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SYNTHESIS AND CHEMISTRY OF N-BENZOYL-O-[N'-BENZOYL-L-PHENYLALANYL]-L-PHENYLALANINOL, THE MAJOR MYCELIAL METABOLITE OF *PENICILLIUM CANADENSE*

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Abstract—The structure of the title compound was established by evidence including total and partial hydrolyses and a stereospecific synthesis. Debenzoylation using triethyloxonium fluoroborate was accompanied by rearrangement to the amidoalcohol 12 (desacetylaspergiaucide).

Culture filtrates of the fungus *Penicillium canadense* afford a number of related lactones including canadensolide and dihydrocanadensolide.[1] We now report the properties and synthesis of the major mycelial constituent, namely the novel ester N - benzoyl - O - [N' - benzoyl - L - phenylalanyl] - L - phenylalaninol (1).

The metabolite was readily obtained from chloroform extracts of *P. canadense* grown in surface culture. It formed needles, $C_{32}H_{30}N_2O_4$, m.p. 210°, $[\alpha]_D$ −98.6° (*c*, 0.76 in pyridine). The IR spectrum showed strong absorption at 1748 cm^{-1} (aliphatic ester) and at 1638 and 1530 cm^{-1} (benzamide) while the NMR and mass spectra showed features consistent with the presence of two benzyl and two benzamide functions.

Total hydrolysis of 1 by heating at 130° with 5 N HCl gave benzoic acid together with L-phenylalaninol (2) and L-phenylalanine. Selective hydrolysis of the ester link using 0.01 M NaOH afforded equal amounts of N - benzoyl - L - phenylalanine (4) and N - benzoyl - L - phenylalaninol (5). These results, together with a detailed analysis of the NMR spectra lead to the structure 1 for the metabolite. This structure was confirmed by synthesis as follows.

The alcohol 5 was prepared by benzoylation of 2. Although the dibenzoyl derivative 6 was also formed under the reaction conditions, this was readily hydrolysed by base to give a further quantity of 5. The key reaction, esterification of this alcohol with 4, was accomplished using N,N'-carbonyldiimidazole.[2] The main product, although showing the same mass spectrum and behaviour upon TLC as the natural ester 1, proved to be a mixture of epimers (identical to that obtained by esterifying 5 with N - benzoyl - DL - phenylalanine). Repeated crystallization of the mixture afforded the epimer 7, m.p. 217°, ν_{max} (KBr) 1735, 1652, 1638 and 1530 cm^{-1}, the mother liquors yielding a sample of the more soluble ester, 1, identical with the natural material. The formation of 7 in the above reaction is probably due to the intermediacy of the readily racemized oxazolone 8.[3] In support of this, the racemate of 8 was detected and isolated as a minor product of the reaction and was shown to afford the same mixture of the esters 1 and 7 when treated with N,N'-carbonyldiimidazole and the alcohol 5.

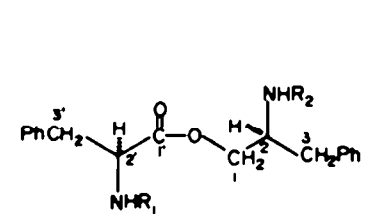
A stereospecific synthesis of 1 was achieved by esterification of 5 with N-CBZ-L-phenylalanine under similar conditions (giving 9), followed by removal of the protecting group and benzoylation of the intermediate amine 10 (Hydrobromide, m.p. 189°). The esterification in this case appeared to have been accompanied by little if any racemization.

It cannot be ruled out that in the biosynthesis of 1, benzoylation of one or more of the amino groups might occur after formation of the ester link. Hence the amine 10, prepared in the above synthesis, may be considered a potential biosynthetic intermediate. Attempts to prepare this or other debenzoyl derivatives of 1 from the natural ester led to the discovery of a novel transformation. Debenzoylation of 1 was carried out under conditions which proved effective for model benzamides, involving treatment with triethyloxonium fluoroborate and then with 3% aq. HOAc.[4] With a large excess of the reagent, a salt of the diamine 11 was formed. When only a slight excess of reagent was used, unexpectedly, the major isolable product was the amidoalcohol 12. Among spectroscopic features indicating the structure of this compound were the carbonyl absorption in the IR, which consisted of bands at 1640 cm^{-1} (benzamide) and at 1660 cm^{-1} (aliphatic amide), and the presence in the mass spectrum of prominent ions resulting from losses of 18 mass units. This product, which separated from various solvents as a gel, readily gave a crystalline benzoate (13), and its structure was confirmed by synthesis from 2 and N - benzoyl - L - phenylalanine azide (15). Although reaction of 2 with the *p*-nitrophenyl ester prepared from 4 also gave 12, this was accompanied by an almost equal amount of its crystalline diastereoisomer 16. The formation of 12 from the ester 1 may occur by acyl rearrangement of the debenzoylation product 17, analogous to rearrangement of O-acylepbedrines.[5]

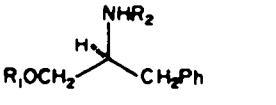
The ^{13}C NMR spectra of various derivatives of 2, obtained during the course of this work showed a consistent pattern (Table 1). The observed shielding effects resulting from O- and N-acylation are contrasted in Table 2.[6]

Recently, the related compound aspergiaucide (18) was isolated from a *Euphorbia* species[7] and from a fungus, *Aspergillus glaucus*,[8] the structure being confirmed by syntheses.[7,8] As expected, the O-acetyl derivative of the amidoalcohol 12, prepared in the present work, was identical with an authentic sample of 18.

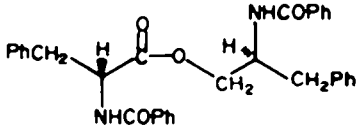
After completion of this work, a metabolite ("asperphenamate"), isolated from the mycelium of *Aspergillus flavipes* was reported to have ester structure 1,[9] and it is



1 $R_1 = R_2 = COPh$
9 $R_1 = CO_2CH_2Ph$, $R_2 = COPh$
10 $R_1 = H$, $R_2 = COPh$
11 $R_1 = R_2 = H$
17 $R_1 = COPh$, $R_2 = H$



2 $R_1 = R_2 = H$
3 $R_1 = R_2 = COMe$
5 $R_1 = H$, $R_2 = COPh$
6 $R_1 = R_2 = COPh$



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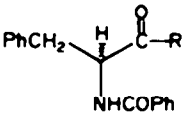
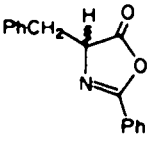


4 R = OH
14 R = $NHNH_2$
15 R = N_3



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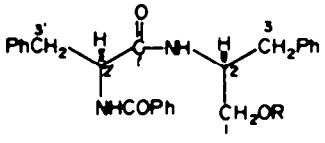

12 R = H
13 R = COPh
18 R = COMe

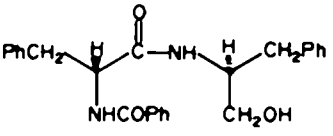

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Table 1. δ_C in C_5D_5N

Compound:	1	2	5	10	12
C-1	65.7	66.7	63.4	67.6	63.1
C-2	51.0	55.4	54.3	51.4	53.7
C-3	37.4	41.3	37.7	37.1	37.6
C-1'	172.4			170.4	171.8
C-2'	55.8			55.3	55.6
C-3'	37.5			37.1	38.6
CONH	167.7 168.5		167.8	167.7	167.7 171.8
aryl C p to CH_2	128.6 127.1	126.3	126.5	126.7 127.7	126.4 126.8
aryl C p to CONH	131.3 131.8		131.2	131.4	131.4
quaternary aryl C	135.0 135.7 138.1 138.6	140.4	136.2 139.8	135.6 135.6 139.1	135.5 138.5 139.5
other aryl C (signals > 2C)	128.1 128.6 128.7 128.8 129.7	128.7 129.8	127.9 128.6 128.7 129.9	128.5 128.7 129.1 129.8 130.1	128.0 128.6 129.9

Table 2. Shielding effects in some acyl derivatives of 2

		C-1	C-2	C-3
-OCOR	(δ_C^{10} - δ_C^{5})	+4.2	-4.0	-0.6
	(δ_C^{1} - δ_C^{5})	+2.3	-3.3	-0.3
-NHCOPh	(δ_C^{5} - δ_C^{2})	-3.3	-1.1	-3.6
-NHCOR	(δ_C^{12} - δ_C^{2})	-3.6	-1.7	-3.7

clear from comparison of the published data that this metabolite id identical to that from *P. canadense*.[1,b] We report details of biosynthetic studies in a separate paper.

EXPERIMENTAL

M.ps were recorded on a Reichert hot stage apparatus and are uncorrected. Spectra were obtained using the following instruments: IR spectra, Perkin–Elmer PE 257 or Perkin–Elmer PE 225; UV spectra, Pye–Unicam SP 800; NMR spectra, Varian T 60, Perkin–Elmer R 32, Varian HA 100 or Varian XL 100; mass spectra, AEI MS-12 or AEI MS-902. CD measurements were kindly obtained by Dr. P. M. Scopes, Westfield College, London. NMR spectral assignments were supported by appropriate decoupling experiments. The IR frequencies of 1 and 7 as KBr discs were calibrated against H_2O vapour.

Isolation of N - *benzoyl* - O - (N' - *benzoyl* - L - *phenylalanyl*) - L - *phenylalaninol* (1). Dried mycelium from 11 day old cultures of the fungus *Penicillium canadense* (CMI 95,493) grown on Czapek-Dox/1% yeast extract medium, was extracted with $CHCl_3$ for 24 hr. After evaporation, crystallization of the residue from EtOH gave the ester 1 (*ca.* 150 mg/l.), m.p. 210°, $[\alpha]_D$ -98.6° (*c*, 0.76, C_5H_5N), $[\alpha]_D$ -78.7° (*c*, 0.14, EtOH), CD $[\theta]_{227}$ -22,770; IR (KBr) 3308s, 3060, 3035, 1748s,[a] 1638s, 1602, 1580, 1530s, 1488s, 1455, 1388, 1350, 1308, 1295, 1273, 1211, 1178, 1155, 1098, 1076, 1028, 1003, 747, 713, 696s cm^{-1}; IR ($CHCl_3$) 3435 cm^{-1} (*e* 530), 1740 (400), 1660 (900); ^1H NMR ($CDCl_3$) 2.95 δ (2H, m, H-3), 3.25 (2H, d, J = 6.5 Hz, H-3'), $-OCH_2CH-$ as an ABX system δ_A 4.1, δ_B and δ_X 4.6, J_{AB} = 12, J_{AX} = 5.0 Hz, 4.95 (1H, m, H-2') 6.8 (2H, m, exchangeable in D_2O–CF_3CO_2D, NH), 7.3 (16H, m, 2Ph), 2PhCONR as 6H m at 7.3 and 4H m at 7.8 ppm; ^{13}C NMR ($CDCl_3$)[c] C-3 and C-3' at 37.3 and 37.5 ppm,[a] C-2' at 50.3, C-2 at 54.6, C-1 at 65.4, 6 aryl carbons *m* or *p* to CH_2R at 126.8 (1C), 127.1 (4C) and 127.3 (1C), 12 aryl carbons at 128.4 (2C), 128.6 (4C), 128.8 (2C), 129.2 (2C) and 129.3 (2C), 2 aryl carbons *p* to CONR at 131.4 and 132.0, 4 quaternary aryl carbons at 133.4, 134.3, 135.9 and 137.2, CONR at 167.2 and 167.5, CO_2R at 171.9 ppm; UV λ_{max} (MeOH) 215 nm (*e* 22,700), 227 (20,020); MS *m/e* 506.2203 (0.05%, M$^+$, $C_{32}H_{30}N_2H_4$ requires: 506.2205), 415 (0.1, M-91), 294 (0.2), 269 (1.5), 251 [3, "ion a", $PhCH_2\dot{C}(NHBz)$=C=O], 224 (3, $PhCH_2\dot{C}HNHBz$), 223 (3, a-28), 148 (33), 146 (91, a-105), 118 (34, a-28-105), 105 (80), 91 (100), 77 (72). (Found: C, 76.11; H, 6.20; N, 5.33. $C_{32}H_{30}N_2O_4$ Requires: C, 75.86; H, 5.97; N, 5.53%).

Hydrolysis of the ester 1 *with acid.* The ester 1 (150 mg) was heated in a sealed glass tube with 5 N HCl (3 ml) at 130° for 36 hr. The residue obtained upon evaporation was shown to contain equal quantities of phenylalanine and phenylalaninol by GLC of a sample which had been subjected to methylation and trifluoroacetylation. From the ether soluble part of the residue, an acidic fraction was obtained consisting of benzoic acid (46 mg, 63%), m.p. 122° from aq MeOH. The ether insoluble material was esterified with sat methanolic HCl (15 ml) and acetylated (Ac_2O–pyridine). Preparative TLC gave (+) - N - acetyl - L - phenylalanine methyl ester (41 mg, 61%), m.p. 90°, lit.[16] m.p. 91°), IR (KBr) 1748, 1645, 1527 cm^{-1}; NMR ($CDCl_3$) 2.10 δ (NAc), 3.20 (2H, d, J = 6 Hz, H-3), 3.80 (OMe), 4.95 (1H, dt, J = 7, 8 Hz, H-2), 6.07 (NH), 7.12 (5H, m, Ph). (Found: C, 65.40; H, 6.86; N, 6.39. Calc. for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33%). Preparative TLC also gave 3 (47 mg, 68%), m.p. 128°, $[\alpha]_D$ -15.5° (*c*, 2.25, $CHCl_3$); IR (KBr) 3900, 1725, 1640, 1602, 1548, 1265, 1255, 750, 698 cm^{-1}; NMR ($CDCl_3$) 1.93 (OAc), 2.10 (NAc), 2.87 (2H, d, J = 7 Hz, H-3), 4.03 (2H, d, J = 5 Hz, H-1), 4.40 (1H, m, H-2), 5.60 (NH), 7.22 (5H, m, Ph). (Found: C, 66.29; H, 7.10; N, 5.73. $C_{13}H_{17}NO$ requires: C, 66.36, H, 7.28; 5.95%). This was identical in all respects with a sample prepared from 2.

Hydrolysis of the ester 1 *with base.* The ester 1 (75 mg) was refluxed with 0.07 M NaOH (21.5 ml) and EtOH (30 ml) for 24 hr. The neutral fraction of the product gave 5 (26.4 mg, 70%), m.p. 171–173° from $CHCl_3$–pet. ether, $[\alpha]_D$ -74.5° (*c*, 0.87, C_5H_5N), while the acidic fraction gave 4 (20 mg, 50%), m.p. 141–142° from aq HOAc, $[\alpha]_D$ -42.2° (*c*, 0.07, MeOH).[11] These products were each identical in all respects with authentic samples.

Hydrazinolysis of the ester 1. The ester 1 was readily cleaved by refluxing with 100% $N_2H_4 \cdot H_2O$ in abs EtOH for 3 hr to give a mixture of 5 and 14, m.p. 197°, identical with an authentic sample.[12]

Preparation of N - *benzoyl* - L - *phenylalaninol* (5). L-Phenylalaninol (4.76 g) was treated with approx equimolar quantity of benzoyl chloride in pyridine for 24 hr at room temp. The neutral fraction of the product was a mixture (3:2) of 5 and 6. After refluxing for 1 hr with *ca.* 0.1 M aq alc. NaOH, almost pure 5 was obtained (4.02 g, 50%), m.p. 171–173° from $CHCl_3$–pet. ether, $[\alpha]_D$ -46.4° (*c*, 0.49, $CHCl_3$), $[\alpha]_D$ -78.5° (*c*, 1.11, C_5H_5N); CD $[\theta]_{226}$ -19,300; IR (KBr) 3210, 1635, 1600, 1580, 1540, 1080, 1050, 1030, 750, 700 cm^{-1}; ^1H NMR (C_5D_5N) 3.3 δ (2H, d, J = 7 Hz, H-3), 4.05 (2H, d, J = 6 Hz, H-1), 4.9 (1H, m, H-2), 6.1 (NH); ^1H NMR ($CDCl_3$) 3.03 δ (2H, d, J = 7 Hz, H-3), 3.79 (2H, d, J = 5 Hz, H-1), 4.4 (1H, m, H-2), 7.27 (5H, m, Ph), PhCONR as 3H m at 7.3 and 2H m at 7.7 ppm; ^{13}C NMR ($CDCl_3$)[c] C-3 at 37.1 ppm, C-2 at 53.4, C-1 at 64.5, 3 aryl carbons *m* or *p* to CH_2R, at 126.9, 6 aryl carbons 128.6 (2C), 128.8 (2C) and 129.3 (2C), 1 aryl carbon *p* to CONR at 131.7. (Found: C, 75.10; H, 6.69; N, 5.53. $C_{16}H_{17}NO_2$ requires: C, 75.27; H, 6.71; N, 5.49%). The yield from 1 in the above procedure was greatly improved by use of an excess of benzoyl chloride in the first stage. The intermediate 6 crystallized from benzene–pet. ether, m.p. 170° (lit.[13] m.p. 169°), IR (KBr) 3310, 1720, 1710, 1635, 1538 cm^{-1}; NMR ($CDCl_3$) 3.10 δ (2H, m, H-3), 4.13 (1H, dd, J = 7, 8 Hz, H-2), 4.45 (2H, d, J = 5 Hz, H-1), 6.63 (1H, d, J = 8 Hz, NH), 7.30 (5H, s, Ph), PhCONR as 3H m at 7.40 and 2H m at 7.70 ppm, $PhCO_2R$ as 3H m at 7.40 and 2H m at 8.02 ppm. (Found: C, 77.10; H, 6.13; N, 3.79. $C_{23}H_{21}NO_3$ requires: C, 76.86; H, 5.89; N, 3.90%).

Esterification of the alcohol 5 *with the acid* 4. The acid 4 (140 mg) and N,N'-carbonyldiimidazole (154 mg) in EtOH-free $CHCl_3$ (250 ml) were stirred at room temp. for 2 hr. The alcohol 5 (243 mg) was then added in six portions at 0.5 hr intervals. Additional $CHCl_3$ (100 ml) was added and the soln stirred overnight. Preparative TLC (1% MeOH–$CHCl_3$) of the neutral fraction of the product gave (i) 8, R_f 0.74 (40 mg), m.p. 70–72° from pet. ether (b.p. 100–120°) lit.[3] m.p. 69–71°); IR (KBr) 1825s, 1810s, 1640s, 1600m, 1580m cm^{-1}; NMR ($CDCl_3$) PhC=NR as 3H m at

[b](*Added in proof*): Asperpheanamate was identical to 1 synthesized via N-CBZ-L-phenylalanine anhydride [A. M. Clark and C. D. Hufford, *Phytochemistry* 17, 552 (1978)].

[c]For ^{13}C NMR (C_5D_5N) see Table 1.

7.4 and 2H m at 7.85 ppm; (ii) a mixture of esters, R_f 0.54 (154 mg, 63% w.r.t. 5 consumed), m.p. 192–197°, IR (KBr) 1748s, 1735s cm^{-1}; Repeated crystallization from EtOH gave the ester 7, m.p. 217°, CD $[\theta]_{365}$ +1056, $[\theta]_{317}$ −3730, $[\theta]_{284}$ −3200, $[\theta]_{235}$ −3200, $[\theta]_{345}$ −2145; IR (KBr) 3304s, 3060, 3035, 1735s, 1652s, 1638s, 1603, 1580, 1538s, 1488s, 1455, 1388, 1341, 1309, 1295, 1286, 1228, 1182, 1155, 1098, 1078, 1028, 1002, 749, 713, 698s cm^{-1}; IR ($CHCl_3$) 3430s, 1735s, 1660s, 1600, 1580 cm^{-1}; ^1H NMR ($CDCl_3$) similar to spectrum of 1, but OCH_2CH as 1H m at 4.15 and 2H m at 4.5 ppm; MS *m/e* 506.2202 (M^+, $C_{32}H_{30}N_2O_4$ requires: 506.2205) and fragmentation pattern similar to that of 1. (Found: C, 75.60; H, 5.91; N, 5.36%). Fractional crystallization of the remaining ester mixture afforded a 10 mg sample of the more soluble ester, 1, m.p. 207° identical to natural material (m.m.p. and IR) (iii) Unreacted alcohol 5, R_f 0.16 (121 mg), m.p. 171–173°.

Treatment of 5 (12.6 mg) with N - benzoyl - DL - phenylalanine (20.4 mg) in the presence of N,N'-carbonyldiimidazole (4.5 mg) in EtOH-free $CHCl_3$ (100 ml) for 7 days at room temp. gave a neutral fraction containing *ca.* 40% of the ester mixture 1 + 7.

N - *Benzoyl* - O - (N' - *CBZ* - L - *phenylalanyl*) - L - *phenylalaninol* (9). N - CBZ - L - Phenylalanine (318 mg) and N,N'-carbonyldiimidazole (220 mg) in EtOH-free $CHCl_3$ (50 ml) were stirred for 1 hr at room temp. The alcohol 5 (348 mg) in EtOH-free $CHCl_3$ (100 ml) was added in 6 portions at 0.5 hr intervals and stirring continued for 4 days. Preparative TLC (1% MeOH-$CHCl_3$) of the neutral fraction of the product gave (i) a colourless oil, R_f 0.67 (33 mg), identified as N - CBZ - L - phenylalanine methyl ester by comparison (IR, NMR, TLC) with a sample prepared by esterification of N - CBZ - L - phenylalanine with MeOH using N,N'-carbonyldiimidazole. (ii) The desired ester 9, R_f 0.54 (186 mg 65% w.r.t. 5 consumed), m.p. 185–187° from EtOH; $[\alpha]_D$ −15.6° (*c*, 0.76, $CHCl_3$), CD $[\theta]_{230}$ −12,870; IR (KBr) 3300s, 3020, 1740s, 1690s, 1630s, 1600, 1575, 1530s cm^{-1}; UV (MeOH) λ_{max} 215 nm (*e* 15,100), 227 (10,700); NMR ($CDCl_3$) 2.9 δ (2H, m), 3.1 (2H, d, J = 7 Hz), 4.05 (1H, m), 4.3–4.7 (3H, br m, H-1 and H-2'), 5.0 (2H, s, CO_2CH_2R), 5.25 (NH), 6.5 (NH), 7.2 (15H, m, 3Ph), PhCONR as 3H m at 7.4 and 2H m at 7.7 ppm.

N - *Benzoyl* - O - (L - *phenylalanyl*) - L - *phenylalaninol hydrobromide* (10·HBr). The ester 9 (172 mg) was stirred with 45% w/v HBr in HOAc (25 ml) at room temp. for 2 hr. Evaporation under reduced pressure and crystallization from EtOH/pet. ether gave 10 as its hydrobromide (138 mg, 89%), m.p. 189°, CD $[\theta]_{314}$ +18,280, $[\theta]_{230}$ −7990, $[\theta]_{206}$ +1160; IR (KBr) 3420s, 3300s, 3020, 2920br, 1745s, 1635s, 1600, 1575, 1530s cm^{-1}; UV (MeOH) λ_{max} 215 nm (*e* 10,300), 226 (11,700); NMR ($CDCl_3$ + CD_3OD) 3.0 δ (2H, d, J = 7.5 Hz), 3.35 (2H, d, J = 7.5 Hz) 4.1–4.7 (4H, m) 6.95 (1H, exchangeable D_2O–DCl, NH), 7.2 (10H, m, 2Ph), PhCONR as 3H m at 7.45 and 2H m at 7.8 ppm,[4] MS *m/e* 403 (0.1%, M^+), 146 (25), 120 (25), 118 (13), 105 (33), 91 (100). (Found: C, 61.99; H, 5.55; N, 6.08; Br, 16.8. $C_{25}H_{27}BrN_2O_3$ requires: C, 62.1; H, 5.58; N, 5.79; Br, 16.6%).

N - *Benzoyl* - O - (N' - *benzoyl* - L - *phenylalanyl*) - L - *phenylalaninol* (1). The salt 7·HBr (34 mg) was stirred with benzoyl chloride (9.4 mg) in dry pyridine (15 ml) at room temp. for 24 hr. Crystallization of the neutral fraction of the product from EtOH gave 1 (23.3 mg, 75%), m.p. 207–208° identical in all respects with the natural material (m.m.p., TLC, IR, MS: M^+ at 506.2205, CD: $[\theta]_{228}$ −22,440).

Debenzoylation of N - (2 - *phenethyl*) - *benzamide*. N - (2 - phenethyl) - benzamide[14] (200 mg) and an excess of $Et_3O^+BF_4^-$ in dry CH_2Cl_2 were stirred under N_2 for 24 hr. After evaporation, the residue was stirred with 3% HOAc in dioxan–H_2O (1:1) for a further 24 hr. Evaporation gave an oil, the neutral fraction of which consisted of unreacted amide (42 mg) and ethyl benzoate (60 mg, 57%). The basic fraction was identified (IR, TLC) as 2-phenylethylamine (79 mg, 92%).

Debenzoylation of N - *benzoyl* - DL - *phenylalanine ethyl ester*. Under conditions analogous to those used in the foregoing reaction, N - benzoyl - DL - phenylalanine ethyl ester (100 mg) gave ethyl benzoate and DL-phenylalanine ethyl ester (42 mg, 84%). The latter was identified by TLC and IR and characterised as its hydrochloride, m.p. 127°.[15]

Debenzoylation of the ester 1. The ester 1 (100 mg) and an excess of $Et_3O^+F_4^-$ in dry CH_2Cl_2 were stirred under N_2 for 24 hr. After evaporation, the residue was stirred with 3% HOAc in dioxan–H_2O (1:1) for a further 24 hr. Evaporation and treatment of the residue with ether gave the difluoroborate salt, m.p. 215–217° of 11. IR (KBr) 3300–2800 br, 1740s, 1590, 1480s cm^{-1}; NMR (CF_3CO_2H) 3.1 (2H, m, H-3), 3.5 (2H, m, H-3') 4.2 (1H, m, H-2), 4.5 (1H, m, H-2'), 4.75 (2H, m, H-1), 7.2–7.5 (10H, m, 2Ph); MS *m/e* 298.1682 (17%, M^+, $C_{18}H_{22}N_2O_2$ requires: 298.1681), 282, 207 (23, M-91), 120 (50, $PhCH_2CH^+NH_2$). (Found: C, 39.38; H, 4.53; N, 4.90. $C_{18}H_{22}N_2O_2 \cdot 2HBF_4 \cdot 4H_2O$ requires: C, 39.59; H, 5.90; N, 5.13%). The ether mother liquor afforded unreacted ester 1 (6 mg) and ethyl benzoate (35 mg, 66%).

Conversion of the ester 1 *into the amidoalcohol* 12. A mixture of 1 (2.05 g) and $Et_3O^+BF_4^-$ (2.73 g) in dry CH_2Cl_2 (500 ml) was stirred under N_2 at room temp. for 24 hr. The oil obtained upon evaporation was taken up in dioxan (100 ml) and stirred with 3% HOAc in H_2O (100 ml) at room temp. for 24 hr. Upon concentration of the mixture, colourless solid ppted and this was filtered off and washed with ether. Preparative TLC gave unreacted 1 (265 mg) and 12 (548 mg, 39% based on ester 1 consumed), m.p. 189–191°, $[\alpha]_D$ −47.7° (*c*, 0.67, C_5H_5N), separating from common crystallization solvents as a gel; IR (KBr) 3600–3200s, 1660s, 1640s, 1600, 1580, 1530s, 750, 700s cm^{-1}; NMR ($CDCl_3$ + CD_3OD) 2.8 δ (2H, m, H-3), 3.15 (2H, m, H-3'), 3.5 (2H, d, J = 5 Hz, H-1), 4.1 (1H, m, H-2), 4.8 (1H, t, J = 7.5 Hz, H-2'), 7.15–7.25 (10H, m, 2Ph), PhCONR as 3H m at 7.5 and 2H m at 7.75 ppm; [4] MS *m/e* 402 (3%, M^+), 384 (8, *M*-18), 293 (13, *M*-18-91), 252 [29, $PhCH_2CH(NHBz)\dot{C}^+{=}O$], 224 (20, $PhCH_2CH^+NHBz$), 105 (100), 91 (24). (Found: C, 74.39; H, 6.77; N, 6.84. $C_{25}H_{26}N_2O_3$ requires: C, 74.60; H, 6.51; N, 6.96%).

The alcohol gave crystalline 13, m.p. 212°, IR (KBr) 3280s, 1720s, 1710s, 1660s (RCONH), 1630s (ArCONH), 1600, 1580, 1540s, 1280s, 750, 710s, 696s cm^{-1}; NMR ($CDCl_3$) 2.85 δ (2H, d, J = 7.1 Hz, H-3'), H-3 as ABX type 2H m (δ_A 3.23, δ_B 3.03, J_{AB} = 13.6 Hz, J_{AX} = 6 Hz, J_{BX} = 8.4 Hz), H-1 as ABX type 2H m (δ_A 4.17, δ_B 4.06, J_{AB} = 11.6 Hz, J_{AX} = 4.6 Hz, J_{BX} = 4.6 Hz), 4.5 (1H, m, H-2), 4.7 (1H, m, H-2'), 6.04 (1H, d, J = 8.4 Hz, NH), 6.79 (1H, d, J = 7.5 Hz, NH), 7.2 (10H, m, 2Ph), PhCONR as 3H m at 7.5 and 2H m at 7.75 ppm, $PhCO_2R$ as 3H m at 7.5 and 2H m at 8.0 ppm; MS *m/e* 506 (0.05%, M^+), 384 (90, M–$PhCO_2H$), 293 (100, M–$PhCO_2H$-91), 279 (23, M–$PhCO_2H$-105), 264 (10), 252 [11, $PhCH_2CH(NHBz)\dot{C}{=}O$], 224 (11, $PhCH_2CH^+NHBz$), 105 (56), 91 (61). (Found: C, 75.85; H, 6.03; N, 5.44. $C_{32}H_{30}N_2O_4$ requires: C, 75.87; H, 5.97; N, 5.53%).

Synthesis of the alcohol 12 *via the azide* 15. The hydrazide 14 (100 mg) in a mixture of HOAc (5 ml), 5 N HCl (30 ml) and EtOAc (20 ml) was stirred vigorously and treated at −5° with a soln of $NaNO_2$ (90 mg) in H_2O (5 ml). After 30 min, the EtOAc layer was separated, washed with cold $NaHCO_3$ aq, and then H_2O. After drying ($MgSO_4$ and $CaCl_2$), the resulting soln of 12 was treated with 2 (50 mg) at 5° for 24 hr. Evaporation and preparative TLC of the resulting oil gave 12 (102 mg, 72%) identical (TLC, IR, NMR, MS) to samples prepared from 1 or as described below. The benzoate 13 or just 8 (Found: C, 75.84; H, 5.78; N, 5.66%) was identical (m.p., m.m.p., IR, NMR) to a sample derived from 1.

Synthesis of the alcohols 12 *and* 16 *from* 4. The acid 4 (37 mg) in Analar EtOAc (5 ml) was treated at 0° with *p*-nitrophenol (25 mg) and N,N'-dicyclohexylcarbodiimide (28 mg) for 30 min before allowing the temp. to rise to room temp. The ppt of dicyclohexylurea was then removed and the soln evaporated to give the *p-nitrophenyl ester* (45 mg, 69%), m.p. 159° from EtOH, $[\alpha]_D$ 0° (*c*, 0.25, $CHCl_3$); IR (KBr) 3320, 1755s, 1638s, 1518s, 1485s, 1344s cm^{-1}; NMR ($CDCl_3$) 3.40 δ (2H, d, J = 7 Hz, H-3), 5.30 (1H, q, J = 7 Hz, H-2), 6.70 (NH), *p*-$NO_2C_6H_4$ as two 2H d, J = 9 Hz at 8.27 and 7.17 ppm respectively. (Found: C, 67.53; H, 4.64; N, 7.29. $C_{22}H_{18}N_2O_5$ requires: C, 67.69; H, 4.65; N, 7.18%).

A mixture of this ester (79 mg) and 2 (30 mg) in Analar EtOAc (5 ml) was allowed to stand at room temp. for 24 hr. After

[4] For ^{13}C NMR (C_5D_5N) see Table 1.

washing with dil NaOH aq, and with H_2O, evaporation gave an oil, preparative TLC of which gave 2 products, the amidoalcohol 12 (35 mg, 34%) as a glassy solid $[\alpha]_D$ −26.2° (*c*, 0.07, $CHCl_3$), identical (m.p., TLC, IR, NMR, MS) with a sample prepared from 1 or 12, and its isomer 16 (30 mg, 29%), m.p. 176° from benzene, $[\alpha]_D$ +34.5° (*c*, 0.15, $CHCl_3$); IR (KBr) 3500–3200br, 3280s, 1650s, 1630s, 1530s cm^{-1}; NMR ($CDCl_3$) 2.68 δ (2H, d, J = 7 Hz, H-3), 3.07 (2H, d, J = 7 Hz, H-3'), 3.60 (3H, m, H-1 and OH), 4.20 (1H, m, H-2), 4.93 (1H, q, J = 7 Hz, H-2'), 6.80 (1H, d, J = 7 Hz, NH), 7.20 (10H, m, 2Ph), PhCONR as 3H m at 7.4 and 2H m at 7.7 ppm. (Found: C, 74.47; H, 6.53; N, 6.91. $C_{25}H_{26}N_2O_3$ requires: C, 74.60; H, 6.51; N, 6.96%).

Preparation of asperglaucide 18 *from the alcohol* 12. The alcohol 12 (25.2 mg) was stirred with Ac_2O (1.5 ml) in dry pyridine (10 ml) overnight at room temp. The resulting acetate crystallized from EtOAc–pet. ether (20.6 mg, 74%), m.p. 185–187°; MS *m/e* 444.2047 (1%, M^+ requires: 444.2049), 384 (11, M–HOAc), 293 (17, M–HOAc-91), 252 [25, $PhCH_2CH(NHBz)\dot{C}{=}O$], 224 (24, $PhCH_2\dot{C}HNHBz$), 172 (13), 105 (100), 91 (25). This was identical (m.m.p., IR, NMR, MS) to an authentic sample of 18 kindly supplied by Prof. R. Thomson University of Aberdeen.

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