Synthesis of β-Amino-acid Peptides by Aminolysis of Substituted Dihydro-1,3-Oxazinones and Amino-protected β-Lactams

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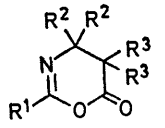
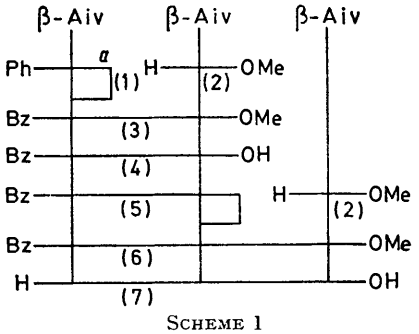
A number of standard peptide coupling methods failed when applied to sterically hindered derivatives of 3-amino-3-methylbutanoic acid. Di- and tri-peptides were made by aminolysis of dihydro-1,3-oxazinones and by this method in conjunction with the pivaloyl mixed anhydride method. *N*-Protected azetidin-2-ones were found to have limited application in β-amino-acid peptide synthesis.

SOME conventional coupling methods failed when applied to 3-amino-3-methylbutanoic acid (β-aminoisovaleric acid, β-Aiv),[1] probably because of steric hindrance at the amino-group. For example the coupling of the benzyloxycarbonylamino-acid (8) and the methyl ester (2) was attempted using isobutyl chloroformate, *NN'*-dicyclohexylcarbodi-imide (DCCI) and Woodward's 'reagent K,' but without success. The DCCI reaction gave only the *N*-acylurea by-product in high yield; with the other reagents the carboxy-component (8) was recovered. We therefore studied the use of dihydro-1,3-oxazinones and *N*-protected azetidin-2-ones (β-lactams) as alternative methods; the latter proved to be of little merit.

The ability of the 2-phenyldihydro-oxazinone (1) to acylate amines, and the structural similarity of such



(1) R^1 = Ph, R^2 = Me, R^3 = H
(1a) R^1 = Ph, R^2R^2 = $[CH_2]_5$, R^3 = H
(1b) R^1 = Ph, R^2 = R^3 = Me



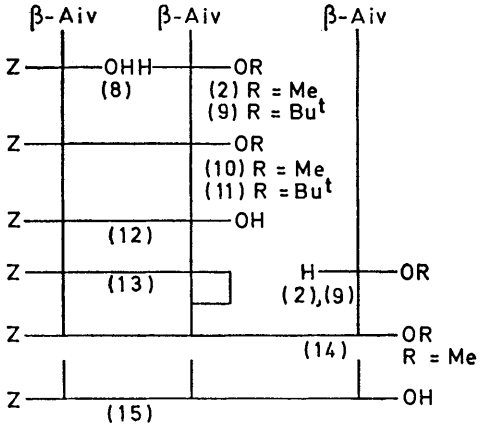
SCHEME 1

[a] Represents the dihydro-oxazinone system.

compounds to oxazolones, the latter having been exploited successfully in the preparation of sterically hindered peptides,[2] suggested to us that dihydro-oxazinone derivatives of 3,3-dialkyl- and 2,2,3,3-tetra-alkyl-β-amino-acids might prove to be useful in coupling reactions.

The 2-phenyldihydro-oxazinone (1)[3] reacted at room temperature with the amino-ester (2) (Scheme 1) to afford the benzoyl dipeptide (3) in low yield. At higher temperatures the product (3) was isolated almost quantitatively. Saponification of the ester (3) gave the protected dipeptide acid (4), which was cyclized with acetic anhydride to the peptide dihydro-oxazinone (5). Compound (5) was difficult to crystallize and was usually used in the crude state, affording good yields of the tripeptide (6) when coupled with the ester (2) under reflux conditions.

Electrolytic cleavage of the benzoyl group[4] from the saponified tripeptide ester (6) gave the linear tripeptide



SCHEME 2

(7), in mediocre yield. T.l.c. showed that some degradation of the peptide chain may have occurred.

It then appeared that other acyl groups were required which would participate in dihydro-oxazinone formation and which would be more susceptible to selective cleavage than the benzoyl group. However we were unable to isolate dihydro-oxazinone derivatives from the trifluoroacetyl and formyl systems, although in the former case an anhydride [$\nu_{max.}$ (film) 1820 and 1710 cm^{-1}] was obtained which reacted with aniline to give β-(trifluoro-acetylamino)isovaleranilide.

The dihydro-oxazinone route (Scheme 2) was nevertheless exploited in conjunction with use of a benzyloxycarbonyl group for *N*-protection, by cyclization of the dipeptide acid (12), derived from the protected peptide methyl (10) or t-butyl ester (11), these being formed in good yield by the pivaloyl mixed anhydride route.[2,5] Acylation of the amino-esters (2) and (9) by the peptide

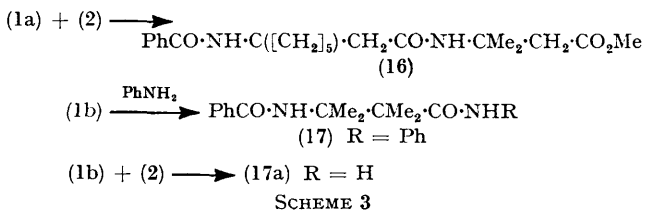
[1] J. Lowbridge and C. N. C. Drey, *Chem. Comm.*, 1970, 791.
[2] M. T. Leplawy, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, 1960, **11**, 39; D. S. Jones, G. W. Kenner, J. Preston, and R. C. Sheppard, *J. Chem. Soc.*, 1965, 6227; G. W. Kenner, J. Preston, and R. C. Sheppard, *ibid.*, p. 6239.
[3] W. Baker and W. D. Ollis, *J. Chem. Soc.*, 1949, 345.
[4] L. Horner and H. Neumann, *Chem. Ber.*, 1965, **98**, 3462.
[5] J. R. Vaughan and R. L. Osato, *J. Amer. Chem. Soc.*, 1951, **73**, 5553; M. Zaoral, *Angew. Chem.*, 1959, **71**, 743.

dihydro-oxazinone (13) proceeded under reflux conditions, yielding the tripeptide methyl ester (14) or t-butyl benzyloxycarbonylbis-β-aminoisovaleryl-β-aminoisovalerate, respectively.

Dissolution of the protected tripeptide t-butyl ester in hydrogen bromide–acetic acid [6] caused extensive degradation of the peptide chain. Alternative attempts to isolate the tripeptide (7) after hydrogenolysis and treatment with trifluoroacetic acid gave an oily product which was tentatively formulated as t-butyl bis-β-aminoisovaleryl-β-aminoisovalerate on the basis of n.m.r. data. Apparently cleavage of the ester group was hindered under the standard conditions, behaviour also observed with t-butyl esters of β-lysine.[7]

Eventually the tripeptide (7) was obtained by hydrogenolysis of the tripeptide derivative (15) isolated from its dicyclohexylammonium salt. However the product was isolated as the hydrochloride; evidently sufficient hydrochloric acid had passed through the washing procedures for this to occur.

Other dihydro-oxazinones (1a and b) were prepared to study the generality of the method. We had noticed in the synthesis of the tripeptides (6) and (14) that as the peptide chain length was extended more severe reaction conditions were required. The same observation was true of the spiro-dihydro-oxazinone (1a), which yielded the protected dipeptide (16) (Scheme 3) after a long reaction.



SCHEME 3

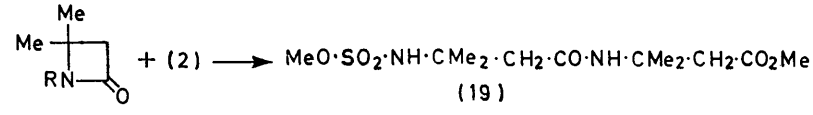
Derivatives of 2,2,3,3-tetramethyl-β-alanine are difficult to synthesize because of steric hindrance.[8] The dihydro-oxazinone (1b) similarly exhibited great reluctance to react with nucleophiles. Prolonged heating with aniline (Scheme 3) eventually yielded the anilide (17), whereas reaction with the ester (2) under similar conditions afforded the amide (17a), presumably arising *via* β-elimination of ammonia from the β-amino-ester (2). In the presence of a solvent, starting materials were recovered.

In view of the known reactivity of azetidin-2-ones, and their ready accessibility,[9] it was decided to investigate their potential in peptide synthesis. We then required a selective *N*-protecting group. Graf [9] has shown that *N*-chlorosulphonylazetidinones may be used to prepare diesters and sulphate ester-amides in high yield, and that these may be preferentially cleaved under acidic conditions yielding amino-esters and amides, respectively.

Reaction of the azetidinone (18) with methyl 3-amino-3-methylbutyrate (2) in methanol gave the dipeptide diester (19) as an oil in reasonable yield (Scheme 4). The reaction demonstrated that the chlorosulphonylazetidinone (18) could be used as an intermediate in acylating the sterically hindered amino-ester (2), but the question of selective cleavage remained open. We felt that extension of this approach was probably unprofitable in view of the lack of crystallinity of the products even when alternative alcohols such as benzyl and *p*-nitrobenzyl were used, and also because of protracted isolation procedures. Furthermore, β-linked peptides are, in our experience, more readily cleaved under acidic conditions than are the corresponding α-systems.

We therefore considered the introduction of typical peptide amino-protecting groups on the free azetidinone (18a). The tertiary bases pyridine and triethylamine were unsuccessful in promoting *o*-nitrophenylsulphenylation, but use of potassium t-butoxide effected a smooth substitution. The resulting sulphenylated lactam (18b) proved a poor acylating derivative, undergoing alkaline hydrolysis with difficulty.

The tosyl group is known to activate [10] both γ- and δ-lactams to nucleophilic agents. Tosylation of the azetidinone (18a) in a similar manner to that yielding the nitrosulphenyl derivative gave two substituted lactams in addition to some polymeric material.[11] One of these was the required derivative (18c) and the other was formulated as the (3-methyl-3-tosylaminobutyryl)-azetidinone (18d), presumably arising by acylation of the lactam anion *via* the tosylazetidinone (18c). However the protected azetidinone (18c) was insufficiently reactive to be used for peptide synthesis, even though aminolysis gave tosyl-β-aminoisovaleranilide.



(18) R = $ClSO_2$
(18a) R = H
(18b) R = Nps
(18c) R = Tos
(18d) R = $TosNH·CMe_2·CH_2·CO$

SCHEME 4

[6] D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 1952, **17**, 1564.
[7] L. I. Rostovtseva, P. D. Resbetov, and A. S. Khokhlov, *Zhur. obshchei Khim.*, 1969, **39**, 96.
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[10] J. Rudinger, *Recent Chem. Progr.*, 1962, **23**, 3.
[11] R. Graf, G. Lohaus, K. Börner, E. Schmidt, and H. Bestian, *Angew. Chem. Internat. Edn.*, 1962, **1**, 481.

EXPERIMENTAL

Solvents were purified and distilled before use. Petrol refers to the fraction of b.p. 60—80°. I.r. spectra were recorded on a Perkin-Elmer 337 or 457 spectrophotometer, and n.m.r. spectra on a Perkin-Elmer (60 MHz) R12 instrument.

In general, neutral products were isolated by washing solutions in ethyl acetate, chloroform, or dichloromethane successively with 0·5M-hydrochloric acid, 5% sodium hydrogen carbonate solution, and water, followed by drying ($MgSO_4$) and evaporation under reduced pressure.

T.l.c. on Merck Kieselgel G (0·25 mm) employed the following solvent systems (v/v): (A) n-butanol–acetic acid–water (4 : 1 : 1), (B) methanol–chloroform–acetic acid (10 : 39 : 1), (C) cyclohexane–chloroform–acetic acid (2 : 8 : 1), (D) ethyl acetate, and (E) chloroform.

Synthesis of β-Aminoisovaleryl-β-aminoisovaleryl-β-aminoisovaleric Acid (7) via *Benzoyl Derivatives.—Methyl β-benzoylaminoisovaleryl-β-aminoisovalerate* (3). 4,5-Dihydro-4,4-dimethyl-2-phenyl-1,3-oxazin-6-one [3] (285 mg, 1·4 mmol) was treated with methyl β-aminoisovalerate [9] (187 mg, 1·43 mmol) in acetonitrile (3 ml) under reflux for 3 h. Removal of solvent and trituration of the residue with petrol yielded the *dipeptide* (455 mg, 97·5%), m.p. 90—94° (97—98° from ether–petrol) (Found: C, 64·8; H, 8·0; N, 8·7. $C_{18}H_{26}N_2O_4$ requires C, 64·7; H, 7·8; N, 8·4%).

β-Benzoylaminoisovaleryl-β-aminoisovaleric acid (4). A solution of the ester (3) (2·7 g, 8·1 mmol) in aqueous acetone (30%; 20 ml) was cooled to 0° and M-sodium hydroxide (8·2 ml) was added gradually. The mixture was stirred at room temperature for 5 h and extracted with ether (10 ml); the aqueous phase was made acid (2M-hydrochloric acid) to Congo Red. The precipitate was filtered off, washed with water, and recrystallized from aqueous acetone (60%) to give the pure N-*protected dipeptide* (2·3 g, 89%), m.p. 151—154°, (153—155° on recrystallization) (Found: C, 63·8; H, 7·3; N, 8·9. $C_{17}H_{24}N_2O_4$ requires C, 63·7; H, 7·6; N, 8·7%).

2-(2-Benzoylamino-2-methylpropyl)-4,5-dihydro-4,4-dimethyl-1,3-oxazin-6-one (5). A solution of the acylaminopeptide (4) (2 g, 6·25 mmol) in acetic anhydride (30 ml) was heated at 110—120° (oil-bath) for 30 min and then evaporated. The oily residue was treated with toluene (3 × 10 ml) and evaporated repeatedly; the crude *oxazinone* (2 g) remained. A sample crystallized from ether–petrol had m.p. 84—87° (Found: C, 67·8; H, 7·2; N, 9·0. $C_{17}H_{22}N_2O_3$ requires C, 67·5; H, 7·3; N, 9·3%).

Methyl β-benzoylaminoisovaleryl-β-aminoisovaleryl-β-aminoisovalerate (6). A solution of the crude oxazinone (5) (1·9 g, 6·3 mmol) and methyl β-aminoisovalerate [9] (825 mg, 6·3 mmol) in acetonitrile (20 ml) was heated under reflux for 8 h, then evaporated. The residue was dissolved in ethyl acetate (100 ml) and washed in the usual way. Evaporation and recrystallization from ethyl acetate–petrol gave the *tripeptide derivative* (1·97 g, 72·5%), m.p. 150—153° (152—153° after recrystallization) (Found: C, 63·6; H, 8·1; N, 9·6. $C_{23}H_{35}N_3O_5$ requires C, 63·7; H, 8·1; N, 9·7%).

β-Benzoylaminoisovaleryl-β-aminoisovaleryl-β-aminoisovaleric acid. The ester (6) (1·9 g, 4·4 mmol) was hydrolysed as described for the isolation of the peptide acid (4) and yielded the *tripeptide derivative* (1·6 g, 87%), m.p. 168—170° [170—171° from aqueous acetone (30%)] (Found: C, 62·9; H, 8·1; N, 10·0. $C_{22}H_{33}N_3O_5$ requires C, 63·0; H, 7·9; N, 10·0%).

β-Aminoisovaleryl-β-aminoisovaleryl-β-aminoisovaleric acid (7). A solution of the foregoing N-protected derivative (1·05 g, 2·5 mmol) and tetramethylammonium chloride (825 mg, 7·5 mmol) in methanol (25 ml) maintained at 10—15° (ice–salt bath) was electrolysed [4] for 1 h between a mercury cathode and a platinum anode (0·8—0·9 A; 25 V). The solution was filtered and evaporated, and the residue dissolved in water (10 ml) and extracted with ethyl acetate–butan-1-ol (1 : 1; 3 × 25 ml). Evaporation of the extract yielded an oil, which was dissolved in water (10 ml) and passed through a column of Dowex 1 resin (OH^- form). When the eluate was neutral, the column was developed with 1% acetic acid, and the product was located at the acid solvent front by t.l.c. These fractions were combined, evaporated to small volume, and passed through a column of Dowex 50 resin (H^+ form), from which the peptide was finally eluted by 3% ammonia. Fractions containing the product, $R_{F(A)}$ 0·65, $R_{F(B)}$ 0·33, were evaporated and the residue crystallized from methanol and ether yielding the *tripeptide* (150 mg, 19%), m.p. 174—176° (182—184° on recrystallization) (Found: C, 57·0; H, 9·3; N, 13·2. $C_{15}H_{29}H_3O_4$ requires C, 57·1; H, 9·3; N, 13·3%).

Synthesis of β-Aminoisovaleryl-β-aminoisovaleryl-β-aminoisovaleric Acid (7) via *Benzyloxycarbonyl Derivatives.—β-Benzyloxycarbonylaminoisovaleric acid* (8) *dicyclohexylammonium salt.* Solutions of benzyloxycarbonyl chloride in acetone (75 ml, 0·15 mol) and sodium hydroxide (75 ml; 2M) were added concurrently during 1 h to a stirred and cooled (0°) solution of β-aminoisovaleric acid [9,12] (16 g, 0·14 mmol) in a mixture of sodium hydroxide solution (75 ml, 2M) and acetone (45 ml). The mixture was stirred at room temperature for 3 h, then the acetone was evaporated off. The aqueous solution was extracted with ether, made acid (2M-hydrochloric acid) to Congo Red, and extracted with ethyl acetate. The organic phase was washed with water, dried, and evaporated yielding the crude product (35 g, 102%) as an oil [$R_{F(C)}$ 0·67]. The oil was dissolved in ethyl acetate (50 ml) and dicyclohexylamine (24·8 g, 0·14 mmol) was added at 0°. The solution was evaporated and the residue recrystallized from ethyl acetate–petrol yielding the *salt* (48 g, 81%), m.p. 128—130° (130—131° on recrystallization) (Found: C, 69·7; H, 9·2; N, 6·2. $C_{25}H_{40}N_2O_4$ requires C, 69·4; H, 9·3; N, 6·5%).

t-Butyl β-aminoisovalerate (9). Oily β-benzyloxycarbonylaminoisovaleric acid (40 g), prepared from the amino-acid [9,12] (18 g, 0·15 mol) as described previously, was dissolved in dichloromethane (50 ml) containing concentrated sulphuric acid (3 ml). Liquid isobutene (400 ml) was added and the mixture set aside in a pressure bottle, at room temperature, for 5 days. The excess of isobutene was allowed to escape; the dichloromethane solution was washed with aqueous 5% sodium hydrogen carbonate and water, dried, and evaporated. The crude product (49·5 g, 105%), chromatographically pure, was a viscous oil, $R_{F(D)}$ 0·90, $R_{F(E)}$ 0·70, $\nu_{max.}$ (film) 3336, 2941, and 1723 cm^{-1}, τ (CCl_4) 2·70 (5H, s, $C_6H_5{\cdot}CH_2$), 4·20br (1H, s, NH), 5·00 (2H, s, $PhCH_2{\cdot}O$), 7·50 (2H, s, $CH_2{\cdot}CO$), and 8·6 and 8·65 (15H, s, Bu^t and CMe_2). The crude benzyloxycarbonyl derivative (49 g) was dissolved in methanol (100 ml) and hydrogenolysed over 10% palladium–charcoal (5 g) for 36 h. Filtration and evaporation yielded a ninhydrin-positive oil (18 g), n_D^{25} 1·4229, $R_{F(A)}$ 0·65. Distillation afforded the *ester* (12 g, 45% based on the amino-acid),

[12] M. D. Slimmer, *Ber.*, 1902, **35**, 400.

b.p. 85° at 20 mmHg, n_D^{25} 1·4246 (Found: C, 62·6; H, 11·2; N, 8·3. $C_9H_{19}NO_2$ requires C, 62·4; H, 11·1; N, 8·1%).

N-(β-*Benzyloxycarbonylaminoisovaleryl*)-NN′-*dicyclohexylurea*. β-Benzyloxycarbonylaminoisovaleric acid (251 mg, 1 mmol) and methyl β-aminoisovalerate [9] (131 mg, 1 mmol) were mixed in acetonitrile (3 ml). The solution was cooled to 0° and dicyclohexylcarbodi-imide (206 mg, 1 mmol) was added with stirring. The mixture was left at room temperature overnight. The *urea* (227 mg, 50%), m.p. 152—154°, was filtered off. A second crop (175 mg, 38%), m.p. 147—151°, was isolated from the filtrate (m.p. 153—155° from cyclohexane–petrol) (Found: C, 68·3; H, 8·8; N, 9·1. $C_{26}H_{39}N_3O_4$ requires C, 68·2; H, 8·6; N, 9·2%).

Reaction of β-*benzyloxycarbonylaminoisovaleric acid with pivaloyl chloride*. Pivaloyl chloride (3·5 g, 28·8 mmol) was added to a stirred and cooled (−5°) solution of β-benzyloxycarbonylaminoisovaleric acid dicyclohexylammonium salt (12·5 g, 28·8 mmol) in acetonitrile (75 ml). The mixture was stirred at −5° for 2 h, then at room temperature for 1 h, filtered, and evaporated. Toluene (20 ml) was added and evaporation was repeated. This procedure was repeated three times yielding an oil (9·3 g, 96%), $\nu_{max.}$ (film) 3395, 2987, 1818, and 1754 cm^{-1}, τ (CCl_4) 2·70 (5H, s, C_6H_5·CH_2), 4·75br (1H, s, NH), 5·00 (2H, s, PhCH_2·O), 7·15 and 7·25 (2H, s, CH_2·CO), 8·60 and 8·65 (6H, s, CMe_2), and 8·60 (9H, s, Bu^t).

β-*Benzyloxycarbonylaminoisovaleryl*-β-*aminoisovaleric acid* (12) *dicyclohexylammonium salt*. (*a*) A solution of the foregoing crude pivalic acid mixed anhydride, derived from β-benzyloxycarbonylaminoisovaleric acid dicyclohexylammonium salt (14·25 g, 33 mmol), in acetonitrile (10 ml) was added gradually to a solution of methyl β-aminoisovalerate [9] (3·93 g, 30 mmol) in acetonitrile (10 ml) under reflux. Heating was continued for 3 h. The solvent was evaporated off and the oily residue dissolved in ethyl acetate. The usual washing procedure and evaporation of the dried ethyl acetate solution yielded crude methyl β-benzyloxycarbonylaminoisovaleryl-β-aminoisovalerate (11 g, 101%) as a viscous oil, $R_{F(D)}$ 0·90, $R_{F(E)}$ 0·50, $\nu_{max.}$ (film) 3338, 2940, 1739, 1724, 1666, and 1514 cm^{-1}, τ (CCl_4) 2·75 (5H, s, C_6H_5·CH_2), 3·50br and 3·85br (2H, 2s, NH), 5·00 (2H, s, PhCH_2·O), 6·43 (3H, s, CO_2Me), 7·30 and 7·60 (4H, s, CH_2·CO), and 8·60 and 8·65 (12H, s, NH·CMe_2).

A solution of this crude dipeptide methyl ester (11 g, 30·3 mmol) was hydrolysed by the procedure described for the isolation of the peptide acid (4), yielding an oil (4·4 g, 42%), $R_{F(C)}$ 0·58. A sample of this (300 mg, 0·86 mmol) was dissolved in ethyl acetate (1 ml) containing dicyclohexylamine (156 mg, 0·86 mmol). Petrol (5 ml) was added and the *salt* (336 mg, 30·5% based on the crude peptide ester), m.p. 124—128°, was filtered off (m.p. 131—133° from ethyl acetate–petrol) (Found: C, 67·7; H, 9·1; N, 7·7. $C_{30}H_{49}H_3O_5$ requires C, 67·8; H, 9·3; N, 7·9%).

(*b*) A solution of the crude mixed pivaloyl anhydride (9 g, 27 mmol) in acetonitrile (10 ml) was added gradually to a solution of t-butyl β-aminoisovalerate (4·5 g, 26 mmol) and triethylamine (2·7 g, 27 mmol) in acetonitrile (10 ml) under reflux. Heating was continued for 5 h, and the solution was filtered and evaporated. The residue was partitioned between ethyl acetate (50 ml) and water (10 ml). The organic phase was separated, washed in the usual way, dried, and evaporated to give crude t-butyl β-benzyloxycarbonylaminoisovaleryl-β-aminoisovalerate (9·6 g, 87·5%) as an oil, $R_{F(D)}$ 0·85, $R_{F(E)}$ 0·45, $\nu_{max.}$ (film) 3337, 2986, 1724, 1667, and 1515 cm^{-1}, τ ($CDCl_3$) 2·65 (5H, s, C_6H_5·CH_2), 3·60 and 3·90 (2H, 2s, NH), 4·90 (2H, s, PhCH_2·O), 7·40 and 7·60 (4H, s, CH_2·CO), and 8·60, 8·63, and 8·65 (21H, s, Bu^t and NH·CMe_2).

The crude dipeptide t-butyl ester (700 mg, 1·72 mmol) was dissolved in trifluoroacetic acid (2 ml) at −5°. The solution was allowed to attain room temperature and stirred for 5 min, then poured on crushed ice (20 g). The aqueous solution was extracted with chloroform; the organic phase was washed with water, dried, and evaporated to an oil (440 mg, 73%), $R_{F(C)}$ 0·58. Dicyclohexylamine (244 mg, 1·35 mmol) was added to a cold (0°) solution of the product in ethyl acetate (1 ml). Dilution with petrol precipitated β-benzyloxycarbonylaminoisovaleryl-β-aminoisovaleric acid dicyclohexylammonium salt (434 mg, 47·5% based on the crude ester), m.p. 133—136°.

Methyl β-*benzyloxycarbonylaminoisovaleryl*-β-*aminoisovaleryl*-β-*aminoisovalerate* (14). β-Benzyloxycarbonylaminoisovaleryl-β-aminoisovaleric acid (9·6 g, 23·6 mmol) was dried overnight *in vacuo* (P_2O_5) and converted into the oily dihydro-oxazinone (7·6 g, 97%) by the method described for the preparation of (5) (heating for 2 h under reflux); $\nu_{max.}$ (film) 3355, 3030, 2984, 2941, 2262, 1785, and 1518 cm^{-1}. A solution of the crude dihydro-oxazinone (166 mg, 0·5 mmol) and methyl β-aminoisovalerate [9] (92 mg, 0·7 mmol) in acetonitrile (5 ml) was heated under reflux for 3 h, then evaporated; the residue was taken up in ethyl acetate (10 ml) and washed in the usual way. Evaporation gave the crude product as an oil, $R_{F(D)}$ 0·70, $R_{F(E)}$ 0·45, which crystallized slowly on trituration with aqueous methanol (80%). Recrystallization from aqueous methanol (50%) gave the *tripeptide methyl ester* (118 mg, 51%), m.p. 79—82°, unchanged on recrystallization [Found (allowing for 4·03% combustion residue): C, 61·8; H, 8·3; N, 9·4. $C_{24}H_{37}N_3O_6$ requires C, 62·2; H, 8·1; N, 9·1%].

β-*Benzyloxycarbonylaminoisovaleryl*-β-*aminoisovaleryl*-β-*aminoisovaleric acid* (15) *dicyclohexylammonium salt*. The crude benzyloxycarbonyl tripeptide methyl ester (14) (13 g) was saponified as described for the methyl ester (4). The oily product (12·5 g), $R_{F(C)}$ 0·58, was dissolved in ethyl acetate (50 ml) and dicyclohexylamine (5 g) was added at 0°. Evaporation left a crystalline residue which was recrystallized from ethyl acetate giving the *salt* (7·3 g, 41%), m.p. 96—100° (100—103° on further recrystallization) (Found: C, 66·5; H, 9·4; N, 8·9. $C_{35}H_{58}N_4O_6$ requires C, 66·6; H, 9·3; N, 8·9%).

β-*Aminoisovaleryl*-β-*aminoisovaleryl*-β-*aminoisovaleric acid* (7) *hydrochloride*. The foregoing tripeptide dicyclohexylammonium salt (6·6 g, 10·5 mmol) was dissolved in a mixture of aqueous 5% sodium hydrogen carbonate (100 ml) and methanol (10 ml) and the solution was extracted with ether. The aqueous phase was made acid (2M-hydrochloric acid) to Congo Red and extracted with chloroform. The organic phase was washed with water, dried, and evaporated yielding an oil (5·1 g), $R_{F(C)}$ 0·50. The crude benzyloxycarbonyl tripeptide was dissolved in methanol (60 ml) and hydrogenolysed over 10% palladium–charcoal (1 g) for 5 h. Filtration and evaporation yielded an oil $R_{F(A)}$ 0·65, $R_{F(B)}$ 0·33, which crystallized under ether. Recrystallization from methanol–ether gave the tripeptide hydrochloride (2·3 g, 62·5%), m.p. 155—160° (156—159° on further recrystallization) (Found: C, 50·8; H, 8·6; N, 11·8%; [M − HCl]$^{+\cdot}$, 315. $C_{15}H_{30}ClN_3O_4$ requires C, 51·2; H, 8·6; N, 11·9%; [M − HCl], 315).

4,5-*Dihydro*-2-*phenyl*-1,3-*oxazine*-4-*spirocyclohexane*-6-*one* (1a) (with S. Adamou and H. Chan). 1-Benzoylaminocyclohexaneacetic acid [13] (10 g, 0·038 mol) was converted by the procedure described for the preparation of (5) (heating under reflux for 3 h) into the *oxazinone derivative* (4·0 g, 43%), m.p. 72—74° (from carbon tetrachloride), raised to 75·5—77° on sublimation (59° and 0·01 mmHg) (Found: C, 74·3; H, 7·2; N, 5·8. $C_{15}H_{17}NO_2$ requires C, 74·1; H, 7·0; N, 5·8%).

Methyl 1-*benzoylaminocyclohexylacetyl*-β-*aminoisovalerate* (16). A solution of the oxazinone (1a) (1·04 g, 4·3 mmol) and methyl β-aminoisovalerate [9] (0·62 g, 4·7 mmol) in acetonitrile was heated under reflux for 10 h, then evaporated. The residue was taken up in ethyl acetate (6 ml) and washed in the usual way. Evaporation gave the crude product which was recrystallized from ethyl acetate–petrol to yield the *dipeptide derivative* (0·69 g, 43%), $R_{F(E)}$ 0·29, m.p. 90—92·5° (raised to 91—92·5°) (Found: C, 67·0; H, 7·8; N, 7·6. $C_{21}H_{30}N_2O_4$ requires C, 67·3; H, 8·1; N, 7·5%).

4,5-*Dihydro*-4,4,5,5-*tetramethyl*-2-*phenyl*-1,3-*oxazin*-6-*one* (1b). *N*-Benzoyl-2,2,3,3-tetramethyl-β-alanine [13] (4 g, 0·016 mol) after heating under reflux for 1 h was dehydrated to the *oxazinone* (2·77 g, 75%) by the procedure described for the isolation of (5); m.p. 66·5—68·5° (from carbon tetrachloride) (68—69° on further recrystallization), $R_{F(E)}$ 0·85 (Found: C, 72·6; H, 7·3; N, 6·3. $C_{14}H_{17}NO_2$ requires C, 72·7; H, 7·4; N, 6·1%).

N-*Benzoyl*-2,2,3,3-*tetramethyl*-β-*alanine anilide* (17). A solution of the oxazinone (1b) (150 mg, 0·7 mmol) and aniline (121 mg, 1·3 mmol; redistilled) in ether (10 ml) was heated with acetic acid (45 mg) for 20 h. The mixture was evaporated and washed in the usual way. Trituration of the crude product with petrol gave the *anilide* (108 mg, 51%), m.p. 162·5—163·5° (from ethyl acetate–petrol), $R_{F(E)}$ 0·23, $R_{F(D)}$ 0·88 (Found: C, 74·3; H, 7·6; N, 8·4. $C_{20}H_{24}N_2O_2$ requires C, 74·0; H, 7·5; N, 8·6%). The anilide was also obtained in 62% yield (m.p. 161·5—162°) by heating the oxazinone (150 mg, 0·7 mmol) in an excess of aniline (1·2 ml), under reflux for 2 h.

Isolation of *N*-*benzoyl*-2,2,3,3-*tetramethyl*-β-*alanine amide* (17a). The oxazinone (1b) (300 mg, 1·3 mmol) and methyl β-aminoisovalerate [9] (340 mg, 2·6 mmol) were heated under reflux for 4 h.* On cooling white crystals were deposited (101 mg), m.p. 168·5—174°. A second crop was obtained by the usual isolation procedure; after trituration with ether (25 mg), recrystallization from ethyl acetate gave the pure *amide*, m.p. 178—179°, $R_{F(D)}$ 0·65 (Found: C, 68·0; H, 8·0; N, 11·4. $C_{14}H_{20}N_2O_2$ requires C, 67·7; H, 8·1; N, 11·3%).

Azetidin-2-*ones as Potential Acylating Reagents.*—*Methyl* β-*methoxysulphonylaminoisovaleryl*-β-*aminoisovalerate* (19). Dry methanol was added to a cooled (0°) and stirred solution of 1-chlorosulphonyl-4,4-dimethylazetidin-2-one [9] (1·98 g, 0·01 mol) in ether (30 ml). A solution of methyl β-aminoisovalerate [9] (1·31 g, 0·01 mol) and triethylamine (1·01 g, 0·01 mol) in dry methanol (3 ml) was added during 20 min. Stirring was continued at room temperature for 3 days, then the solution was filtered and evaporated. The residue was washed in the usual way, and the product, an oil (2·0 g, 62%), chromatographed over silica in ethyl acetate. The *peptide*, obtained as an oil, (1·2 g, 41%) was chromatographically pure, $R_{F(D)}$ 0·88 (Found: C, 58·2; H, 5·9; N, 12·0. $C_{17}H_{21}N_3O_3S$ requires C, 58·8; H, 6·1; N, 12·1%), $\nu_{max.}$ (film) 3350, 2950, 1780, 1660, and 1540 cm^{-1}, τ ($CDCl_3$) 3·05 (1H, s, NH), 3·65 (1H, s, NH), 6·18 (3H, s, $MeO{\cdot}SO_2$), 6·33 (3H, s, OMe), 7·18 (2H, s, $CH_2{\cdot}CO$), and 7·63 (2H, s, $CH_2{\cdot}CO$).

4,4-*Dimethyl*-1-(o-*nitrophenylthio*)*azetidin*-2-*one* (18b). A stirred solution of 4,4-dimethylazetidin-2-one [9] (500 mg, 5 mmol) in t-butyl alcohol (10 ml) was treated with potassium t-butoxide (570 mg, 5 mmol), under dry nitrogen, followed 5 min later by a solution of *o*-nitrophenylsulphenyl chloride (960 mg, 5 mmol) in benzene (40 ml) during 20 min. The mixture was stirred at room temperature for 1 h, kept at 0° overnight, washed with water (2 × 20 ml), dried, and evaporated leaving a yellow solid (1·15 g), $R_{F(E)}$ 0·09, 0·46, and 0·88, $R_{F(D)}$ 0·48, 0·82, and 0·92. The component of $R_{F(E)}$ 0·46 and $R_{F(D)}$ 0·82 was separated by passage through a column of silica with chloroform as solvent to give the *azetidinone* (730 mg, 58%), m.p. 142·5—144° (145—145·5° from ethyl acetate) (Found: C, 52·25; H, 4·9; N, 11·3; S, 12·7. $C_{11}H_{12}N_2O_3S$ requires C, 52·4; H, 4·8; N, 11·1; S, 12·7%).

β-(o-*Nitrophenylthioamino*)*isovaleric acid dicyclohexylammonium salt*. A solution of the foregoing lactam (200 mg, 0·8 mmol) and sodium hydroxide (160 mg, 4·0 mmol) in acetonitrile–water (30 ml; 2 : 1) was heated under reflux for 2 h. Acetonitrile was evaporated off and the aqueous solution made acid to Congo Red with potassium hydrogen sulphate (1M) and extracted with ethyl acetate. The extract was washed with water, dried, and evaporated to small bulk. Dicyclohexylamine (159 mg, 0·88 mmol) was added at 0° and the *salt* crystallized (yield 214 mg, 59%), m.p. 156·5—158·5 (decomp.) [raised to 159—160° (decomp.)] (Found: C, 61·0; H, 8·0; N, 9·1. $C_{23}H_{37}N_3O_4S$ requires C, 61·2; H, 8·3; N, 9·3%).

4,4-*Dimethyl*-1-*tosylazetidin*-2-*one* (18c). 4,4-Dimethylazetidin-2-one [9] (1·0 g, 10 mmol) and tosyl chloride (1·92 g, 10 mmol) were treated with potassium t-butoxide (1·13 g, 10 mmol) in t-butyl alcohol as described for the preparation of the nitrophenylsulphenyl derivative (18b). Overnight a white precipitate (900 mg; m.p. >250°) separated; the organic layer was evaporated, leaving a white waxy solid (2·40 g). A sample of this (1·70 g) was triturated † with ethyl acetate (100 ml) and the solution was washed with water and dried. On evaporation a yellow oil (1·10 g) (a five-component mixture) was recovered, $R_{F(E)}$ 0·0, 0·07, 0·17, 0·48, 0·92. A sample of the oil (0·84 g) was chromatographed over silica in ethyl acetate. The band of $R_{F(E)}$ 0·92 was identified as tosyl chloride (0·29 g). The eluate corresponding to material of $R_{F(E)}$ 0·48 gave the *azetidinone* (0·14 g, 10%), m.p. 97—100° (102—102·5° from ethyl acetate) (Found: C, 56·9; H, 6·1; N, 5·5; S, 12·4. $C_{12}H_{15}NO_3S$ requires C, 56·9; H, 5·9; N, 5·5; S, 12·7%). Material of $R_{F(E)}$ 0·07 was identified by comparison of i.r. spectra as 4,4-dimethylazetidin-2-one [9] (23 mg).

Isolation of 4,4-*dimethyl*-1-(3-*methyl*-3-*tosylaminobutyryl*)-*azetidin*-2-*one* (18d). Evaporation of the eluate containing the compound of $R_{F(E)}$ 0·17 from the foregoing chromatographic separation gave the crystalline *azetidinone* (0·26 g, 27%) m.p. 98—101° (101·5—102° from ethyl acetate) (Found: C, 57·8; H, 7·1; N, 7·9. $C_{17}H_{24}N_2O_4S$ requires C, 57·9; H, 6·9; N, 8·0%).

* In the presence of a solvent the starting materials were recovered.

† A water-insoluble polymeric substance was recovered (0·19 g), $\nu_{max.}$ (Nujol) 3320, 1660, and 1550 cm^{-1}.[11]

[13] L. W. Hartzel and J. J. Ritter, *J. Amer. Chem. Soc.*, 1949, **71**, 4130.

β-*Tosylaminoisovaleranilide*. A solution of the foregoing azetidinone (45 mg, 0·2 mmol) and aniline (33 mg, 0·4 mmol) in ethyl acetate (9 ml) was heated under reflux for 34 h; t.l.c. then showed the absence of starting material. The mixture was washed in the usual way, dried, treated with charcoal, and filtered; evaporation of the filtrate gave the *anilide* (41 mg, 67%), m.p. 152—153° (from ethyl acetate) (Found: C, 62·2; H, 6·6; N, 8·4. $C_{18}H_{22}N_2O_3S$ requires C, 62·4; H, 6·4; N, 8·1%).

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