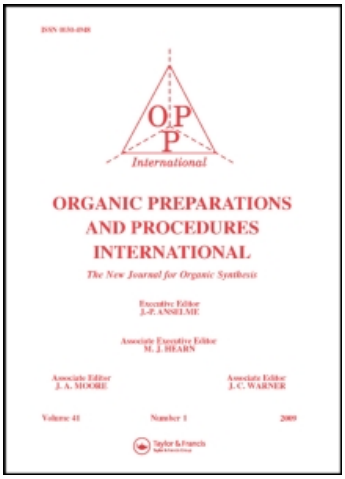

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SYNTHESIS OF PHENYLACETYL-L-CYSTEINYL-D-VALINE DISULFIDE. A BIOSYNTHETIC PRECURSOR OF PENICILLING

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SYNTHESIS OF PHENYLACETYL-L-CYSTEINYL-D-VALINE DISULFIDE. A BIOSYNTHETIC PRECURSOR OF PENICILLIN G

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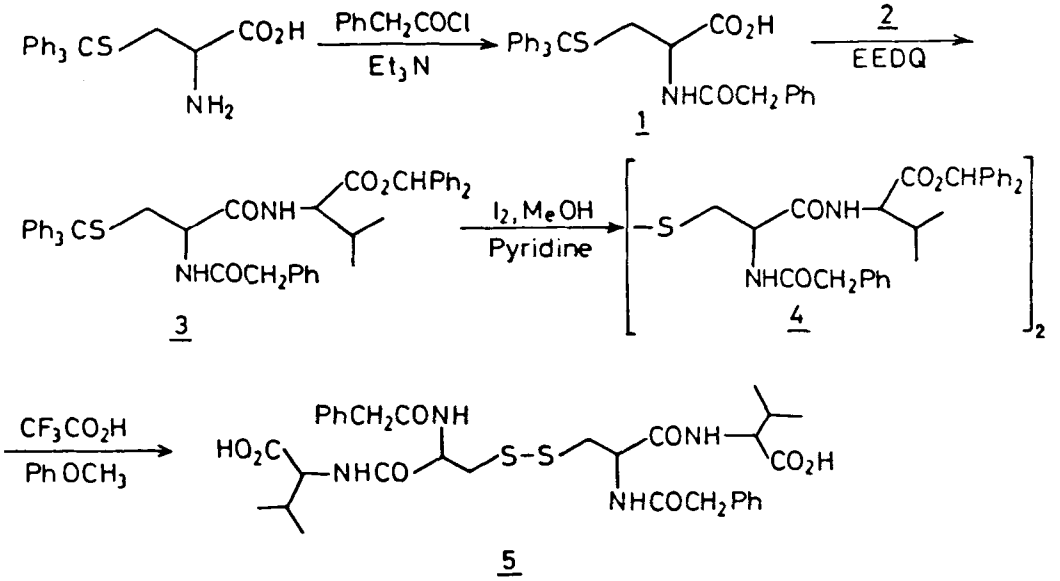
Phenylacetyl-L-cysteinyl-D-valine (PVC) is of interest because Penicillin is produced through enzymatic cyclization from it.[1] In order to expand the number of the chemical pathways which may lead to further understanding of the biosynthesis of Penicillin G, we undertook the synthesis of PVC disulfide.

Phenylacetyl-S-trityl-L-cysteine,(1) obtained by reaction of phenylacetyl chloride with S-trityl-L-cysteine, was treated with the p-toluenesulfonic acid salt of D-Valine benzhydryl ester (2) using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoleine (EEDQ) as an activator to yield the benzhydryl ester of phenylacetyl-L-cysteinyl-D-valine (3).



Removal of the trityl group using iodine in methanol and pyridine provided the benzhydryl ester of PVC disulfide (4), which was treated with trifluoroacetic acid and anisole to yield the PVC disulfide(5).

EXPERIMENTAL SECTION

Phenylacetyl chloride and S-trityl-L-cysteine was supplied by Sigma Chemical Co. Other chemicals were obtained from Merck in analytical pure grade. Melting points were determined on a Buchi apparatus and are uncorrected. The IR spectra were recorded on a Beckman Acculab 4 Spectrophotometer using KBr disk. The ^{1}H-NMR spectra were determined on a Bruker 80 Mhz instrument using Me4Si as internal standard. Microanalyses and mass spectrum were performed by Instituto de Quimica Organica. Universidad de Murcia. Optical rotation were obtained on a Perkin Elmer 2401 polarimeter. HPLC chromatographies were performed using a Constametric II LDC pump, a Rheodyne 7120 LDC injector fitted with a 20 µl loop and a Waters Model 441 UV Detector. Only spectrally pure solvents and deionized water were used in HPLC. Solvents were distilled before use and were dried as described literature procedures.

Phenylacetyl-S-trityl-L-cysteine (1).- To a suspension of S-trityl-L-cysteine (4.4g, 12 mmol) in chloroform (92 ml) containing triethylamine (2.7g, 26.4 mmol) cooled in ice, was added a solution of phenylacetyl chloride (1.8g, 12 mmol) in chloroform (20 ml). The mixture was stirred at 0-5° for 15 min. and at room temperature for 24 hrs. Water was added (100 ml) and pH was adjusted to 1.5 with 5N aqueous HCl. The aqueous phase was removed and the organic phase was washed with saturated sodium chloride (100 ml), dried over anhydrous sodium sulfate and concentrated to give 4.9 g. (85%) of a white crystalline solid mp. 60-62°; $[\alpha]_D$ = +21.8° (c 2, CH_3OH); IR (KBr):1720 cm^{-1}; ^{1}H -NMR($CDCl_3$): δ 8.47 (1H, s), 7.30 (20H, m), 4.30 (1H, m), 3.52 (2H, s), 2.64 (2H, d, 5.5)

Anal. Calcd. for $C_{30}H_{27}NO_3S$: C, 74.84; H, 5.61; N, 2.91 Found: C, 74.44; H, 5.40; N, 3.20

D-Valine Benzhydryl Ester p-Toluenesulfonic Acid Salt(2)[2].- Yield 86%, lit.[2,3] 77%, 81%; mp. 177-179°; lit.[2] 175-177°; $|\alpha|_D$ = +21° (c 2, CH_3OH), lit.[2] +20.2° (c 2, CH_3OH); IR (KBr): 1742 cm^{-1};^{1}H-NMR ($CDCl_3$): δ 7.64 (2H, d, 8.2), 7.20-7.50 (10H, m), 6.80-7.10 (4H, m), 4.0 (1H, d 3.9), 2.24 (3H, s), 0.84 (3H, d, 6.9) 0.75 (3H, d, 6.9).

Anal. Calcd for $C_{25}H_{29}NO_5S$: C, 65.93; H, 6.37; N, 3.08 Found: C, 65.60; H, 6.60; N, 3.13

Phenylacetyl-S-trityl-L-cysteinyl-D-valine Benzhydryl Ester (3).- To a solution of 1 (4.7 g, 9.8 mmol) in ethyl acetate (50 ml), were added 2 (4.6 g, 10.8 mmol), N-methylmorpholine (1.1 g, 10.8 mmol), EEDQ (2.5 g, 10.8 mmol); the mixture was stirred at room temperature for 48 hrs. Ethyl acetate (50 ml) was added and the mixture was successively extracted with 10% potassium hydrogen sulfate (3 x 80ml), saturated sodium chloride (1 x 80 ml). The organic phase was dried over anhydrous sodium sulfate and recrystallized from ethyl acetate: petroleum ether (1:1) to yield 4.6 g (64%), mp. 147-149°; $[\alpha]_D$ = -15° (c 1, CH_3OH): IR (KBr): 1740, 1635, 1530 cm^{-1};^{1}H-NMR (DMSO-d_6): δ 7.30

(3OH, m), 6.80 (1H, s), 4.90 (1H, m), 3.50 (2H, s), 2.64 (2H, d, 5.5).

Anal. Calcd for $C_{48}H_{46}N_2O_4N_4S$: C, 77.21; H, 6.16; N, 3.75

Found : C, 77.01; H, 6.30; N, 3.95

Bis-phenylacetyl-L-cysteinyl-bis-D-Valine Dibenzhydryl Ester (4).- The detritylation of fully protected PVC (3) was carried out as described by Wolfe.[2] Yield 70%, mp. 169-170°; $[\alpha]_\Delta$ = +14°(c 1, CH_3OH); IR (KBr): 1730, 1640, 1530 cm^{-1};^{1}H-NMR (DMSO-d_6): δ 7.30-7.50 (15H, m), 6.80 (1H, s), 4.90 (1H, d, 5.2), 3.50 (2H, s,), 2.90 (2H, t, 5.2), 2.70 (1H, d, 5.4), 1.80 (1H, m), 0.80 (2H, d, 6.5), 0.70 (3H, d, 6.5).

Anal. Calcd for $C_{58}H_{62}N_4O_8S_2$: C, 69.18; H, 6.16; N, 5.67

Found : C, 69.09; H, 6.20; N, 5.87

PVC Disulfide (5).- Compound (4) (2.0 g, 2 mmol) was added to a mixture of trifluoroacetic acid (20 ml) and anisole (2 ml). The mixture was stirred for 1 hr at room temperature and the trifluoroacetic acid was distilled off. The product was dissolved in water (40 ml) and diethyl ether (80 ml). The pH was adjusted to 8 with 5N aqueous NaOH and the aqueous phase removed. The other phase was washed with water (20 ml). The combined aqueous fraction was concentrated and 1N HCl added to pH 2. A precipitate was obtained and absolute ethanol (10 ml) was added. After 10 hrs in the refrigerator, the precipitate was collected as a white crystalline solid. The compound was purified by reverse phase HPLC on a C_{18} (Microbondapack) at λ =230 nm with 0.05 M ammonium phosphate/ methanol (55:45) as mobile phase to yield 1.1 g (78%) mp. 240°; $[\alpha]_D$ = -17° (c 1, CH_3OH); IR (KBr): 1720, 1640, 1530 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.20 (5H, s), 4.92 (1H, m), 3.50 (2H, s), 2.92 (2H, t, 5.2), 2.72 (1H, d, 5.4), 1.84 (1H, m), 0.80 (3H, d, 6.5), 0.70 (3H, d, 6.5).

Anal. Calcd. for $C_{32}H_{42}N_4O_8S_2$. 1.5 H_2O : C, 54.78; H, 5.99; N, 7.98

Found: C, 54.69; H, 5.76; N, 7.74

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