The Biosynthesis of Fungal Metabolites. Part VIII.[1] Identification of *N*-benzoyl-L-phenylalanyl-L-phenylalaninol Acetate, a Metabolite of *Aspergillus glaucus*

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Asperglaucide, a metabolite of *Aspergillus glaucus*, is shown by spectroscpoc methods, chemical degradation, and synthesis to be *N*-benzoyl-L-phenylalanyl-L-phenylalaninol acetate (II)

A NEW metabolite, asperglaucide, $C_{27}H_{28}N_2O_4$, is produced in relatively large amounts by the fungus *Aspergillus glaucus*, strains I.M.I. 53242 and 53243. Since the i.r. spectrum of the metabolite contained strong bands at 1 730 and 1 650 cm^{-1}, suggestive of ester and amide functions, respectively, it was decided to investigate the structure by hydrolytic procedures.

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be effected by coupling *N*-benzoylphenylalanine with phenylalaninol by, for example, the dicyclohexylcarbodiimide–*N*-hydroxysuccinimide method.[3,4] However, it is known that methods of this type produce racemisation of the *N*-benzoylphenylalanine residue *via* oxazolone formation.[5] Since *N*-benzyloxycarbonyl-L-phenylalanine

as a natural product;* the only record of a naturally occurring derivative seems to be a fungal polypeptide of unknown structure which gives phenylalaninol on hydrolysis.[6] The antibiotic chloramphenicol, which can be regarded as a derivative of phenylalaninol, appears to be the closest analogy.[7]

EXPERIMENTAL

Unless otherwise stated, i.r. spectra were measured with a Perkin-Elmer 257 instrument for solutions in chloroform, u.v. spectra with a Unicam SP 8000 spectrophotometer, ^1H and ^{13}C n.m.r. spectra with Varian HA-100 and XL-100-15FT instruments, respectively, for solutions in deuteriochloroform containing tetramethylsilane as internal standard, and optical rotations with an ETL-NPL automatic polarimeter. Mass spectra were measured at 70 eV with an A.E.I. MS12 spectrometer and accurate masses with an MS9 instrument. Silica gel GF (Merck) was used for t.l.c. M.p.s were determined with a Kofler hot-stage instrument.

Isolation of Asperglaucide (II).—*Aspergillus glaucus*, I.M.I. strain 53242 or 53243, was grown from a spore suspension in static culture for 15 days at 30 °C in flat vessels (*ca.* 1 l), each containing modified Czapek–Dox medium (500 ml; 5% sucrose and 5% sodium chloride). The dried mycelium was ground and continuously extracted with chloroform. The residue from this extraction was dissolved in the minimum amount of hot chloroform, and an excess of light petroleum (b.p. 60—80°) was added to precipitate a white solid. Several recrystallisations from ethyl acetate gave *asperglaucide* (II) (*ca.* 150 mg l^{-1}) as needles, m.p. 185—186°; $[\alpha]_D^{24}$ −39.9° (*c* 2.02 in $CHCl_3$); $\nu_{max.}$ 3 400 (NH), 1 730 (C=O), and 1 650 (C=O) cm^{-1}; $\lambda_{max.}(CH_2Cl_2)$ 238 nm (log ε 3.9); δ_C 20.7 (CO·*C*H_3), 37.4 (Ph*C*H_2), 38.5 (Ph*C*H_2), 49.5 (NH·*C*H·*C*H_2), 55.0 (NH·*C*H·CO), 64.6 (*C*H_2·O), 126.5—131.6 (7 discrete signals corresponding to the 15 aromatic CH), 136.6, 136.7, and 167.0 (aromatic *C*·C), and 170.5 (2 × C=O); *m/e* 444 (2%, $C_{27}H_{28}N_2O_4$), 384 (13, $C_{25}H_{24}N_2O_2$), 353 (6, $C_{20}H_{21}N_2O_4$), 323 (5, $C_{20}H_{21}NO_3$), 311 (8, $C_{18}H_{19}N_2O_3$), 293 (18, $C_{18}H_{17}N_2O_2$), 279 (6, $C_{18}H_{19}N_2O$), 269 (18, $C_{16}H_{17}N_2O_2$), 263 (2, $C_{18}H_{17}NO$), 252 (88, $C_{16}H_{14}NO_2$), 232 (12, $C_{13}H_{14}NO_3$), 224 (72, $C_{15}H_{14}NO$), 176 (9, $C_{11}H_{12}O_2$), 172 (30, $C_{11}H_{10}NO$), and 77 (100, C_6H_5), *m** *ca.* 332 (444 → 384), 274 (353 → 311), 235 (444 → 323), 199 (252 → 224), 163 (444 → 269), and 127.5 (232 → 172) (Found: C, 72.9; H, 6.4; N, 6.4. $C_{27}H_{28}N_2O_4$ requires C, 73.0; H, 6.4; N, 6.3%).

Deacetylasperglaucide (III).—Asperglaucide (660 mg) in chloroform (10 ml) was treated with a solution of sodium (38 mg) in methanol (0.9 ml) at room temperature for 3 h. After cooling to 0 °C the precipitate was collected, washed with water, and crystallised from aqueous ethanol to give *deacetylasperglaucide* (III) (500 mg), needles, m.p. 189—190° (with sintering from 179°); $[\alpha]_D^{24}$ −83.4° (*c* 1.04 in Me_2N·CHO), $\nu_{max.}$ 3 550 (OH), 3 400 (NH), and 1 650 cm^{-1} (Found: C, 74.3; H, 6.3; N, 7.0%; M^+, 402. $C_{25}H_{26}N_2O_3$ requires C, 74.6; H, 6.5; N, 7.0%; *M*, 402).

Acid-catalysed Hydrolysis of Asperglaucide.—Asperglaucide (750 mg) dispersed in 6N-hydrochloric acid (60 ml) was kept at 100 °C for 30 h. Extraction of the mixture with diethyl ether–ethyl acetate (1 : 1) gave benzoic acid, which separated from water as prisms, m.p. and mixed m.p. 121—122°. The aqueous residues were evaporated to dryness, water (100 ml) was added, and the resultant solution was neutralised with sodium hydrogen carbonate and saturated with sodium chloride. Isolation in ethyl acetate and purification by preparative t.l.c. [ethyl acetate–methanol (1 : 1)] gave L-phenylalaninol (I) (75 mg), which was purified *via* the hydrogen oxalate salt [8] [needles, m.p. 177—178° (from ethanol)]. The regenerated parent amine gave needles, m.p. 89—91°, $[\alpha]_D^{24}$ −27.2° (*c* 1.02 in EtOH) (lit.,[8] m.p. 90—91°, $[\alpha]_D$ −24.7°; lit.,[2] m.p. 91—93°, $[\alpha]_D$ −25.6°), identical (mixed m.p. and i.r. spectrum) with a sample prepared according to the literature procedure,[2] m.p. 91—92°, $[\alpha]_D$ −26.0° (*c* 9.09 in EtOH) (Found: H, 8.6; N, 9.3%).

A similar hydrolysate was subjected to amino-acid analysis (JEOL JLC-6AH instrument); phenylalanine was detected.

N-Benzyloxycarbonyl-L-phenylalanyl-L-phenylalaninol (V).—*N*-Benzyloxycarbonyl-L-phenylalanine (IV) (1.30 g), purified *via* the dicyclohexylamine salt [m.p. 154—156° (lit.,[9] 155—156°)], dicyclohexylcarbodi-imide (0.78 g), *N*-hydroxysuccinimide (0.84 g), and L-phenylalaninol (I) (0.43 g) were stirred in dimethylformamide (50 ml) at room temperature for 50 h. After addition of acetic acid (4 drops) to destroy the excess of carbodi-imide reagent, the mixture was cooled to 0 °C, filtered to remove dicyclohexylurea, and evaporated. A solution of the residue in ethyl acetate (70 ml) was washed successively with 2N-citric acid (10 ml), 2N-sodium hydrogen carbonate (10 ml), and water (10 ml) and then dried and evaporated to give *N-benzyloxycarbonyl-L-phenylalanyl-L-phenylalaninol*, which separated from toluene as needles (1.10 g), m.p. 158—159°, $[\alpha]_D^{24}$ −46.9° (*c* 1.38 in MeOH), $\nu_{max.}$ 3 550 (OH), 3 400 (NH), 1 710 (C=O of urethane), and 1 670 cm^{-1} (C=O of amide) (Found: C, 72.1; H, 6.5; N, 6.5%; M^+, 432. $C_{26}H_{28}N_2O_4$ requires C, 72.2; H, 6.5; N, 6.5; *M*, 432).

N-Benzyloxycarbonyl-L-phenylalanyl-L-phenylalaninol Acetate (VI).—The alcohol (V) (0.80 g) was treated with acetyl chloride–acetic acid (1 : 1) (30 ml) for 1 h at 24 °C. The reagent was removed *in vacuo* and the residue in ethyl acetate was washed with 2N-sodium hydrogen carbonate (10 ml) and water (10 ml); the solution was then dried and evaporated. The residue separated from ethyl acetate–ether as needles (0.90 g) of the *acetate*, m.p. 139—140°, $[\alpha]_D^{24}$ −24.1° (*c* 1.94 in $CHCl_3$), $\nu_{max.}$ 3 400 (NH), 1 720 (C=O of ester and urethane), and 1 670 cm^{-1} (C=O of amide) (Found: C, 71.0; H, 6.2; N, 6.2. $C_{28}H_{30}N_2O_5$ requires C, 70.9; H, 6.4; N, 5.9%).

L-Phenylalanyl-L-phenylalaninol Acetate Toluene-p-sulphonate Salt (VII).—The acetate (VI) (0.85 g) and toluene-*p*-sulphonic acid (0.35 g) in acetic acid (30 ml) were shaken under hydrogen at room temperature and atmospheric pressure in the presence of palladium–charcoal (5%; 80 mg) for 20 h until the uptake ceased. Isolated in the usual way, the *salt* (VII) formed needles (0.88 g) from ethanol–ether, m.p. 224—225°, $\nu_{max.}$ 1 730, 1 670, 1 120,

* *Note added in proof.* It has been reported recently that *N*-acetyl-L-phenylalanyl-L-phenylalaninol has been isolated from cultures of *Emericellopsis salmosynnemata* (A. D. Argoudelis, S. A. Mizsak, and L. Baczynskyj, *J. Antibiotics*, 1975, **28**, 733).

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[7] For biosynthesis see D. Gottlieb, in 'Antibiotics. Volume II. Biosynthesis,' eds. D. Gottlieb and P. D. Shaw, Springer-Verlag, New York, 1967, p. 37.
[8] J. H. Hunt and D. McHale, *J. Chem. Soc.*, 1957, 2073.
[9] E. Klieger, E. Schröder, and H. Gibian, *Annalen*, 1961, **640**, 157.

and 1 010 cm^{-1} (Found: C, 63.5; H, 6.0; N, 5.3. $C_{27}H_{32}N_2O_6S$ requires C, 63.3; H, 6.3; N, 5.5%).

N-*Benzoyl*-L-*phenylalanyl*-L-*phenylalaninol Acetate* (II).—The salt (VII) (0.74 g) suspended in chloroform (20 ml) was treated successively with benzoyl chloride (0.24 g) and triethylamine (0.86 g). After shaking at room temperature for 30 min, the mixture was evaporated *in vacuo* and the residue in ethyl acetate (80 ml) was washed successively with water (10 ml), 2N-citric acid (10 ml), 2N-sodium hydrogen carbonate (10 ml), and water (10 ml). The solution was then dried and evaporated, and the residue crystallised from ethyl acetate to give needles (0.72 g) of the acetate (II), m.p. and mixed m.p. with asperglaucide, 185—186°, $[\alpha]_D^{24}$ −40.0° (*c* 1.98 in $CHCl_3$) (Found: C, 73.0; H, 6.2; N, 6.4%).

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