The Formation of 2-Benzyloxyoxazol-5(4*H*)-ones from Benzyloxycarbonylamino-acids [1]

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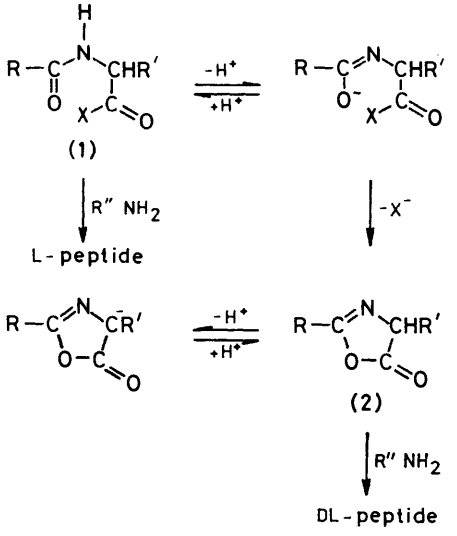
The cyclodehydration of benzyloxycarbonylamino-acids by successive treatment with acid chloride-forming reagents and triethylamine has been shown to give 2-benzyloxyoxazol-5(4*H*)-ones, not *N*-benzyloxycarbonylaziridinones as reported previously. The 2-benzyloxyoxazol-5(4*H*)-ones, which are the first 2-alkoxyoxazol-5(4*H*)-ones to be described, are more easily attacked by nucleophiles at position 5 and less easily ionised at position 4 than their well known 2-aryl and 2-alkyl analogues.

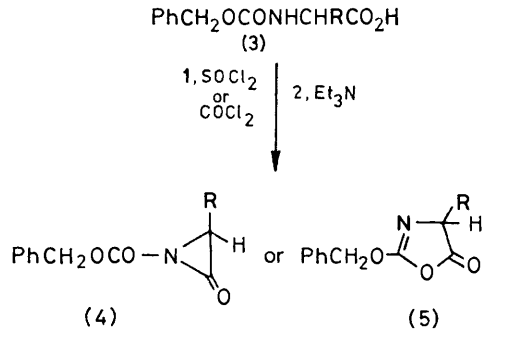
DERIVATIVES (1) of acetylamino-acids, benzoylamino-acids, and acylpeptides which are activated for peptide bond formation undergo ready cyclisation to give oxazol-5(4*H*)-ones (2), this process competing with aminolysis by an external nucleophile. The oxazol-5(4*H*)-ones (2) thus formed are themselves acylating agents and so lead ultimately to formation of peptides but their racemisation through stabilised anions is rapid and any peptide produced in this way is not stereochemically homogeneous (Scheme 1). Alkoxycarbonyl-



SCHEME 1

amino-acids, however, can be activated and coupled without any risk of racemisation in all except a few special cases, where side-chain intervention occurs.[2]

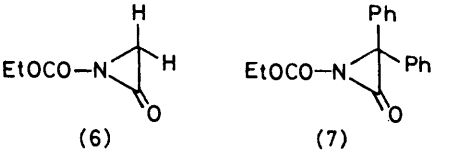
Miyoshi [3,4] has described the cyclodehydration of benzyloxycarbonylamino-acids (3) to give activated heterocycles (Scheme 2) which react rapidly with amino-esters to yield, after appropriate isolation procedures including recrystallisation, optically pure peptides. An alkoxycarbonylaziridinone structure (4; R = $PhCH_2$) was assigned to the crystalline intermediate derived from benzyloxycarbonyl-L-phenylalanine (3; R = $PhCH_2$) following a detailed study of its i.r., ^1H n.m.r., and mass spectra. However, all the spectroscopic evidence for (4; R = $PhCH_2$) is also consistent with the 2-benzyloxyoxazol-5(4*H*)-one structure (5; R = $PhCH_2$), and the ambiguity is not easily resolved as no close analogy for either has been substantiated. The only *N*-alkoxycarbonylaziridinone reported previously is (6), which was said [5] to have been isolated after ruthenium tetraoxide oxidation of *N*-ethoxycarbonylaziridine, but it appears [6]



SCHEME 2

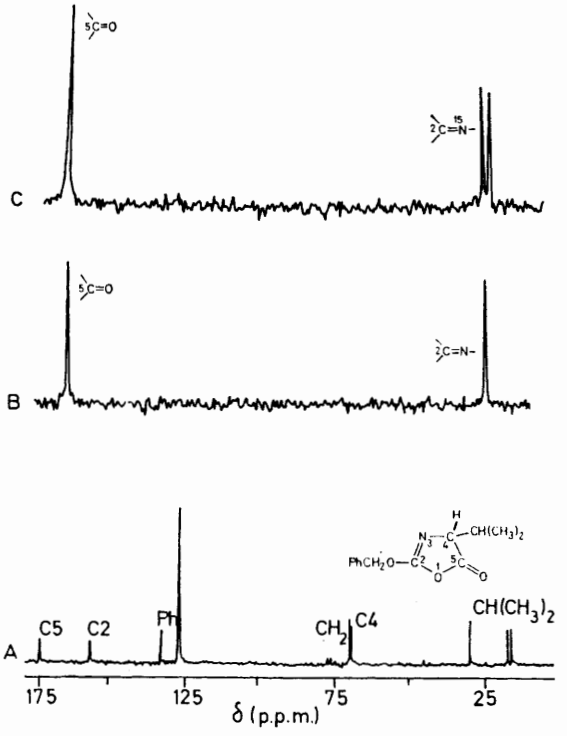
that the compound actually isolated was ethyl carbamate. So far as analogies for (5) are concerned, no 2-alkoxyoxazol-5(4*H*)-ones have been described.

Miyoshi favoured (4) largely on the grounds that aminolysis gave optically pure peptides, this being held to rule out (5) because the formation of oxazol-5(4*H*)-ones in peptide synthesis commonly leads to racemisation. This is a *non sequitur*, however: whether racemisation occurs must depend on the relative rates of racemisation and ring opening [7] and there is no reason to suppose that (5) would necessarily be the same as previously encountered oxazol-5(4*H*)-ones in this respect.



We now present unambiguous evidence that the products of the cyclodehydration shown in Scheme 2 are in fact 2-benzyloxyoxazol-5(4*H*)-ones. In the ^{13}C n.m.r. spectra of the derivatives obtained from [^{15}N]-labelled benzyloxycarbonyl-DL-phenylalanine and -valine, ^{13}C–^{15}N coupling was observed for one only of the two low-field carbons in each case.[8] The Figure shows this for the valine derivative. Whilst this is clearly consistent with these compounds having structures (5; R = $PhCH_2$ and $CHMe_2$ respectively), as these each have but one sp^2

carbon atom attached directly to nitrogen, it rules out structures of type (4) since these have two. All the so-called benzyloxycarbonylaziridinones reported by Miyoshi which we have prepared have similar i.r. characteristics and are presumably actually 2-benzyloxyoxazol-5(4*H*)-ones: other compounds such as the supposed α-lactam (7),[9] which was prepared by Miyoshi's method, also require re-examination.



Completely proton-decoupled ^{13}C n.m.r. spectra: A, [^{14}N]-2-benzyloxy-4-isopropyl-DL-oxazol-5(4*H*)-one, full spectrum; B, as A expanded to show the two low-field carbon signals only; C, as B for the [^{15}N]oxazol-5(4*H*)-one

Comparison of 2-alkyl- and 2-alkoxy-thiazol-5(4*H*)-ones has shown [10] that the members of the 2-alkoxy-series are substantially more difficult to ionise at position 4 than are their 2-alkyl counterparts. This proved to apply in the oxazol-5(4*H*)-ones as well: the oxazol-5(4*H*)-one derived from benzyloxycarbonyl-L-phenylalanine was racemised by tertiary base much more slowly than were the oxazol-5(4*H*)-ones obtained from acetyl- and benzoyl-L-phenylalanine (Table 1). This alone

TABLE 1

Second-order rate constants for the racemisation of 2-substituted-4-benzyloxazol-5(4*H*)-ones by equivalent amounts of tertiary amines, at a concentration of 0.025M in chloroform at 293 K

2-Substituent	k_2 for triethylamine as base/ l mol^{-1} s^{-1}	k_2 for di-isopropylamine as base/ l mol^{-1} s^{-1}
Ph	>1	3.6
CH_3	>1	1.7×10^{-1}
$PhCH_2O$	2.6×10^{-2}	4.2×10^{-3}

TABLE 2

Second-order rate constants for the formation of 4-bromo- and 4-nitro-anilides by reaction of 2-substituted- 4-benzyloxazol-5(4*H*)-ones with equivalent amounts of 4-bromo- or 4-nitro-anilines, in deuteriochloroform solution at 310.5 K, at a concentration of 0.17M

2-Substituent	k_2 for reaction with 4-bromoaniline/ l mol^{-1} s^{-1}	k_2 for reaction with 4-nitroaniline/ l mol^{-1} s^{-1}
Ph	8.4×10^{-5}	$<10^{-6}$
CH_3	2.1×10^{-3}	3.3×10^{-4}
$PhCH_2O$	2.0×10^{-2}	5.5×10^{-3}

would have sufficed to explain Miyoshi's finding that his compounds underwent aminolysis to give optically pure products but the scales are tilted even more in favour of coupling without racemisation in the case of the 2-benzyloxyoxazol-5(4*H*)-ones by the fact that they are also much more reactive as acylating agents than are oxazol-5(4*H*)-ones of the familiar kind (Table 2).

2-Aryl- and 2-alkyl-oxazol-5(4*H*)-ones undergo a range of useful reactions at position 4 in which the anion is an intermediate. Our attempts to carry out reactions of this type on the 2-benzyloxyoxazol-5(4*H*)-one (5; R = $PhCH_2$) were largely fruitless because of its diminished ease of ionisation and greater sensitivity to nucleophiles.[11]

Kemp has shown [12] that the base-catalysed formation of oxazol-5(4*H*)-ones is a two-step process involving a rate-determining intramolecular reaction of the anion formed by ionisation of the amide function (Scheme 1). In considering the effect of varying the amide function on the ease of cyclisation the factors to focus upon are therefore the acidity of the N–H and the nucleophilicity of the conjugate base. In both respects the cyclisation of an activated benzyloxycarbonylamino-acid would be predicted to be more difficult than that of the corresponding acetyl or benzoyl derivative. We are not aware of any published quantitative comparison of the N–H acidity of amides and carbamates, but Bordwell has made such a comparison by the application of his methods [13] to the benzyloxycarbonyl- and benzoyl-derivatives of glycine benzyl ester in dimethyl sulphoxide, for which he finds pK values of 22.0 and 21.2, respectively. In addition to this, urethane groups have been shown [14] to be less nucleophilic at oxygen than amides. It was therefore not unexpected that we should be unable to bring about the formation of (5; R = $PhCH_2$) from (3; R = $PhCH_2$) with any treatment milder than those indicated in Scheme 2.

In conclusion, it is misleading to say, as many texts and reviews have, that benzyloxycarbonylamino-acids can be coupled without racemisation because of their inability to form oxazol-5(4*H*)-ones. These protected amino-acids can be converted into oxazol-5(4*H*)-ones, although only under restricted and forcing conditions, but the derived oxazolones are highly reactive acylating agents of relatively high optical stability: even if they were formed under coupling conditions they would not be expected to lead to racemised peptides.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. N.m.r. spectra were recorded with deuteriochloroform as solvent and tetramethylsilane as internal standard (a) for protons, with a Perkin-Elmer R32 spectrometer operating at 90 MHz and 310.5 K and (b) for ^{13}C, on a Bruker WH90 instrument operating at 22.6 MHz and 305 K with solutions of *ca.* 50% concentration (assignments were confirmed by observation of C–H coupling but only the completely proton-decoupled spectra are reported). Mass spectra were recorded on a Varian CH7 spectrometer operating at 70 eV.

2-Benzyloxy-4-benzyl-L-oxazol-5(4H)-one.— Thionyl chloride (0.8 ml, 11 mmol) was added to a stirred solution of *N*-benzyloxycarbonyl-L-phenylalanine (2.99 g, 10 mmol) in ether (25 ml) at −30°. After 5 min, triethylamine (5.2 ml, 40 mmol) was added dropwise over 30 min with stirring. After a further 10 min, the mixture was filtered and evaporated to give an oil, which was dissolved in ether (15 ml). The ether solution was filtered and evaporated, and the residue was triturated with light petroleum (2 × 35 ml). Evaporation of the triturate gave the required product as an oil (1.2 g, 43%). Crystallisation from light petroleum gave the oxazolone as needles, m.p. 73—73.5°; $[\alpha]_D^{20}$ −34.5° (*c* 1 in THF) {lit.,[3] m.p. 73.5°, $[\alpha]_D^{20}$ −36° (*c* 1 in THF)}; $\nu_{max.}$ ($CHCl_3$) 1 835, 1 690, 1 460, 1 390, 1 325, 1 090, 900, and 710 cm^{-1}; δ_H 7.38 (5 H, s, ArH), 7.23 (5 H, m, ArH), 5.31 (2 H, s, $PhCH_2O$), 4.57 (1 H, m, N–CH), 3.15 (2 H, m, $PhCH_2C$); δ_C 175.2 (O–C=O), 158.3 (O–C=N), 135.5—127.0 (aryl carbons), 71.6 ($PhCH_2O$), 66.7 (N–CH), and 37.5 p.p.m. ($PhCH_2C$); *m/e* 281 (calculated mol. wt. 281) (Found: C, 72.6; H, 5.45; N, 5.1. Calc. for $C_{17}H_{15}NO_3$: C, 72.6; H, 5.4; N, 5.0%).

The DL-oxazolone was prepared similarly. Recrystallisation from ether–light petroleum gave the *DL-oxazolone* as needles of m.p. 52—53° with the same spectroscopic properties as the L-form (Found: C, 72.35; H, 5.3; N, 4.7%). The 75% [^{15}N]-labelled DL-oxazolone was prepared in the same manner from 75% [^{15}N]-labelled benzyloxycarbonyl-DL-phenylalanine and obtained as needles of m.p. 52—53°; δ_C as above with the exception of 158.3 (d, J_{CN} 6.1 Hz, N=C–O) and 66.7 p.p.m. (d, J_{CN} 2.4 Hz, N–CH).

2-Benzyloxy-4-isopropyl-DL-oxazol-5(4H)-one.— Thionyl chloride (0.8 ml, 11 mmol) was added to a stirred solution of *N*-benzyloxycarbonyl-DL-valine (2.51 g, 10 mmol) in ether (25 ml) at −30°. After 5 min, triethylamine (5.2 ml, 40 mmol) was added dropwise over 30 min with stirring. After a further 10 min, the mixture was filtered and evaporated to give an oil, which was dissolved in ether (15 ml). The ether solution was filtered and evaporated and the residue was triturated with light petroleum (2 × 35 ml). Evaporation of the triturate gave the required oxazolone as an oil (1.4 g, 60%), $\nu_{max.}$ ($CHCl_3$) 1 840, 1 685, 1 460, 1 390, 1 320, 1 100, and 910 cm^{-1}; δ_H 7.35 (5 H, m, ArH), 5.32 (2 H, s, $PhCH_2O$), 4.12 (1 H, d, *J* 4.5 Hz, N–CH), 2.13 (1 H, m, $CHMe_2$), and 0.94 (6 H, dd, Me_2C); δ_C (see Figure), 174.9 (CO–O), 157.95 (N=C–O), 134.2 (aryl carbon), 128.5—128.2 (aryl carbons), 71.2 ($PhCH_2$), 70.5 (N–CH), 30.6 ($CHMe_2$), and 18.2 and 16.8 (Me_2C); *m/e* 233 (calculated mol. wt. 233). The 95% [^{15}N]-labelled oxazolone, prepared from 95% [^{15}N]-labelled benzyloxycarbonyl-DL-valine in the same manner, was obtained as an oil; δ_H ($CDCl_3$) as above with the exception of 4.12 (1 H, dd, J_{HH} 4.5, J_{NH} 1.3 Hz, N–CH); δ_C (see Figure) as above with the exception of 157.95 (d, J_{CN} 6.1 Hz, N=C–O), 71.2 (d, J_{CN} 3.7 Hz, $PhCH_2$), and 70.5 (d, J_{CN} 2.4 Hz, N–CH).

Racemisation Rates.—2-Methyl-4-benzyl-L-oxazol-5(4*H*)-one and 2-phenyl-4-benzyl-L-oxazol-5(4*H*)-one were prepared by the general method of Siemion.[15] The second-order rate constants for racemisation of the oxazolones by tertiary amines in chloroform at 293 K were determined by measuring at intervals the optical rotations of solutions in chloroform (dried over $CaCl_2$) which were 0.025M with respect to both oxazolone and tertiary amine. Excellent and reproducible second-order plots were obtained.

Aminolysis Rates.—The second-order rate constants for ring-opening of the oxazolones by anilines in chloroform at 310.5 K were determined by measuring the rate of decrease of the integral of a suitable ^1H n.m.r. signal of the oxazolone for a 0.17M solution of the oxazolone in deuteriochloroform containing one equivalent of the 4-substituted aniline. For the 2-benzyloxy- and 2-phenyl-oxazolones the anilide (the anilide products were subsequently isolated and characterised) was the only product observed; in the case of the 2-methyloxazolone a second product which decomposed on attempted isolation was observed. Allowance was made for this in the calculation of the rate constant for anilide formation.

Acylaminoacylanilides.—The following anilides were isolated from the reaction mixtures obtained from aminolysis rate investigations. All had spectroscopic properties in accord with the structures stated. *Benzoyl-DL-phenylalanine 4-bromoanilide* had m.p. 265° (Found: C, 62.5; H, 4.7; Br, 18.8; N, 6.6. $C_{22}H_{19}BrN_2O_2$ requires C, 62.4; H, 4.5; Br, 18.9; N, 6.6%), *acetyl-DL-phenylalanine 4-bromoanilide*, m.p. 260° (Found: C, 56.9; H, 4.9; Br, 21.6; N, 7.8. $C_{17}H_{17}BrN_2O_2$ requires C, 56.5; H, 4.7; Br, 22.1; N, 7.75%), *benzyloxycarbonyl-DL-phenylalanine 4-bromoanilide* m.p. 175—176° (Found: C, 60.7; H, 4.7; Br, 17.3; N, 6.3. $C_{23}H_{21}BrN_2O_3$ requires C, 60.9; H, 4.7; Br, 17.6; N, 6.1%), *acetyl-DL-phenylalanine 4-nitroanilide*, m.p. 264—267° (Found: C, 62.4; H, 5.4; N, 12.7. $C_{17}H_{17}N_3O_4$ requires C, 62.4; H, 5.2; N, 12.8%), and *benzyloxycarbonyl-DL-phenylalanine 4-nitroanilide*, m.p. 210—211° (Found: C, 65.8; H, 5.1; N, 9.8. $C_{23}H_{21}N_3O_5$ requires C, 65.9; H, 5.05; N, 10.0%).

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