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THE SYNTHESIS OF CYCLO-(*R*-β-PHENYL-β-ALANYL-L-PROLYL-)$_2$, A PEPTIDE ISOLATED FROM *ROCCELLA CANARIENSIS*

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Abstract— A comparison between the lichen substance roccanin and the synthetic peptide cyclo-(*R*-β-phenyl-β-alanyl-L-prolyl-)$_2$ demonstrated their identity. The main feature of the synthesis involved stepwise addition of active esters of amino acids, activation of the tetrapeptide using *p*-nitrophenol and dicyclohexylcarbodiimide, followed by cyclization in pyridine.

THE cyclic tetrapeptide roccanin has previously been isolated from the lichen *Roccella canariensis* Darb. and assigned the structure cyclo-(*R*-β-phenyl-β-alanyl-L-prolyl-)$_2$.[1] In order to confirm this structure, an unambiguous synthesis of the peptide has now been performed.

For the synthesis of roccanin a linear tetrapeptide with protected amino group Z(OMe)-*R*-β-Phe-L-Pro-*R*-β-Phe-L-Pro† (VII) was prepared. Starting from free proline, active esters were added 3 times to give a step by step elongation as shown schematically in Fig 1. A proline residue was selected as the C-terminal amino acid because of its well-known resistance to racemization. The very labile *p*-methoxybenzyloxycarbonyl group seemed advantageous to protect the amino function in the final step. The carboxyl group of compound VII was activated by means of *p*-nitrophenol and dicyclohexylcarbodiimide to give VIII. After removal of the amino protecting group, intermediate IX was cyclized.

R-β-Phenyl-β-alanine was prepared as described earlier.[1] The optical purity of the product was demonstrated by the Manning and More procedure.[1,2]

Several synthetic schemes were tried before the approach outlined below was adapted. Most of them were carried out using β-alanine instead of *R*-β-phenyl-β-alanine. A first attempt starting with L-proline methyl ester[3] gave crystalline Z(OMe)-β-Ala-L-Pro-OMe. However, this compound, in our hands, gave rise to difficulties on saponification. Similar complications are cited by Schröder and Lübke.[4] A second attempt with β-alanine using *p*-nitrobenzyl ester for carboxyl protection of proline was carried through stepwise to *t*-butyloxycarbonyl-tetrapeptide *p*-nitro-

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† Abbreviations: β-Phe = β-phenyl-β-alanine; Pro = proline; β-Ala = β-alanine; Z = benzyloxycarbonyl; z(OMe) = *p*-methoxybenzyloxycarbonyl; ONp = *p*-nitrophenyl ester; OTcp = 2, 4, 5-trichlorophenyl ester; TFA = trifluoroacetic acid.

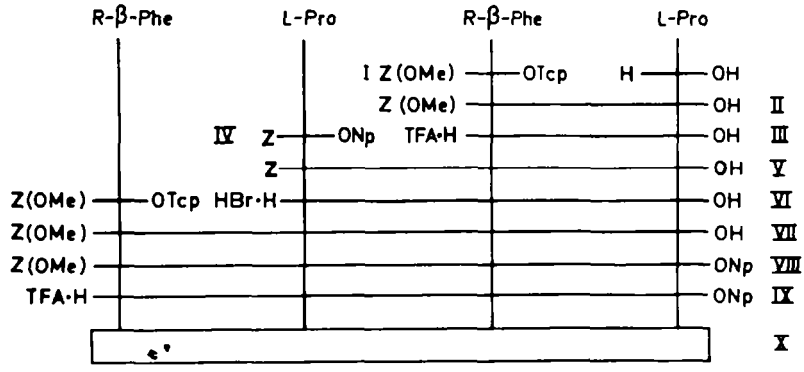

FIG 1.

benzyl ester without giving a single crystalline intermediate. A third attempt was made without any carboxyl protecting group for proline. Stepwise addition of amino acids as active esters then gave crystalline Z(OMe)-β-Ala-L-Pro but oily Z-L-Pro-β-Ala-L-Pro and Z(OMe)-β-Ala-L-Pro-β-Ala-L-Pro. Repeated attempts to convert the latter compound to the corresponding *p*-nitrophenyl ester using di-(*p*-nitrophenyl) sulphite[5] in pyridine invariably resulted in dark solutions. The use of *p*-nitrophenol and dicyclohexylcarbodiimide, however, seemed to work better. After removal of the *p*-methoxybenzyloxycarbonyl group with trifluoroacetic acid in the presence of anisole and cyclization in pyridine, an oily product was obtained. Mass spectroscopy analysis indicated the structure expected.

The final approach to the synthesis of roccanin started with treatment of *R*-β-phenyl-β-alanine with *p*-methoxybenzyloxycarbonyl azide[6] by the pH-stat technique of Schnabel[7] to give Z(OMe)-*R*-β-Phe. This compound was converted to Z(OMe)-*R*-β-Phe-OTcp (I) using dicyclohexylcarbodiimide and 2,4,5-trichlorophenol as described for a similar compound by Bodanszky *et al.*[8] The dipeptide II (Fig 1) was prepared by the reaction of I with L-proline in the presence of triethylamine. Removal of the *p*-methoxybenzyloxycarbonyl group from II by treatment with trifluoroacetic in glacial acetic acid[10] to give the tripeptide hydrobromide (VI). Z(OMe)-*R*-β-Phe-ester[9] (IV) with III in the presence of triethylamine gave the benzyloxycarbonyltripeptide (V) as an oil.

The benzyloxycarbonyl group was removed by treatment with hydrogen bromide in glacial acetic acid[10] to give the tripeptide hydrobromide (VI). Z(OMe)-*R*-β-Phe-OTcp (I) was then coupled with the triethylammonium salt of VI to give the amino protected tetrapeptide VII. This derivative had also to be used as an oil. It was converted to the *p*-nitrophenyl ester (VIII) by the action of *p*-nitrophenol and dicyclohexylcarbodiimide. The removal of the *p*-methoxybenzyloxycarbonyl group from VIII was accomplished as before yielding the tetrapeptide *p*-nitrophenyl ester trifluoroacetate (IX).

The cyclization of IX was carried out at high dilution in pyridine. The cyclic peptide (X) could be isolated as a colourless crystalline compound, sparingly soluble in most organic solvents.

Different batches of X recrystallized from glacial acetic acid all analyzed for a monohydrate. The water of crystallization could be removed by sublimation.

The synthetic cyclo-(*R*-β-Phe-L-Pro-)$_2$ was identical with the lichen constituent roccanin, as shown by identical melting points, infrared spectra, mass spectra and optical rotations.

EXPERIMENTAL

All m.ps are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. All steps of the syntheses were checked by mass spectrometry with an LKB 9000 mass spectrometer and TLC on silica gel. The amino acid compositions of the di-, tri-, and tetrapeptides, after hydrolysis for 24 hr at 110° in sealed evacuated tubes, were verified with a Beckman Spinco automatic amino acid analyzer.[1]

Z(OMe)-R-β-*Phe*. A mixture of *R*-β-phenyl-β-alanine[1] (50 mmol) and *p*-methoxybenzyloxycarbonyl azide[6] (55 mmol) in dioxane (25 ml) and water (25 ml) was titrated with 4N NaOH using a pH-stat (pH ≈ 9·9) for 15 hr at room temp. The soln was washed with ether, acidified with solid citric acid to pH 3 (z(OMe)-β-Phe started to precipitate at pH 6) and then extracted with EtOAc. The extract was washed with water, dried ($MgSO_4$) and evaporated to dryness. The residue was recrystallized from EtOAc–light petroleum; yield 81%, m.p. 123–124°, $[\alpha]_D^{25} = +20{\cdot}5 \pm 0{\cdot}5$ (c = 1, DMSO). (Found: C, 65·49; H, 5·83; N, 4·10. Calc. for $C_{18}H_{19}NO_5$: C, 65·64; H, 5·82; N, 4·25%).

Z(OMe)-β-*Ala* was prepared by an analogous procedure; yield 87%, m.p. 90–91°. (Found: C, 56·88; H, 5·99; N, 5·51. Calc. for $C_{12}H_{15}NO_5$: C, 56·91; H, 5·97; N, 5·53%).

Z(OMe)-R-β-*Phe-OTcp* (I). Z(OMe)-*R*-β-Phe (25 mmol) and 2,4,5-trichlorophenol (30 mmol) were dissolved in EtOAc (70 ml) and dicyclohexylcarbodiimide (5 g) was added at 0°. After stirring for 4 hr at room temp, AcOH (0·25 ml) was added to destroy unreacted dicyclohexylcarbodiimide, and the N,N′-dicyclohexylurea, which precipitated was removed by filtration. The solvent was evaporated *in vacuo*. The solid product was recrystallized from EtOH; yield 84%, m.p. 115–116°, $[\alpha]_D^{25} = +18{\cdot}2° \pm 0{\cdot}5°$ (c = 1, DMSO). (Found: C, 56·62; H, 3·97; Cl, 20·77; N, 2·75. Calc. for $C_{24}H_{20}Cl_3NO_5$: C, 56·66; H, 3·96; Cl, 20·90; N, 2·75%).

Z(OMe)-β-*Ala-OTcp* was prepared by an analogous procedure; yield 71%, m.p. 85–86°. (Found: C, 50·04; H, 3·73; Cl, 24·61; N, 3·22. Calc. for $C_{18}H_{16}Cl_3NO_5$: C, 49·97; H, 3·73; Cl, 24·58; N, 3·24%).

Z(OMe)-R-β-*Phe*-L-*Pro*-R-β-*Phe*-L-*Pro-ONp* (VIII). To a suspension of L-proline (18 mmol) in water (3 ml) and dimethylformamide (15 ml) I (15 mmol) was added at 0° together with enough triethylamine and then stirred for 15 hr at 37°. The residue after evaporation was suspended in sat $NaHCO_3$ and washed with EtOAc, acidified with solid citric acid to pH 3, and extracted with EtOAc. The extract was washed with water, dried ($MgSO_4$) and evaporated *in vacuo*. The product II was obtained as an oil; yield ≈ 100%.

II (10 mmol) mixed with anisole (2·5 ml) was treated with trifluoroacetic acid (12 ml) at −5°. After 30 min the soln was evaporated *in vacuo* at 0° and the residue was treated with ether at 0° to give a semi-solid dipeptide trifluoroacetate (III). V was obtained by reacting III with Z-L-Pro-ONp[9] (IV) as described in the case of II; yield ≈ 100%.

Compound V (10 mmol) dissolved in glacial AcOH (20 ml) was allowed to react with a soln (20 ml) of HBr in the same solvent (45%, w/v) for 1 hr. When the residue after evaporation *in vacuo* was treated with ether, VI was obtained as a hygroscopic semi-solid product. VII was obtained by reacting I with VI following the procedure described in the case of II; yield of oily product ≈ 70%.

Compound VII was converted to the corresponding *p*-nitrophenyl ester (VIII) as described in the case of I, yield of oily product ≈ 90%.

Cyclo-(R-β-*Phe*-L-*Pro*-)$_2$ (X). Trifluoroacetic acid (7·5 ml) was added at −5° to VIII (6 mmol) and anisole (1·5 ml). The soln became a dark red colour, which disappeared after a few min. After 30 min the soln was evaporated *in vacuo* at 0° and the residue was treated with ether at 0°. The tetrapeptide *p*-nitrophenyl ester trifluoroacetate (IX) was dissolved in dimethylformamide (12 ml) containing glacial AcOH (0·4 ml). This soln was added drop-wise to 3000 ml pyridine and kept at 55–60° for 5 hr. After standing for 20 hr at room temp the pyridine was evaporated to give an oily residue. To purify this it was dissolved in EtOAc and washed with $NaHCO_3$ aq. The cyclic tetrapeptide (X) precipitated from the EtOAc as yellow crystals. The ppt was washed with HCl, water and ether and recrystallized from glacial AcOH; yield 20%, m.p. 319–320°, $[\alpha]_D^{25} = -91{\cdot}6° \pm 1°$ (c = 1, DMSO). (Found: C, 66·4; H, 6·7; N, 10·9. Calc. for $C_{28}H_{32}N_4O_4 + H_2O$: C, 66·38; H, 6·77; N, 11·06%). Roccanin: m.p. 319–320°, $[\alpha]_D^{25} = -92{\cdot}0 \pm 1°$ (c = 1, DMSO). (Found: C, 66·3; H, 6·7; N, 10·9%).

Z(OMe)-β-Ala-L-*Pro* was synthesized in analogy with II and obtained as crystals after purification over the dicyclohexylammonium salt; yield 64%, m.p. 116–116·5° (from EtOAc), $[\alpha]_D^{25} = -33{\cdot}4° \pm 0{\cdot}5°$ (c = 1, DMSO). (Found: C, 58·22; H, 6·32; N, 7·92. Calc. for $C_{17}H_{22}N_2O_6$: C, 58·28; H, 6·33; N, 8·00%).

Dicyclohexylammonium salt, yield 81%, m.p. 130° (from EtOAc), $[\alpha]_D^{25} = -11{\cdot}1° \pm 0{\cdot}5°$ (c = 1, DMSO). (Found: C, 65·04; H, 8·46; N, 7·26. Calc. for $C_{29}H_{45}N_3O_6$; C, 65·51; H, 8·47; N, 7·90%).

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