Synthesis of Quinoxaline Peptides by the Solid Phase Method

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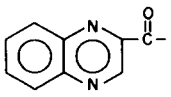
The 2-quinoxalinecarbonyl moiety (**1**) occurs in the quinomycins and triostins, two related families of quinoxaline peptide antibiotics (1,2). The quinoxaline antibiotics have been reported active against gram-positive bacteria (3), certain tumors (4), and to inhibit RNA synthesis (5).

The most practical method for the introduction of the 2-quinoxalinecarbonyl group onto the amino group of an α-amino acid or peptide has been by acylation of the amino function with 2-quinoxalinecarbonyl chloride (**2**) in alkaline media (6,7). Use of standard peptide coupling procedures for the preparation of quinoxaline peptides has given, in general, unsatisfactory results (6). This paper reports the synthesis of quinoxaline peptides by application of the Merrifield solid phase method (8).



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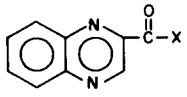

2 x = Cl
3 x = OH

The introduction of the 2-quinoxalinecarbonyl group was accomplished by coupling 2-quinoxalinecarboxylic acid (**3**), using *N,N'*-dicyclohexylcarbodiimide (9) in methylene chloride-dimethylformamide, to a previously deprotected amino acid or peptide resin ester. Use of excess reagents, as is standard for the solid phase method (8), apparently leads to satisfactory incorporation of the quinoxaline moiety onto the peptide resin despite unfavorable formation of *N*-acylurea products reported (6) to occur in coupling reactions involving the acid **3**. In the present study, the coupling reaction was carried out two consecutive times to assure maximum introduction of the quinoxalinecarbonyl group. Cleavage of the quinoxaline peptide was accomplished by treatment of the resin with hydrogen bromide in trifluoroacetic acid (8).

N-(2-Quinoxalinecarbonyl)-L-valine (**4**) was prepared by removal of the protecting group in *N-t*-butyloxycarbonyl-L-valine resin ester, followed by coupling with 2-quinoxalinecarboxylic acid (**3**), and cleavage of the product from the resin. The quinoxaline amino acid **4** was indistinguishable by thin layer chromatography, ultraviolet spectra, and mixture melting point from an authentic sample of *N*-(2-quinoxalinecarbonyl)-L-valine (7).

The quinoxaline tripeptide, *N*-(2-quinoxalinecarbonyl)-L-alanyl-*S*-benzyl-L-cysteinyl-L-valine (**5**), and the quinoxaline tetrapeptide, *N*-(2-quinoxalinecarbonyl)-L-seryl-L-alanyl-*S*-benzyl-L-cysteinyl-L-valine (**6**), likewise were prepared from *N-t*-butyloxycarbonyl-L-valine resin ester by the solid phase method. The amino acids, as the corresponding *N-t*-butyloxycarbonyl derivatives, were coupled in sequence to the resin by the carbodiimide method prior to introduction of the quinoxalinecarbonyl group. The quinoxaline tetrapeptide (**6**) can be considered as a desmethyl analog related to the quinoxaline tetrapeptide moiety present in triostin A (2).

Qxc-Val-OH **4** Qxc-Ala-Cys(Bzl)-Val-OH **5**

Qxc-Ser-Ala-Cys(Bzl)-Val-OH **6**

Qxc = 2-Quinoxalinecarbonyl (**1**)

The quinoxaline peptides **5** and **6** were homogeneous upon thin layer chromatography and were characterized by elemental analyses and corresponding spectral data. The ultraviolet spectra showed maxima typical (6,7) of the quinoxaline chromophore at 205, 244, 318, and 326 mμ. The nmr spectra of the quinoxaline peptides **5** and **6** also were consistent with the proposed structures. The H-3 proton of the quinoxaline heterocycle appeared as a singlet at τ 0.53, while the other quinoxaline ring protons appeared as a multiplet centered at approximately τ 2.0. The remainder of the peaks in each spectrum were readily assignable to the various proton groupings of the peptide component (see Experimental).

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Ultraviolet spectra were recorded on a Bausch and Lomb 505 spectrophotometer. The nmr spectra were recorded at 60 Hz on a Varian A-60 spectrometer. Solvents were removed *in vacuo* on a Buchler rotary evaporator at bath temperatures below 40°. Thin layer chromatographic data were obtained upon Brinkmann Silica Gel F_{254}

pre-coated plates in the following ascending solvent systems: Rf_A, chloroform-methanol-acetic acid (85:10:5), Rf_B, acetone-acetic acid (98:2). The solid phase reactions were carried out in a 80 x 30 mm reaction vessel shaken at 26 rpm. The *N-t*-butyloxycarbonylamino acids and resin ester (Schwarz Bio Research) used were commercially available. The dimethylformamide used in the coupling reactions was purified prior to use (8c). Dioxane was passed through a column of neutral alumina prior to use. Other solvents employed were of analytical grade.

General Procedure for Preparation of Quinoxaline Peptides.

The *N-t*-butyloxycarbonyl derivatives of *S*-benzyl-L-cysteine, L-alanine, and *O*-benzyl-L-serine were used in the appropriate sequence in the synthesis of the peptides described herein. *N-t*-Butyloxycarbonyl-L-valine resin ester (3.0 g., 1.2 mmoles) was placed in the reaction vessel and the following cycle of deprotection, neutralization, and coupling was carried out for the introduction of each residue: (1) wash with 3 x 25 ml. of dioxane; (2) cleavage of *N-t*-butyloxycarbonyl group by treatment with 30 ml. of 4 *N* hydrogen chloride in dioxane; (3) wash with 3 x 25 ml. of dioxane; (4) wash with 3 x 25 ml. of chloroform; (5) neutralization with 3 ml. of triethylamine in 27 ml. of chloroform for 10 minutes; (6) wash with 3 x 25 ml. of chloroform; (7) wash with 3 x 25 ml. of methylene chloride; (8) addition of 3.0 mmoles of *N-t*-butyloxycarbonylamino acid in 20 ml. of methylene chloride followed by shaking for 5 minutes; the quinoxalinecarboxylic acid **3** (3.0 mmoles), prepared according to Schultz, *et al.* (7), was added in 20 ml. of 2:1 methylene chloride-dimethylformamide; (9) addition of 2.48 ml. (3.0 mmoles) of a 33% solution of *N,N'*-dicyclohexylcarbodiimide in methylene chloride followed by a 2.5 hours reaction period; (10) wash with 3 x 25 ml. of methylene chloride.

After the first coupling reaction with 2-quinoxalinecarboxylic acid, the resin was treated as follows: (1) wash with 3 x 25 ml. of methylene chloride; (2) wash with 2 x 25 ml. of glacial acetic acid; (3) wash with 2 x 25 ml. of absolute ethanol; (4) wash with 3 x 25 ml. of methylene chloride; (5) a second treatment with 3.0 mmoles of 2-quinoxalinecarboxylic acid in 20 ml. of 2:1 methylene chloride-dimethylformamide with 5 minutes of mixing; (6) addition of 3.0 mmoles of *N,N'*-dicyclohexylcarbodiimide followed by a 2.5 hours reaction period; (7) repeat of above wash procedures given in steps (1) through (4); (8) resin filtered, washed with methylene chloride, and dried in vacuum desiccator over potassium hydroxide.

Cleavage of Quinoxaline Peptide from Resin.

The resin was suspended in anhydrous trifluoroacetic acid (10 ml./g. resin) contained in cleavage vessel. In the cleavage of the peptide **6**, a 15-fold excess of DL-methionine was added (11). Anhydrous hydrogen bromide was passed through the suspension for 90 minutes. The trifluoroacetic acid solution was removed by filtration and the resin washed with 3 portions trifluoroacetic acid (10 ml./g. resin). The filtrates were combined and the solvent removed *in vacuo*. The residue was taken up two times in 20-30 ml. 2:1 methanol-water and the solvent evaporated *in vacuo*. The solid obtained was triturated with ether in the cases of **4** and **5**, while with **6** the solid was suspended in water, filtered, and triturated with ether. The solid was dried in a vacuum desiccator over potassium hydroxide.

N-(2-Quinoxalinecarbonyl)-L-valine (**4**).

The solid material obtained from 2.0 g. (0.8 mmole) of resin was recrystallized from 95% ethanol-water to give 130 mg. (59%) of product, m.p. 191-193°, mixed m.p. 191-193°, lit. (7) 196.5-197.5°: Rf_A 0.70; λ max (95% ethanol), 205, 244, 318, 326 mμ, lit. (7) λ max (95% ethanol), 207, 244, 317, 327 mμ; nmr (DMSO-d_6) τ 0.56 (s, quinoxaline H-3), 1.50 and 1.70 (two s, N–H and O–H), 2.10 (m, quinoxaline H-5 to H-8), 5.65 (m, α-hydrogen), 7.82 (m, methine hydrogen), 9.15 (d, non-equivalent isopropyl hydrogens).

N-(2-Quinoxalinecarbonyl)-L-alanyl-*S*-benzyl-L-cysteinyl-L-valine (**5**).

From 3.0 g. (1.2 mmoles) of resin was obtained 0.43 g. (67%) of **5**: one recrystallization from ethanol gave material melting at 221-223°: tlc Rf_A 0.55, Rf_B 0.45; λ max (95% ethanol), 206 (ε 48,000), 243 (ε 46,200), 317 (ε 8,300), 327 mμ (ε 8,300); $[\alpha]_D^{29}$ + 61° (c 1.0, DMF); nmr (trifluoroacetic acid) τ 0.53 (s, 1H, quinoxaline H-3), 1.30 (m, 1H, O–H), 1.93 (m, 4H, quinoxaline H-5 to H-8), 2.50 (m, 1H, N–H), 3.15 (m, 7H, N–H and *S*-benzyl aromatic), 5.78 (m, 3H, α-hydrogens), 6.65 (s, 2H, *S*-benzyl), 7.45 (m, 2H, cysteinyl methylene), 8.10 (m, 1H, valyl methine), 8.73 (d, 3H, alanyl methyl), 9.42 (d, 6H, valyl isoproyl hydrogens). An analytical sample was prepared by recrystallization from ethanol, m.p. 220-222°.

Anal. Calcd. for $C_{27}H_{31}N_5O_5S$ (537.6): C, 60.4; H, 5.81; N, 13.1. Found: C, 60.3; H, 5.91; N, 12.8.

N-(2-Quinoxalinecarbonyl)-L-seryl-L-alanyl-*S*-benzyl-L-cysteinyl-L-valine (**6**).

Three g. (1.2 mmoles) of *N-t*-butyloxycarbonylvaline resin ester, treated as described above, yielded 0.44 g. (58%) of the quinoxaline tetrapeptide **6**, m.p. 204-207°: tlc Rf_A 0.37, Rf_B 0.16; λ max (95% ethanol), 206 (ε 43,800), 243 (ε 43,100), 317 (ε 7,230), 326 mμ (ε 7,400); $[\alpha]_D^{27}$ + 11° (c 2, DMF); nmr (DMSO-d_6) τ 0.52 (s, 1H, quinoxaline H-3), 1.2-2.2 (brd m, 9H, N–H, O–H and quinoxaline H-5 to H-8), 2.80 (s, 5H, *S*-benzyl aromatic), 5.60 (brd m, 4H, α-hydrogens), 6.20 (m superimposed upon s at 6.30, ≈ 2H, seryl methylene), 6.30 (s, ≈ 2H, *S*-benzyl), 7.33 (m, 2H, cysteinyl methylene), 8.15 (m, 1H, valyl methine), 8.80 (d, 3H, alanyl methyl), 9.25 (d, 6H, valyl isopropy hydrogens). An analytical sample was prepared by recrystallization from ethanol-ethyl acetate, m.p. 205.5-207°.

Anal. Calcd. for $C_{30}H_{34}N_6O_7S$ (622.7): C, 57.8; H, 5.51; N, 13.5. Found: C, 58.0; H, 5.43; N, 13.3.

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