IVb).

EXPERIMENTAL

Unless otherwise stated, i.r. spectra were determined for Nujol mulls and u.v. spectra were measured in methanol. N.m.r. spectra were determined on a Varian Associates A 60 spectrometer with tetramethylsilane as internal standard; parentheses after τ-values contain relative intensity and multiplicity of signal. Molecular weights were determined on A.E.I. MS 9 mass spectrometer. Silica gel for chromatography was Hopkin and Williams M.F.C. and light petroleum had b. p. 60—80°.

Isolation of 2-Pyruvoylaminobenzamide.—(*a*) *Penicillium chrysogenum* (ACC 1482) was grown at 25° in Thompson bottles each containing 1 l. of an aqueous medium made up from Cerelose (5%), sodium nitrate (0·8%), potassium dihydrogen phosphate (0·01%), magnesium sulphate heptahydrate (0·05%), potassium chloride (0·05%), ferrous sulphate (0·001%), and minor-element concentrate (0·1%). After incubation for 15 days the contents of the bottles were filtered and the filtrate (77 l.) was extracted with ethyl acetate (5 × 7 l.). The extract was concentrated and the resulting precipitate was crystallised from ethyl acetate to give 2-*pyruvoylaminobenzamide* (Ia) as needles (5·6 g.), m. p. 181—184°, $[\alpha]_D$ 0° (Found: C, 58·2; H, 5·0; N, 13·6%; *M*, 206. $C_{10}H_{10}N_2O_3$ requires C, 58·2; H, 4·9; N, 13·6%; *M*, 206), $\nu_{max.}$ 3408m, 3227br, 1730m, 1691s, 1661s, 1608w, 1592s, and 1523s cm.$^{-1}$, $\lambda_{max.}$ 211, 247, and 302 mμ (ε 22,000, 10,300, and 6000), τ (in [D_6]-dimethyl sulphoxide) —1·7 (1, br), 1·4 (1, dd), 1·7 (1, br), 4·5 (4, m), and 7·55 (3, s).

The mother-liquor from the precipitated amide was evaporated to dryness to give a brown semi-solid (3·6 g.) which was chromatographed on silica gel. Elution with chloroform gave a solid (280 mg.) which crystallised from acetone to give 2-acetyl-3*H*-quinazolin-4-one (see below) (129 mg.). Elution with chloroform–ethyl acetate (9 : 1 and 5 : 1) gave a solid (1·02 g.) from which was obtained further amide (Ia) (500 mg.)

(*b*) *P. notatum* (ACC 26) was grown at 25° in Glaxo vessels on Raulin–Thom medium (250 ml. per flask). After incubation for 7 days the culture filtrate (4·6 l.) was acidified with hydrochloric acid and extracted with ethyl acetate to give a gummy residue (540 mg.) which was chromatographed on silica gel. Elution with chloroform–benzene (1 : 1) gave a solid (46 mg.) which was crystallised to give 2-pyruvoylaminobenzamide, m. p. 185—188°.

Acid Hydrolysis of 2-Pyruvoylaminobenzamide.—The amide (Ia) (20 mg.) in 3N-hydrochloric acid (10 ml.) was heated under reflux for 2 hr. The cooled solution was washed with ethyl acetate, basified with sodium hydrogen carbonate solution, and extracted with ethyl acetate to give a gummy solid (15 mg.) which was chromatographed on silica gel to give anthranilic acid (8 mg.), m. p. 143—145°, identical with an authentic sample.

2-*Acetyl*-3H-*quinazolin*-4-*one* (IIa).—(a) *From* 2-*pyruvoylaminobenzamide.* The amide (Ia) (57 mg.) in 2N-sodium hydroxide (15 ml.) was heated under reflux for 2 hr. The cooled mixture was acidified and extracted with ethyl acetate to give a yellow oil (45 mg.) which solidified and was recrystallised from acetone to give 2-*acetyl*-3H-*quinazolin*-4-*one* (IIa) (33 mg.) as prisms, m. p. 197—200° (decomp.) (Found: C, 63·5; H, 4·5; N, 14·8%; *M*, 188. $C_{10}H_8N_2O_2$ requires C, 63·8; H, 4·3; N, 14·9%; *M*, 188), $\nu_{max.}$ 3160w, 1706m, 1666s, and 1594m cm.$^{-1}$, $\lambda_{max.}$ 230 and 303 mμ (ε 16,700 and 8800); τ (in deuteriochloroform) 1·7 (1, m), 2·3 (3, m), and 7·25 (3, s).

(b) *From* 2-(1-*hydroxyethyl*)-3H-*quinazolin*-4-*one.* 8N-Chromic acid (0·8 ml.) was added dropwise to a solution of the alcohol (IIb) (250 mg.) in acetone. The mixture was then diluted with water and extracted with ethyl acetate to give a solid (130 mg.) which was chromatographed on silica gel. Elution with chloroform gave the ketone (Ia) (60 mg.), identical with the material obtained above.

Reduction of 2-Pyruvoylaminobenzamide with Sodium Borohydride.—Sodium borohydride (65 mg.) was added portionwise to the amide (Ia) (54 mg.) in methanol (5 ml.) and the mixture was set aside at room temperature overnight. The solution was diluted with water and extracted with ethyl acetate to give a pale yellow solid (34 mg.) which was recrystallised from acetone–light petroleum to give 2-(1-hydroxyethyl)-3*H*-quinazolin-4-one (IIb) (17 mg.)

[1] M. Uskokovic, J. Iacobelli, V. Toome, and W. Wenner, *J. Org. Chem.*, 1964, **29**, 582.

as needles, m. p. 178—180° (Found: C, 63·1; H, 5·3; N, 15·0%; *M*, 190. Calc. for $C_{10}H_{10}N_2O_2$: C, 63·1, H, 5·3; N, 14·7%; *M*, 190), identical with the synthetic material below; τ (in [D_6]acetone) 1·8 (1, m), 2·3 (3, m), 4·9 (1, q), and 8·4 (3, d).

Treatment of the Benzoxazepine (III) *with Ammonia.*—(*a*) (According to Uskokovic *et al.*[1]). The benzoxazepine (13·9 g.) in methanol (1·3 l.) saturated with ammonia was set aside at room temperature for 7 days. The solvent was evaporated *in vacuo* and the resulting syrup was chromatographed on silica gel. Elution with benzene–chloroform (3 : 1) gave a solid which was crystallised from ethyl acetate–light petroleum to give *methyl* N-(2-*hydroxypropionyl*)*anthranilate* (IVa) (6·1 g.) as prisms, m. p. 97—98° (Found: C, 59·5; H, 6·1; N, 6·2%; *M*, 223. $C_{11}H_{13}NO_4$ requires C, 59·2; H, 5·9; N, 6·3%; *M*, 223), $\nu_{max.}$ 3290m, 3200m, 1708s, 1662s, and 1586s cm.$^{-1}$, τ (in deuteriochloroform) −1·7 (1, br), 1·3 (1, dd), 2·0 (1, dd), 2·5 (1, m), 2·8 (1, m), 5·6 (1, q), 6·1 (3, s), 6·35 (1, br), and 8·45 (3, d).

Elution of the column with ethyl acetate–chloroform (1 : 1) gave a solid which was crystallised from acetone–light petroleum to give 2-(1-hydroxyethyl)-3*H*-quinazolin-4-one (IIb) (3·8 g.).

(*b*) A suspension of the benzoxazepine (III) (500 mg.) in ammonium hydroxide (*d* 0·88, 150 ml.) was shaken for 2 hr. and the resulting solution was set aside at room temperature for 7 days. The solution was evaporated to dryness *in vacuo* and the residue was taken up in ethyl acetate and washed with water, dried, and evaporated to give a solid (470 mg.) which was crystallised from acetone–light petroleum to give the quinazolinone (IIb) (340 mg.), m. p. 179—182°.

Catalytic Reduction of 2-Pyruvoylaminobenzamide.—The amide (Ia) (20 mg.) in ethyl acetate (5 ml.) was shaken with hydrogen in the presence of palladium–charcoal (5%; 20 mg.) for 60 hr. Removal of the catalyst and solvent gave a gum which was chromatographed on silica gel. Elution with ethyl acetate gave 2-(2-*hydroxypropionyl-amino*)*benzamide* (Ib) as a gum which slowly crystallised but which could not be recrystallised (Found: *M*, 208. $C_{10}H_{12}N_2O_3$ requires *M*, 208), $\nu_{max.}$ ($CHCl_3$) 3405m, 3355m, 3275m, 1688s, 1609w, 1579m, and 1524s cm.$^{-1}$, τ (in deuteriochloroform–trifluoroacetic acid) 1·7 (1, m), 2·6 (6, m), 5·4 (1, q), and 8·4 (3, d).

The hydrogenation product forms an *acetate* (Ic), m. p. 134—135° (Found: C, 57·7; H, 5·8; N, 11·2%; *M*, 250. $C_{12}H_{14}N_2O_4$ requires C, 57·6; H, 5·6; N, 11·2%; *M*, 250), $\nu_{max.}$ 3358m, 3251w, 3172m, 1750s, 1691s, 1668vs, 1626m, 1600m, 1538s, and 1516s cm.$^{-1}$, $\lambda_{max.}$ 217, 253, 300 mμ (ε 22,000, 13,500, and 3750).

Synthesis of 2-Pyruvoylaminobenzamide.— Pyruvoyl chloride (0·03 ml.; prepared by warming pyruvic acid with thionyl chloride) was added to a solution of anthranilamide (48 mg.) in chloroform containing a few drops of pyridine. After 3 hr., the chloroform was removed and the residue was taken up in ethyl acetate and washed with water, 3N-hydrochloric acid, water, sodium hydrogen carbonate solution, and water, dried, and evaporated to give a solid (28 mg.) which was recrystallised from ethyl acetate–light petroleum to give 2-pyruvoylaminobenzamide (22 mg.), m. p. and mixed m. p. 179—181°.

Synthesis of 2-(2-Acetoxypropionylamino)benzamide.—2-Acetoxypropionyl chloride[2] (0·15 ml.) was treated with anthranilamide (100 mg.) for 30 min. as described above to give 2-(2-acetoxypropionylamino)benzamide (Ic) (108 mg.), m. p. and mixed m. p. 133—135°.

Methyl N-*Pyruvoylanthranilate* (IVb).—8N-Chromic acid (2 ml.) was added dropwise to a solution of the hydroxy-ester (IVa) (1 g.) in acetone. The mixture was diluted with water and extracted with ethyl acetate to give a low-melting solid (950 mg.) which was chromatographed on silica gel. Elution with benzene and crystallisation of the product from light petroleum gave *methyl* N-*pyruvoylanthranilate* (IVb) (892 mg.) as fine needles, m. p. 111—112° (Found: C, 59·4; H, 5·1; N, 6·2. $C_{11}H_{11}NO_4$ requires C, 59·7; H, 5·0; N, 6·3%), $\nu_{max.}$ 3240w, 1728m, 1705s, 1687s, 1592s, and 1530s cm.$^{-1}$, τ (in deuteriochloroform) −2·2 (1, br), 1·3 (1, dd), 2·0 (1, dd), 2·5 (2, m), 6·05 (3, s), and 7·45 (3, s).

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[2] E. M. Filachione, J. H. Lengel, and C. H. Fisher, *J. Amer. Chem. Soc.*, 1944, **66**, 494.