# Studies in Mycological Chemistry. Part XXX [1] and Last. Isolation and Structure of Purpuride, a Metabolite of *Penicillium purpurogenum* Stoll

By **T. J. King, John C. Roberts,*** and **D. J. Thompson,** Department of Chemistry, The University, Nottingham NG7 2RD

The isolation and characterisation of purpuride, a metabolite of *Penicillium purpurogenum* Stoll, is recorded. Its structural elucidation, by direct *X*-ray analysis and determination of the chirality of one of the asymmetric centres in the molecule, is described. Purpuride is the *N*-acetyl-L-valine ester (1) of 1α-hydroxydrim-8-en-12,11-olactone.

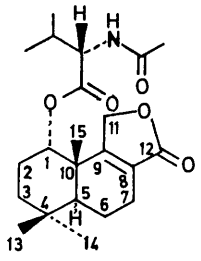
In the course of processing extracts from the dried mycelium of a strain of *Penicillium purporogenum* Stoll (with the main objective of producing the pigments purpurogenone [2] and deoxypurpurogenone [3]) we isolated, on one occasion only, a small quantity (12 mg) of a colourless, crystalline material for which we propose the trivial name purpuride. We now describe the elucidation of the structure of this compound.

Purpuride, $C_{22}H_{33}NO_5$ (mass spectrometry) dissolved in aqueous ethanol to form a neutral solution. I.r. ($\nu_{max.}$ 1756s $cm^{-1}$) and u.v. [$\lambda_{max.}$ 216·5 nm (log ε 4·08)] data indicated [4,5] the presence of an αβ-unsaturated γ-lactone. Additionally, the i.r. spectrum showed (i) strong absorption at 1736 $cm^{-1}$, suggesting the presence of an aliphatic ester group, and (ii) moderately strong absorptions at 3300, 1642, and 1554 $cm^{-1}$, characteristic of a secondary amide function.

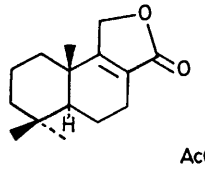
Since the amount of material available to us was now minimal (*ca.* 5 mg) we attempted a structural elucidation by direct *X*-ray crystallographic analysis. The *X*-ray measurements were made on a crystal with the approximate dimensions 0·5 × 0·4 × 0·4 mm, by use of a Hilger-Watts four-circle diffractometer. Mo-$K_\alpha$ Radiation was used and reflexions with θ between 1 and 30° were counted; a total of 1487 measurements were deemed to be significantly above the background. The structure was elucidated by use of the program MULTAN, developed at the Universities of York and Louvain in conjunction with the suite of programs of Dr. Ahmed and his collaborators of the N. R. C., Ottawa. A Fourier synthesis based on that of phases produced by MULTAN which had the highest figure of merit, revealed a complete structure with relative stereochemistry shown (1). Refinement by block-

[1] Part XXIX, G. M. Holmwood and J. C. Roberts, *J. Chem. Soc.* (*C*), 1971, 3899.
[2] (*a*) T. J. King, J. C. Roberts, and D. J. Thompson, *Chem. Comm.*, 1970, 1499; (*b*) J. C. Roberts and D. J. Thompson, *J. Chem. Soc.* (*C*), 1971, 3488.
[3] J. C. Roberts and D. J. Thompson, *J. Chem. Soc.* (*C*), 1971, 3493.
[4] S. M. Kupchan, Y. Aynehchi, J. M. Cassady, H. K. Schnoes, and A. L. Burlingame, *J. Org. Chem.*, 1969, **34**, 3867.
[5] L. Dorfman, *Chem. Rev.*, 1953, **53**, 125.

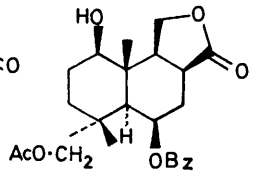
diagonal least-squares gave a final conventional $R$ value of 7·5% with all atoms treated anisotropically but with no contribution included from the hydrogen atoms. The bond-lengths and angles are within the usual limits found in such structures. Details of the cell



(1)



(2)



(3)

parameters, atomic positional and thermal parameters, bond-lengths and angles, and observed and calculated structure factors are given in Supplementary Publication No. SUP 20566 (11 pp., 1 microfiche).*

The result obtained does not specify the absolute configuration, so we attempted to solve this problem in the following way. Acid hydrolysis of purpuride (1·6 mg) yielded a solution which, after thorough extraction with ether, was investigated by paper chromatography. The presence of valine was established (matching $R_F$ values in three different solvent systems) but there was also present another (unidentified) ninhydrin-reactive substance which always showed much higher $R_F$ values. The valine in the hydrolysis product was not oxidisable [6] by D-amino-acid oxidase and hence was L-valine. Confirmation of the chirality of the valine by the o.r.d. method [7] was precluded by the scarcity of material.

The structure and absolute configuration of purpuride is therefore as shown (1). The spectral properties expected for a compound of this structure are in good agreement with those found.

So far as we are aware, purpuride is the first natural product known to be an ester formed from a terpenoid alcohol and an amino-acid (in this case *N*-acetylated). The terpenoid alcohol portion of purpuride is closely related to the plant product, confertifolin (2) [$\nu_{max.}$ ($CCl_4$) 1769 $cm^{-1}$, $\lambda_{max.}$ (EtOH) 217 nm (log ε 4·07] [8] and is also, but less closely, related to pebrolide (3), a metabolite of *P. brevi-compactum*.[9] The isolation of a compound, $C_{27}H_{41}NO_7$ (' antibiotic SL 3238 '), from a strain of *P. purpurogenum* (NRRL 3364) has been described [10] but no structural details have been disclosed.

## EXPERIMENTAL

Details concerning physical measurements and chromatography (column and thin-layer) have been given previously.[2b,3]

*Isolation and Properties of Purpuride.*—*Penicillium purpurogenum* Stoll (Centraalbureau voor Schimmelcultures, Baarn, Holland, No. 257.37) was grown and extracted as previously described.[11] Dried mycelium (300 g) yielded pigmented material (10 g) which was chromatographed on a magnesium sulphate column to give ' fraction (iii) ' [see ref. 2*b*] as a red solid (200 mg). Preparative t.l.c. of this solid [3] gave, on one occasion only, three (instead of the usual two) bands. The slowest-running band (yellow) yielded material which crystallised from ethyl acetate to give *purpuride* (12 mg) as large, colourless prisms, m.p. 200—201° (Found: $M^+$, 391·2350. $C_{22}H_{33}NO_5$ requires $M$, 391·2357), $\lambda_{max.}$ (EtOH) 216·5 nm (log ε 4·08), $\nu_{max.}$ (KBr) 3300, 2980, 1756, 1736, 1642, 1554, 1250, and 1240 $cm^{-1}$.

*Hydrolysis of Purpuride and Identification of Valine.*—To a solution of purpuride (1·6 mg) in a few drops of ethyl acetate was added 5N-hydrochloride acid (1 ml). The ethyl acetate was evaporated off and the resulting suspension was heated in a sealed, evacuated tube for 4 h. The product was diluted with water (7 ml) and was extracted with ether (5, 4, and 4 ml). The aqueous portion was taken to dryness *in vacuo* and the residue was dissolved in water (0·25 ml). The presence of valine in this solution was established in the following way.

Spots of the solution and spots of aqueous solutions of L- and D-valine hydrochlorides (2 mg $ml^{-1}$) on Whatman No. 1 papers were exposed to ammonia vapour and were then dried. The chromatograms were developed with the following (ascending) solvent systems: (i) n-butanol–acetic acid–water (4 : 1 : 5 v/v/v; upper layer), (ii) chloroform–methanol–aqueous 17% ammonia (2 : 2 : 1 v/v/v; upper layer), and (iii) the lower layer from system (ii). Two ninhydrin-reactive substances were always apparent in the chromatograms of the hydrolysis product. The two valines and one of the components in the hydrolysis product showed identical $R_F$ values in each system [0·52, 0·83, and 0·14 in systems (i)—(iii), respectively]. The second component in the hydrolysis product showed $R_F$ 0·85, 0·97, and 1·0 in solvents (i)—(iii).

*Determination of the Chirality of the Valine.*—L-Valine, the hydrolysis product of purpuride, and D-valine (all as their hydrochlorides, *ca.* 2·5 μg of each) were chromatographed, after exposure to ammonia vapour, on the same sheet of paper in solvent system (i). The dried paper was treated [6] with D-amino-acid oxidase [*ca.* 2 mg of the enzyme

* For details of Supplementary Publications see Notice to Authors No. 7 in *J. Chem. Soc.* (*A*), 1970, Issue No. 20.

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[10] G. P. 2,005,976/1970 (*Chem. Abs.*, 1970, **73**, 108,258m).

[11] J. C. Roberts and C. W. H. Warren, *J. Chem. Soc.*, 1955, 2992.

preparation (Sigma Chemical Co.) in twice its volume of M/15-phosphate buffer of pH 8·3] and was kept in a water-saturated atmosphere of oxygen at 37° for 2 h. The paper was dried at 37° and sprayed with ninhydrin (0·2% in ethanol). The chromatogram, after 20 min at 40—60°, revealed that the D-amino-acid had been destroyed but that the L-valine and the valine of the hydrolysis product had been unaffected. The last mentioned valine was therefore L-valine.

We thank the S.R.C. for a maintenance grant (to D. J. T.).

[2/1937 *Received*, 14*th August*, 1972]