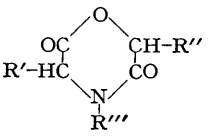
497. 2 : 5-*Diketomorpholines, Their Synthesis and Stability.*

By A. H. Cook and S. F. Cox.

Various substituted α′-bromoacyl-α-amino-acids and the corresponding hydroxy-amino-acids (*e.g.*, I) have been prepared and shown to yield lactones (II) with varying ease depending on substitution and steric configuration. These effects are ascribed to electronic influences of the substituents on lactonisation and to steric hindrance respectively. Biological results with some of the lactones described are appended.

It was reported (Cook, Cox, and Farmer, this vol., p. 1022; *Nature*, 1948, **162**, 61) that lateritiin, an antibiotic produced by *F. lateritium*, was converted by treatment with sodium hydroxide into a hydroxy-acid (I) which lactonised spontaneously to form (II; R′ = R″ = Pr^i, R‴ = Me). The present work was concerned with factors influencing the formation of this practically unknown diketomorpholine ring system, and with the effect of various alkyl groups on some of its properties. The lactones which were prepared were also examined for possible

$Pr^i{\cdot}CH(OH){\cdot}CO{\cdot}NMe{\cdot}CHPr^i{\cdot}CO_2H$
(I.)



(II.)

antibacterial activity, in the hope of throwing some light on the mode of action of lateritiin. The latter is chemically unreactive and it has been suggested that its biological properties might be due to one or other of its breakdown products. The activity was not shown by the natural lactone but it was possible that the solubility or stability of the latter might have been unfavourable for penetration of the bacterial cell, and the considerable variation in these two properties in the synthetic lactones appeared to justify their examination.

Chadwick and Pascu (*J. Amer. Chem. Soc.*, 1943, **65**, 392) during the attempted resolution of *N*-α-bromopropionylglycine found that salts of this compound deteriorated on being kept and, by heating the sodium salt *in vacuo*, were able to isolate a compound which they formulated as 3 : 6-diketo-2-methylmorpholine (II; R′ = R‴ = H, R″ = Me). When this procedure was repeated using *N*-α-bromo*iso*valerylglycine, 3 : 6-*diketo*-2-iso*propylmorpholine* (II; R′ = R‴ = H, R″ = Pr^i) sublimed from the reaction mixture. This compound was soluble in hot water, in which it hydrolysed rapidly giving, on evaporation, N-α-*hydroxy*iso*valerylglycine* which could be sublimed in a high vacuum without change. In the same way, the sodium salt of *N*-α-bromo*iso*valerylsarcosine yielded 3 : 6-*diketo*-4-*methyl*-2-iso*propylmorpholine* (II; R′ = H, R″ = Pr^i, R‴ = Me), a water-soluble product which hydrolysed when warmed in aqueous solution. *N*-α-Hydroxy*iso*valerylsarcosine was obtained in this way as an oil which lost its acidic properties on being kept for 5 days at 20° or on distillation in a high vacuum, by reverting into the lactone. This reacted readily with alcoholic ammonia to give *N*-α-*hydroxy*iso*valerylsarcosine amide.*

The bromoacylamino-acids used in this preliminary work were prepared by the method of Fischer and Schenkel (*Annalen*, 1907, **354**, 13) which involved treating a salt of the amino-acid with an acid chloride in ice-cold aqueous solution in the presence of excess of alkali. This procedure was unsatisfactory with the more complex *N*-alkylamino-acids, as condensation was slow with the result that considerable hydrolysis of the acid chloride occurred at the same time. It was found convenient to shake the acid chloride with two equivalents of amino-acid suspended in dry chloroform whereupon a good yield of the desired product was obtained, and this procedure was used in all subsequent preparations. N-*Chloroacetyl*-N-*methylvaline*, obtained in this way, was converted into 2 : 5-*diketo*-4-*methyl*-3-iso*propylmorpholine* (II; R′ = Pr^i, R″ = H, R‴ = Me) by heating its sodium salt in a high vacuum. This was quickly hydrolysed, on heating it in aqueous solution, to *N*-hydroxyacetyl-*N*-methylvaline from which it was recovered after several weeks at room temperature or on distillation in a high vacuum.

None of the compounds prepared so far contained more than one asymmetric centre but the next product had two asymmetric centres and so existed as two diastereoisomers. Crude N-α-*bromo*iso*valeryl*-N-*methylvaline* was obtained from the condensation as an oil which decomposed on attempted distillation in a high vacuum. It was purified by dissolving it in sodium carbonate solution whereupon a small amount of neutral material was removed, and on acidification the bromo-acid was recovered as a colourless oil which partly crystallised after several

weeks. Repeated recrystallisation gave a pure material which was almost certainly one of the two diastereoisomers, but for the preparation of the corresponding hydroxy-acid the crude condensation product was used. This was heated under reflux with aqueous pyridine for several hours and a product was obtained which was already partly lactonised. The neutral material, which amounted to approximately 50% of the whole, was separated and heated under reflux in aqueous methanol for a further 5 hours whereupon hydrolysis occurred only to the extent of 20%; the unhydrolysed material was isolated as an oil which crystallised on being kept and recrystallisation gave 2 : 5-diketo-4-methyl-3 : 6-di*iso*propylmorpholine (II; R′ = R″ = Pr^{i}, R‴ = Me), m. p. 82°, which proved to be identical with the DL-LD-form of this compound obtained previously (Cook, Cox, and Farmer, *loc. cit.*). The acidic material obtained in the pyridine hydrolysis lactonised slowly and the lactone formed was largely the other (DD-LL) diastereoisomer as on being heated under reflux in aqueous methanol for 5 hours it was reconverted almost completely into the hydroxy-acid. This behaviour may be explained in terms of the steric configurations of the two forms of the molecule. The DL-LD-lactone has, in consequence of its definition, the configuration in which the two *iso*propyl groups are on opposite sides of the plane of the morpholine ring and in aqueous solution an equilibrium involving hydrolysis and lactonisation is established favouring the latter. The DD-LL-form of the lactone appears to be sterically hindered so that, although hydrolysis of the two forms takes place at comparable rates, lactonisation of the DD-LL-form is much slower and the equilibrium in aqueous solution favours the hydroxy-acid. It was of some interest incidentally to see whether the *amide* of N-α-*hydroxy*isov*aleryl*-N-*methylvaline* could be cyclised as easily as the free acid; the amide was prepared by the action of alcoholic ammonia on the DL-LD-diastereoisomer of the lactone but sublimed unchanged in a high vacuum.

*N-iso*Propylvaline condensed with chloracetyl chloride to form N-*chloracetyl*-N-iso*propyl-valine* which on being kept in sodium carbonate solution was converted into 2 : 5-*diketo*-3 : 4-*di*-iso*propylmorpholine* (II; R′ = R‴ = Pr^{i}, R″ = H) which hydrolysed only slowly on being heated under reflux in aqueous methanol. Crude N-α-*bromo*isov*aleryl*-N-iso*propylvaline* was prepared in the usual way but, when an attempt was made to purify it by solution in dilute sodium carbonate, approximately 50% of the material lactonised within 5 minutes to form DL-LD-2 : 5-*diketo*-3 : 4 : 6-*tri*iso*propylmorpholine* (II; R′ = R″ = R‴ = Pr^{i}) whilst the remainder was unaffected after a further hour in the alkaline solution. This difference between the two diastereoisomers must be attributed to the steric factor already discussed. The DL-LD-lactone was unaffected by boiling aqueous methanol but was hydrolysed on being left with aqueous alcoholic sodium hydroxide and the hydroxy-acid which was formed lost its acidic properties after 24 hours at 20°.

The results given above suggested that addition of alkyl groups to the diketomorpholine ring system increased its stability and also the ease of its formation, but did not indicate the way in which this effect was operating. It was therefore decided to study the result of further small variarions in structure, and lactonisation of the bromoacylamino-acids in sodium carbonate solution was selected as the most suitable reaction for investigation. It was necessary to use the crude bromo-acids obtained from the condensations as any attempt to recrystallise these would almost certainly have resulted in a partial separation of the two diastereoisomers. This crude material contained a neutral product formed during the condensation; in the case of the *N*-methylamino-acids, it was possible to remove this at the beginning of the reaction, but with the *N-iso*propylamino-acids lactonisation was too rapid for this to be done. This neutral material consisted partly of a substance which decomposed on attempted distillation in a high vacuum but it also contained some of the lactone which must have been formed by the reaction of the bromacylamino-acid with the excess of amino-acid present during the early stages of the condensation.

The results obtained in these experiments are set out in Table I in which the percentages are computed from the amounts isolated by simple extraction with ether. No claim is made that they provide information of the exactitude obtained in kinetic studies but it is nevertheless possible to reach a number of conclusions about the course of the reaction. It may be assumed that the lactonisation is an internal S_N2 reaction; if it were an S_N1 reaction with ionisation of the bromine as the rate-controlling step, this process would presumably be independent of the steric configuration of the molecule and the diastereoisomer in which lactonisation is sterically hindered should be converted into the corresponding hydroxy-acid whereas in fact the material which is not lactonised is recovered unchanged. Viewed as an S_N2 reaction, the process should involve inversion at the carbon atom concerned and, although no optically active compound has been studied in sodium carbonate solution, it has been shown (Cook, Cox, and Farmer, *loc. cit.*)

TABLE I.

Lactonisation of α-bromoacylamino-acids in two equivalents of N-*sodium carbonate solution at* 20°.

	* Lactone formed after 5 mins.	Additional lactone formed after 1 hr.	Residual acid.
N-α-Bromo-*n*-butyryl-*N*-methylvaline	15%	21%	53%
N-α-Bromo-*n*-valeryl-*N*-methylvaline	13	15	59
N-α-Bromo*iso*valeryl-*N*-methylvaline	10	6	74
N-α-Bromo-*n*-butyryl-*N*-methylnorvaline	7	11	74
N-α-Bromo-*n*-valeryl-*N*-methylnorvaline	4	9	82
N-α-Bromo*iso*valeryl-*N*-methylnorvaline	2	3	89
N-α-Bromo-*n*-butyryl-*N*-*iso*propylvaline	57	—	34
N-α-Bromo-*n*-valeryl-*N*-*iso*propylvaline.....	63	—	29
N-α-Bromo*iso*valeryl-*N*-*iso*propylvaline	53	—	39
N-α-Bromo-*n*-butyryl-*N*-*iso*propylnorvaline	53	—	40
N-α-Bromo-*n*-valeryl-*N*-*iso*propylnorvaline	57	—	36
N-α-Bromo*iso*valeryl-*N*-*iso*propylnorvaline	54	—	42

* Includes neutral impurity in starting material.

that in the similar process of heating L-α-bromo*iso*valeryl-*N*-methyl-DL-valine with pyridine and water, replacement of the bromine atom occurs with inversion and without appreciable racemisation. In this process, the bromo-acid is subject to two competing reactions, hydrolysis to *N*-α-hydroxy*iso*valeryl-*N*-methylvaline and direct lactonisation; moreover, the two products are also subject to interconversion (see above). It must therefore be concluded that removal of the bromine atom by both of these processes is accompanied by inversion and that the lactonisation of the hydroxy-acid and hydrolysis of the lactone are characterised by retention of configuration. Addition of alkyl substituents to the bromoacylamino-acid appears to affect the lactonisation in two ways; first there is an acceleration due to their electron-releasing properties which aids the separation of the bromine ion, and secondly a retardation due to steric hindrance. In the case of one diastereoisomer, the steric effect is overwhelmingly predominant but with the other, the net effect is governed by the position of the alkyl group concerned. In the amino-acid portion of the molecule, the electronic effect is more important so that valine derivatives lactonise faster than norvaline compounds, and substituted *N*-*iso*propyl-amino-acids lactonise faster that substituted *N*-methylamino-acids. In the case of the alkyl group attached to the carbon atom carrying the bromine atom, however, the steric effect becomes more important. This is easy to understand when it is remembered that an S_N2 reaction involves a transition state with five groups in the vicinity of the carbon atom under attack so that bulky constituents must be expected to cause some retardation.

As the lactonisation of the α′-hydroxyacyl-α-amino-acids involves retention of optical configuration, it must take place by a mechanism different from that discussed above. It seems unprofitable to speculate on the mechanism of this process without further experimental evidence.

Several of the lactones prepared during this work were examined for antibacterial activity in the Experimental Medicine Unit of Glaxo Laboratories Ltd. whom we thank for these facilities. Examined by the method of serial dilution in broth or serum-broth they inhibited the growth of *Staph. aureus* and *Esch. coli* only slightly even at a concentration of 1/100. *Esch. coli*, *Staph. aureus*, *B. adherans*, and *Bact. typhi* were not inhibited when streaked across agar plates prepared with the lactones in a concentration of 1/500; haemolytic *Strep.* and *Strep. pneumoniæ* were inhibited by the lactones in similar concentration on blood-agar plates. Clearly the lactones show none of the biological activity of lateritiin.

EXPERIMENTAL.

Reactions with Glycine.—*N*-α-Bromo*iso*valerylglycine (Fischer and Schenkel, *loc. cit.*) (5 g.) was dissolved in 2N-sodium hydroxide (10·5 c.c.) and evaporated to dryness *in vacuo*; the sodium salt obtained in this way was heated at 200° and 10^{-5} mm. whereupon 3 : 6-*diketo*-2-iso*propylmorpholine* (2 g.; 60%) slowly sublimed out of the reaction. It recrystallised from chloroform as colourless needles, m. p. 150° (Found : C, 53·6; H, 7·2; N, 8·9. $C_7H_{11}O_3N$ requires C, 53·5; H, 7·0; N, 8·9%). This product (1 g.) was heated in water (3 c.c.) on the steam-bath for 30 minutes and evaporated *in vacuo*; the residue was N-α-*hydroxy*iso*valerylglycine* (1·1 g.) which crystallised from water as colourless prisms, m. p. 160° (Found : C, 48·2; H, 7·4; N, 7·8. $C_7H_{13}O_4N$ requires C, 48·0; H, 7·4; N, 8·0%).

Reactions with Sarcosine.—*N*-α-Bromo*iso*valerylsarcosine (Levene, Simms, and Pfaltz, *J. Biol. Chem.*, 1926, **70**, 262) (5 g.) was dissolved in 2N-sodium hydroxide (10 c.c.) and evaporated to dryness *in vacuo*; when the residue was heated at 150° and 10^{-5} mm., 3 : 6-*diketo*-4-*methyl*-2-iso*propylmorpholine* (2·5 g., 73%) distilled from the reaction as an oil which crystallised on cooling to give colourless prisms,

m. p. 49—51° (Found : N, 8·1. $C_8H_{13}O_3N$ requires N, 8·2%). The lactone (1 g.) was heated in water (3 c.c.) on the steam-bath for 30 minutes and then diluted with ether (100 c.c.). After drying ($MgSO_4$) for 30 minutes, the solvent was removed *in vacuo* leaving an acid oil which reverted to the lactone during 5 days at 20° in an open flask. The lactone (1 g.) in methanol (10 c.c.) was added to liquid ammonia (5 c.c.) and left at 0° for 16 hours. Evaporation gave N-α-*hydroxy*isovalerylsarcosine *amide* (1·1 g.) which crystallised from methanol as colourless cubes, m. p. 139° (Found : C, 51·1; H, 8·8; N, 14·5. $C_8H_{16}O_3N_2$ requires C, 51·1; H, 8·5; N, 14·9%).

Reactions with N-*Methylvaline*.—*N*-Methylvaline (8 g.) was suspended in dry chloroform (40 c.c.) cooled in ice, and chloroacetyl chloride (3·4 g.) was added. The mixture was shaken for 1 hour during which the temperature was allowed to rise to 20°. Most of the chloroform was then removed *in vacuo* and the residue was extracted with ether (100 c.c.) and water (20 c.c.). The ether layer was washed with water (10 c.c.), dried, and evaporated to give N-*chloroacetyl*-N-*methylvaline* as a gum which crystallised from benzene as colourless flakes, m. p. 112° (yield: 4·4 g., 70%) (Found : C, 46·2; H, 6·5; N, 6·5. $C_8H_{14}O_3NCl$ requires C, 46·3; H, 6·8; N, 6·8%). This product (3 g.) was dissolved in 2N-sodium hydroxide (7·5 c.c.) and evaporated to dryness *in vacuo*; when heated at 120° and 10^{-5} mm., 2 : 5-*diketo*-4-*methyl*-3-iso*propylmorpholine* (1·8 g.; 72%) distilled as a colourless oil (Found : N, 8·1. $C_8H_{13}O_3N$ requires N, 8·2%). The lactone (1 g.) was dissolved in water (3 c.c.) and heated on the steam-bath for 30 minutes; it was then diluted with ether (100 c.c.), dried ($MgSO_4$) for 30 minutes, and evaporated *in vacuo*. *N*-Hydroxyacetyl-*N*-methylvaline was obtained as a gum which lactonised on distillation in a high vacuum or after several weeks in an open flask at 20°.

N-Methylvaline (8 g.) was suspended in dry chloroform (40 c.c.) cooled in ice, and α-bromo*iso*valeryl chloride (6·1 g.) was added. The mixture was shaken for 1 hour during which the temperature was slowly allowed to rise to 20° and then the chloroform was removed *in vacuo*. Ether (100 c.c.) and water (20 c.c.) were added and after the mixture had been well shaken the ether layer was extracted with N-sodium carbonate (50 c.c.) and washed with water (10 c.c.). The combined alkaline solution was immediately acidified and re-extracted with ether (2 × 50 c.c.) evaporation of the solvent *in vacuo* gave a colourless oil (7·2 g., 80%). After several weeks, crystallisation took place and several recrystallisations from benzene–light petroleum gave N-α-*bromo*isovaleryl-N-*methylvaline* as colourless flakes, m. p. 120° (Found : C, 45·0; H, 7·0; N, 4·5. $C_{11}H_{20}O_3NBr$ requires C, 44·9; H, 6·8; N, 4·8%). Crude *N*-α-bromo*iso*valeryl-*N*-methylvaline (8 g.) was heated under reflux with pyridine (15 c.c.) and water (20 c.c.) for 2 hours and the solution was concentrated by evaporation until most of the pyridine was removed. Ether (100 c.c.) and 2N-hydrochloric acid (20 c.c.) were added and, after the solution had been shaken, the ether layer was extracted with N-sodium carbonate (30 c.c.) followed by water (10 c.c.). The oil (2·5 g.), obtained on evaporation of the ether, was heated under reflux in methanol (10 c.c.) and water (10 c.c.) for 5 hours, and 2·3 g. of neutral material were recovered (a similar result was obtained when another sample was heated under reflux for 15 hours). This crystallised on being kept, and recrystallisation from light petroleum gave colourless cubes, m. p. 82°, which proved to be identical with the DL-LD-diastereoisomer of 2 : 5-diketo-4-methyl-3 : 6-di*iso*propylmorpholine obtained previously (Cook, Cox, and Farmer, *loc. cit.*). The sodium carbonate solution and washings were acidified and extracted with ether (2 × 100 c.c.); evaporation gave a colourless gum (2·6 g.). After 2 weeks at 20° in an open flask, this yielded 1·4 g. of neutral oil which when heated under reflux for 5 hours in aqueous methanol was hydrolysed to the extent of 90%.

DL-LD-2 : 5-Diketo-4-methyl-3 : 6-di*iso*propylmorpholine (2 g.) in methanol (15 c.c.) was added to liquid ammonia (10 c.c.) and left at 0° for 16 hours. Evaporation gave N-α-*hydroxy*iso*valeryl*-N-*methylvaline amide* (2·1 g.) which crystallised from benzene–light petroleum as colourless prisms, m. p. 120° (Found : N, 12·0. $C_{11}H_{22}O_3N_2$ requires N, 12·2%).

Reactions with N-iso*Propylvaline*.—Chloroacetyl chloride (3·5 g.) was added to *N-iso*propylvaline (10 g.) in dry chlorform (50 c.c.) and after the solution had been shaken for 1 hour the product was isolated as in the preparation of *N*-chloroacetyl-*N*-methylvaline. Recrystallisation from benzene gave N-*chloroacetyl*-N-iso*propylvaline* (6 g., 83%) as colourless prisms, m. p. 105° (Found : C, 51·3; H, 7·7; N, 6·0. $C_{10}H_{18}O_3NCl$ requires C, 51·0; H, 7·6; N, 5·9%). When this acid (2 g.) was dissolved in N-sodium carbonate (20 c.c.) and left at 20° for 1 hour, separation of the acidic and neutral products yielded unchanged starting material (1 g.) and 2 : 5-*diketo*-3 : 4-*di*iso*propylmorpholine* (0·75 g., 44%). This distilled at 100°/10^{-5} mm. and crystallised on being kept to form colourless prisms, m. p. 53° (Found : N, 7·0. $C_{10}H_{17}O_3N$ requires N, 7·0%).

*N-iso*Propylvaline (10 g.) in dry chloroform (50 c.c.) was cooled in ice and shaken with α-bromo*iso*-valeryl chloride (6·2 g.) for 1 hour, the temperature being allowed to rise slowly to 20°. Most of the chloroform was then removed *in vacuo*, and ether (100 c.c.) and water (20 c.c.) were added. The ether layer was shaken with N-sodium carbonate (50 c.c.) for 5 minutes and washed with water (10 c.c.), the sodium carbonate solution and washings being acidified and extracted with ether. The acidic product was DD-LL-N-α-*bromo*iso*valeryl*-N-iso*propylvaline* (3·2 g., 39%) which crystallised from light petroleum as colourless prisms, m. p. 126° (Found : C, 48·9; H, 7·7; N, 4·4. $C_{13}H_{24}O_3NBr$ requires C, 48·4; H, 7·5; N, 4·3%). The neutral product (3·2 g., 53%) distilled at 100°/10^{-5} mm. with partial decomposition but redistilled smoothly to give DL-LD-2 : 5-*diketo*-3 : 4 : 6-*tri*iso*propylmorpholine* as colourless prisms, m. p. 48—50° (Found : N, 5·9. $C_{13}H_{23}O_3N$ requires N, 5·8%).

Lactonisation in Sodium Carbonate Solution.—The bromo-acids were prepared by the condensation of the appropriate acid chloride and amino-acid in dry chloroform, using the procedure described above. After removal of the chloroform *in vacuo*, ether and water were added and the ether layer was shaken for 5 minutes with N-sodium carbonate solution (2 equivalents) and washed with a little water. After a further hour, the aqueous solution was re-extracted with ether, and the bromo-acid recovered by acidification. The lactone obtained in this way from *N*-α-bromo*iso*valeryl-*N*-methylvaline was substantially the DL-LD-diastereoisomer and it seems likely that all the products obtained in this way are the DL-LD-forms. The bromo-*N-iso*-propylamino-acids could be purified without difficulty but the bromo-*N*-methyl-amino-acids crystallised only after being left for several weeks. The products prepared in this way are listed in Table II.

TABLE II.

Bromo-acid.	M. p.	Found, N, %.	Reqd. N, %.	Corresponding lactone. M. p.	Found, N, %.	Reqd. N, %.
N-α-*Bromo*-n-*butyryl*-N-*methylvaline*	99—100°	4·9	5·0	40°	7·2	7·0
N-α-*Bromo*-n-*valeryl*-N-*methylvaline*	oil	—	—	oil	6·5	6·6
N-α-*Bromo*-n-*butyryl*-N-*methylnorvaline*	105	4·8	5·0	61	7·2	7·0
N-α-*Bromo*-n-*valeryl*-N-*methylnorvaline*	77	4·6	4·8	78	6·7	6·6
N-α-*Bromo*isov*aleryl*-N-*methylnorvaline*	122	4·9	4·8	45	6·8	6·6
N-α-*Bromo*-n-*butyryl*-N-iso*propylvaline*	112	4·3	4·5	43—45	6·1	6·2
N-α-*Bromo*-n-*valeryl*-N-iso*propylvaline*	96	4·0	4·3	45—46	5·7	5·8
N-α-*Bromo*-n-*butyryl*-N-iso*propylnorvaline* ...	110—112	4·4	4·5	68	6·2	6·2
N-α-*Bromo*-n-*valeryl*-N-iso*propylnorvaline* ...	107	4·2	4·3	36—38	5·9	5·8
N-α-*Bromo*isov*aleryl*-N-iso*propylnorvaline* ...	117	4·3	4·3	oil	5·7	5·8

Grateful acknowledgment is made of the award of an Australian National University Scholarship (to S. F. C.) which made participation in this work possible. We also thank Sir Ian Heilbron, D.S.O., F.R.S., for his interest and encouragement.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
S. KENSINGTON, LONDON, S.W.7.

[*Received, May 7th*, 1949.]