Versimide, a Metabolite of *Aspergillus versicolor*

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The structure of versimide, a metabolite of *Aspergillus versicolor* has been established as methyl (+)-(*R*)-α-(methylsuccinimido)acrylate.

ASPERGILLUS VERSICOLOR is noted for the production of complex xanthones, anthraquinones,[1] and a γ-pyrone.[2] Examination[3] of a strain of *A. versicolor* led to the isolation of a new optically active liquid metabolite, $C_9H_{11}NO_4$, herein called versimide. The structure of versimide has been established as methyl (+)-(*R*)-α-(methylsuccinimido)acrylate (1).

The n.m.r. spectrum (in CCl_4) of versimide showed the following signals; two 1H singlets at τ 3·53 and 4·22, ascribed to a terminal methylene group; a 3H singlet at τ 6·23 from a methoxy-group; a 3H multiplet in the range τ 6·8—8·0; and a 3H doublet centred at τ 8·65 (*J* 7 Hz) derived from a secondary methyl group. When the n.m.r. spectrum of versimide was measured in deuterium oxide the two down-field protons appeared as a pair of doublets (*J* 1·1 Hz). This coupling was not observed in any other solvent. Dihydroversimide, $C_9H_{13}NO_4$ was formed by the catalytic reduction of versimide and possessed two secondary methyl groups (τ 8·64, 3H, *J* 7 Hz and 8·45, 3H, *J* 7 Hz); a methoxy-group (τ 6·28); 3H multiplet (τ 6·8—8·0) in which one proton was coupled to one of the secondary methyl groups; and a 1H quartet (τ 5·2, *J* 7 Hz). Hydrolysis of dihydroversimide with concentrated hydrochloric acid at 100° for 16 hr. gave alanine hydrochloride and (*R*)-methylsuccinic acid.[4] Hydrolysis of versimide under similar conditions gave the same acid.

This evidence suggested the imide structure (2; R = Me) dihydroversimide and thus versimide as the acrylate (1).

Examination of the i.r. spectra of versimide ($\nu_{max.}$ 1780w and 1720 cm.$^{-1}$) and its reduction product ($\nu_{max.}$ 1790w and 1720s cm.$^{-1}$) confirmed the presence of a five-membered imide,[5] while absorptions at $\nu_{max.}$ 1720, 1645, and 1200 cm.$^{-1}$ in versimide and at $\nu_{max.}$ 1750 cm.$^{-1}$ in dihydroversimide supported the conclusion that versimide was an acrylate.[6] Further evidence for the imide structure (1) was obtained by the hydrolysis of dihydroversimide at 100° for 4 hr. to give an acid $C_8H_{11}NO_4$, whose spectral properties (i.r., and n.m.r.)

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[1] E. Bullock, J. C. Roberts, and J. G. Underwood, *J. Chem. Soc.*, 1963, 4179; J. S. E. Holker and S. A. Kagal, *Chem. Comm.*, 1968, 1574; E. Bullock, D. Kirkaldy, J. C. Roberts, and J. G. Underwood, *J. Chem. Soc.*, 1963, 829; J. C. Roberts and P. Roffey, *ibid.*, 1965, 3666; J. H. Birkinshaw, J. C. Roberts, and P. Roffey, *J. Chem. Soc.* (*C*), 1966, 855; J. S. E. Holker, S. A. Kagal, L. J. Mulheirn, and P. M. White, *Chem. Comm.*, 1966, 911; P. Roffey and M. V. Sargent, *ibid.*, 1966, 913; P. Roffey, M. A. Sargent, and J. A. Knight, *J. Chem. Soc.* (*C*), 1967, 2328; Y. Hatsuda, T. Hamasaki, M. Ishida, and S. Yoshikawa, *Agric. and Biol. Chem.* (*Japan*), 1969, **33**, 131; T. Hamasaki, M. Renbutsu, and Y. Hatsuda, *ibid.*, 1967, **31**, 1513; T. Hamasaki, Y. Hatsuda, N. Terashima, and M. Renbutsu, *ibid.*, 1967, **31**, 11; T. Hamasaki, Y. Hatsuda, N. Terashima, and M. Renbutsu, *ibid.*, 1965, **29**, 166, 696.

[2] A. K. Dhar and S. K. Bose, *Tetrahedron Letters*, 1969, 4871.

[3] B.P. 1,187,070/1970.

[4] J. A. Mills and W. Klyne, *Progr. Stereochem.*, 1954, **1**, 203; Schreiber and Ripperger, *Annalen*, 1962, **655**, 114.

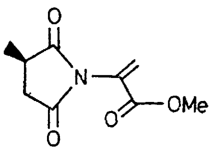
[5] K. Nakanishi, 'Infrared Absorption Spectroscopy,' Holden and Day, San Francisco, 1962, p. 47.

[6] Ref. 4, p. 44.

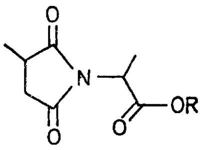
were very similar to those of dihydroversidmie (2; R = Me). It was concluded that the acid was α-(methylsuccinimido)propionic acid (2; R = H) since on methylation with diazomethane it gave dihydroversimide.

Synthesis of racemic α-(methylsuccinimido)propionic acid (2; R = H) and its methyl ester (2; R = Me) confirmed the structures of the hydrolysis product of dihydroversimide and of versimide respectively. Methylsuccinic anhydride was condensed with alanine at 180—200° and the product (2; R = H) was treated with diazomethane to form methyl α-(methylsuccinimido)-propionate, whose properties (i.r., n.m.r.) corresponded to those of dihydroversimide.

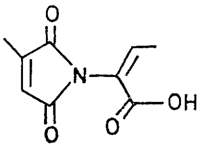
The only other reported[7] example of a naturally occurring imide is pencolide, which is an acid isolated from *Penicillium multicolor*. The material was shown to be *cis*-α-citraconimido-crotonic acid (3).[8]



(1)



(2)



(3)

EXPERIMENTAL

N.m.r. spectra were measured with a Varian A60-A instrument with deuteriochloroform as solvent unless stated otherwise and tetramethylsilane as internal standard. M.p.s were determined on a Kofler hot-stage apparatus and i.r. spectra were recorded on a Perkin-Elmer 137 spectrophotometer.

Versimide (1).—Versimide, the solution of which has been described[3] elsewhere, is a colourless viscous oil, soluble in organic solvents except light petroleum. It was purified by molecular distillation at 60°/0·003 mm., n_D^{21} 1·4896, $[\alpha]_D^{20}$ +22·3° (*c* 2 in $CHCl_3$), $\nu_{max.}$ (film) 1780 and 1720 cm.$^{-1}$; τ (CCl_4) 8·65 (d, 3H, CH_3CH, *J* 7 Hz), 6·8—8·0 (m, 3H, COCH_2CHCO), 6·23 (s, 3H, OCH_3), 4·22 (s, 1H, =C*H*H), and 3·53 (s, 1H, =CH*H*); τ (D_2O) 8·63 (d, 3H, CH_3CH, *J* 6·8 Hz), 6·5—7·7 (m, 3H, CO$CHCH_2$CO), 6·16 (s, 3H, OCH_3), 3·90 (d, 1H, =C*H*H, *J* 1·1 Hz), and 3·22 (d, 1H, =CH*H*, *J* 1·1 Hz) [Found: C, 54·3; H, 5·6; N, 6·9%; *M* (mass spectrum) 197. $C_9H_{11}NO_4$ requires C, 54·8; H, 5·6; N, 7·1%; *M* 197·2].

Hydrogenation of Versimide.—A solution of versimide (0·64 g.) in acetic acid (20 ml.) was hydrogenated in the presence of Adams' catalyst (100 mg.) at room temperature. The catalyst was removed and the solvent evaporated to give a colourless oil (0·6 g.) which was distilled in yield *dihydroversimide* b.p. 110—120° (air bath)/0·035 mm. $\nu_{max.}$ (film) 1790 1750 and 1720 cm.$^{-1}$; τ 8·64 (d 3H, CH_3CH, *J* 7 Hz), 8·45 (d, 3H, CH_3CH, *J* 7 Hz), 6·28 (s, 3H, OCH_3), 6·8—8·0 (m, 3H, CO$CHCH_2$CO), and 5·2 (q, 1H, CH_3C*H*, *J* 7 Hz) (Found: C, 54·05; H, 6·55; N, 7·0. $C_9H_{13}NO_4$ requires C, 54·25; , 6·6; H N, 7·05%).

Hydrolysis of Dihydroversimide.—(*a*) A solution of dihydroversimide (0·265 g.) in concentrated hydrochloric acid (4 ml.) was kept at 100° for 16 hr., cooled, diluted with water (4 ml.), and extracted with ethyl acetate (3 × 20 ml.). The ethyl acetate extract was washed with saturated brine, dried ($MgSO_4$), and evaporated to form a colourless solid (140 mg.), which was identified (i.r. and n.m.r.) as (+)-(*R*)-methylsuccinic acid, m.p. 109—110° [from ethyl acetate–light petroleum (b.p. 60—80°)] (lit.,[4] m.p. 112—114°), $[\alpha]_D^{25}$ +7·5° (*c* 2·7, H_2O) (Found: C, 45·4; H, 6·05. Calc. for $C_5H_8O_4$: C, 45·4; H, 6·05%). The aqueous solution on evaporation formed a colourless solid identical with a sample of (±)-alanine hydrochloride (i.r., n.m.r., and t.l.c.)

(*b*) A similar reaction carried out for 4 hr. at 100° again gave alanine hydrochloride, but from the ethyl acetate extract a colourless gum was isolated which solidified and was characterised as α-(*methylsuccinimido*)*propionic acid*, m.p. 99—100° [prisms from ethyl acetate–light petroleum (60—80°)] (Found: C, 51·2; H, 6·0; N, 7·1. $C_8H_{11}NO_4$ requires C, 51·9; H, 6·0; N, 7·59%), $\nu_{max.}$ (Nujol) 3500—2400, 1780, 1750, and 1675 cm.$^{-1}$; τ 8·65 (d, 3H, CH_3CH, *J* 7 Hz), 8·42 (d, 3H, CH_3CH, *J* 7 Hz) 6·75—7·95 (m, 3H, CO$CHCH_2$CO), 5·14 (m, 1H, CH_3C*H*CO_2H, *J* 7 Hz) and −0·20 (s, 1H, CO_2*H*, exchanges with D_2O).

Hydrolysis of Versimide.—Hydrolysis of versimide (146 mg.) with concentrated hydrochloric acid (2 ml.) in the above manner produced (+)-(*R*)-methylsuccinic acid, m.p. 104—106° raised to 109—110° [from ethyl acetate–light petroleum (b.p. 60—80°)] (33 mg.) identical to that produced from dihydroversimide.

α-(*Methylsuccinimido*)*propionic Acid.*—Racemic methylsuccinic anhydride (5·93 g., 0·4 mole) and (±)-alanine (4·93 g., 0·4 mole) were mixed together and heated at 180—190° for 1·5 hr. The clear melt obtained solidified on cooling and a portion (1 g.) was crystallised from ethyl acetate–light petroleum (b.p. 60—80°) to give α-(*methylsuccinimido*)*propionic acid*, m.p. 104—105° (Found: C, 52·1; H, 6·1; N, 7·45. $C_8H_{11}NO_4$ requires C, 51·9; H, 6·0; N, 7·55%), identical (i.r., n.m.r.) to that obtained by the hydrolysis of dihydroversimide.

Methyl α-(*Methylsuccinimido*)*propionate* (*Racemic Dihydroversimide*).—A portion of the above acid (3·3 g.) was treated with diazomethane in ether to yield methyl α-(*methylsuccinimido*)*propionate*, as a colourless oil (2·7 g.), b.p. 85—98°/0·05 mm. (Found: C, 53·95; H, 6·4; N, 6·4. $C_9H_{13}NO_4$ requires C, 54·25; H, 6·6; N, 7·05%), which was identical (i.r. and n.m.r.) to dihydroversimide.

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[7] J. H. Birkinshaw, M. G. Kalyanpur, and C. E. Stickings, *Biochem. J.*, 1963, **86**, 237; J. K. Sutherland, *ibid.*, 1963, **86**, 283.

[8] A. G. Brown and T. C. Smale, *Chem. Comm.*, 1969, 1489.