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ACTIVATION OF THE AMIDE GROUP BY ACYLATION

HYDROXY- AND AMINOACYL INCORPORATION IN PEPTIDE SYSTEMS*

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Abstract—A new reaction, the hydroxy- and aminoacyl incorporation in aliphatic and alicyclic N-hydroxy-(amino)acyl amides is described in detail. The reaction affording linear or cyclic peptides and depsipeptides proceeds *via* formation of cyclols. Its course depends on the nucleophilicity of the HO- and NH_2-groups, the electrophilicity of the amide carbonyl and, with cyclic amides, on the size of the ring. The cyclols which sometimes can be isolated display a number of unique properties, in particular, a tendency to undergo transformation into acylamides or macrocycles. Hydroxy- and aminoacyl incorporation is significant in the synthesis of peptides and depsipeptides. For example, it has been utilized in the synthesis of the antibiotic serratamolide and its analogues. A discussion of the biochemical and biogenetic implications of the reaction is given.

IN THE chemical and biochemical studies of peptides and proteins until recently little attention has been given to the possible activation of the usually inert, resonance stabilized amide group. However, there are many cases when this group can become reactive under very mild conditions. Such activation may play a significant part in the behaviour of peptide systems. The existence of reactions such as enzymatic hydrolysis of amides or transpeptidation clearly show that Nature has the means of greatly augmenting the reactivity of the amide group.

Very often nucleophilic reactions of amides take place under mild conditions without the presence of enzymes—for example, the reaction between the amide and hydroxy groups in phalloidine[2] and griseoviridin[3] and the amide and amino groups in bacitracin A[4] and in the polymyxin M,[5] in addition to the numerous cases of intramolecular N → O, N → S and N → N migration of acyl residues[6] which can be represented by the general scheme (1) ⇄ (2).

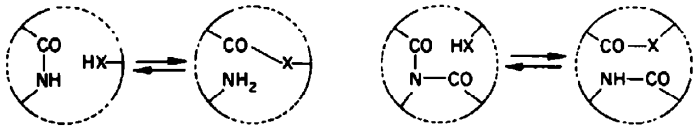
When the required steric conditions are fulfilled, the ease of such reactions depends either on activity of the attacking nucleophilic group or activation of the amide. The latter can be brought about by a lowering of the electron density of the amide carbon as for instance by O-protonation or by incorporation of an electron accepting substituent, as for example by N-acylation of the amide.

* This paper summarizes our studies on this subject earlier published as short communications or discussed in various reports (see Ref. 1).

[1] M. M. Shemyakin and V. K. Antonov, *Pure Appl. Chem.* **9**, 75 (1964).
[2] Th. Wieland, *Pure Appl. Chem.* **6**, 339 (1963).
[3] M. C. Fallona, P. de Mayo and A. Stoesse, *Canad. J. Chem.* **72**, 394 (1964).
[4] W. Hausmann, J. R. Weisiger and L. C. Craig, *J. Amer. Chem. Soc.* **77**, 723 (1955).
[5] A. B. Silaev, *Antibiotiki* **5**, No. 6, 3 (1960).
[6] L. Cohen and B. Witkop, *Angew. Chem.* **73**, 253 (1961).

It is well known that N-acylamides are very much more reactive with respect to nucleophilic reactants than ordinary amides. Thus, they readily undergo hydrolysis and aminolysis, including the intramolecular reaction.[7] Our studies have shown that in accordance with their electrophilicity the carbonyl groups of N-acylamides are comparable with those of aldehydes and ketones.

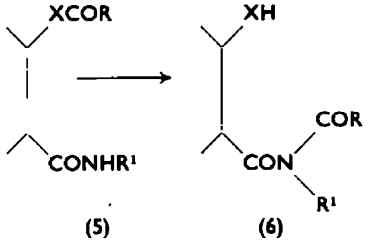
Since the intramolecular nucleophilic reactions of the N-acylamide groupings (3) → (4) are accompanied by an increase in the number of amide or ester groups in the molecule these reactions may form the basis of an entirely new approach to the synthesis of peptides and depsipeptides.



(1) (2) (3) (4)

X=O, S, NH, etc.

On the other hand, it is quite possible that N-acylation may be a means of activating amide groups in biological systems, although at present little is known about the formation of N-acylamides *in vivo*. Possible precursors of N-acylamides in the organism may be acyloxy- and acylthio peptide and protein derivatives of the type (5), wherein transfer of the acyl group to the amide nitrogen with the formation of N-acylamides (6) can take place in the case of closely associated ester and amide groups. Grounds for such an assumption is supported by the numerous facts of ester–amide interactions, leading either to imides, or to products of their further transformation.



(5) (6)

X = O or S

As early as the 1900's Titerley *et al.*[8–11] and Auwers[12] showed that O-acyl derivatives of salycylylamide are prone to isomerize to N-acyl derivatives. This reaction was later extended by Brenner[13] to include O-aminoacyl derivatives of salicylylamides. It is well known that imide formation takes place in peptides containing glutamic and aspartic acid residues.[14] Intermediate formation of N-acylamides apparently takes

[7] Th. Wieland and H. Urbach, *Liebigs Ann.* **613**, 84 (1958).
[8] A. W. Titerley and J. McConnan, *J. Chem. Soc.* 1207 (1905).
[9] J. McConnan and A. W. Titerley, *J. Chem. Soc.* 1318 (1906).
[10] A. W. Titerley and W. L. Hicks, *J. Chem. Soc.* 908 (1909).
[11] A. W. Titerley, *J. Chem. Soc.* 1419 (1906).
[12] K. Auwers, *Ber. Dtsch. Chem. Ges.* **38**, 3256 (1905); *Ibid.* **40**, 3506 (1907).
[13] M. Brenner, *CIBA Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity*, p. 157. London (1958).
[14] E. Sondheimer and R. W. Holley, *Nature, Lond.* **173**, 773 (1954).

place in the S → N and O → N migrations of the acyl groups investigated by Wieland *et al.*[15] and by Botvinik *et al.*[16,17] Finally, in connection with the possibility of N-acylamides formation in biological systems, mention must be made of the enzymatic synthesis of N-carboxybiotin[18] and also of the existence of such active biological acylating agents as thioesters such as coenzyme A and mixed anhydrides with phosphoric acid derivatives. All these considerations give a sound backing to the assumption that N-acylamides probably play an active part in biochemical processes.

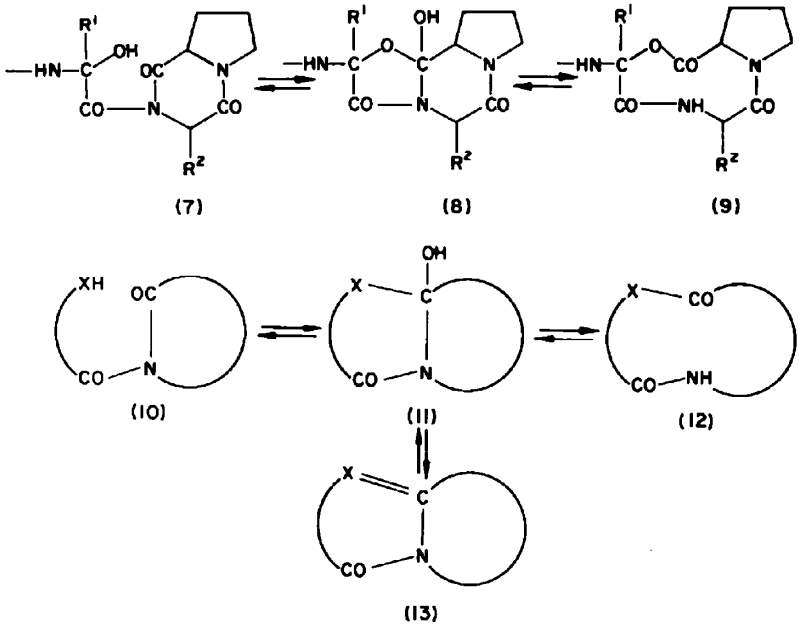
The appearance of N-acylamide groups in peptide systems may cause fundamental changes in the structure of the latter by virtue of intramolecular interactions of these groups with various nucleophilic groups. The possibility of such isomeric or tautomeric interconversions among N-hydroxyacylamides, cyclols and depsipeptides (7) ⇄ (8) ⇄ (9) was mentioned for the first time in 1960 at the Basel Peptide Symposium on the problem of synthesizing the ergot alkaloid peptide moiety.[19] Similar reasoning later served as the basis for the synthesis of ergotamine by Hofmann *et al.*[20]

In the course of our studies, we found that these conversions are of a more universal character and represent a new general reaction for incorporating hydroxy-, amino- and mercapto acids* into the peptide chain or ring with the formation of linear or cyclic peptides, depsipeptides or thiodepsipeptides (10) → (11) → (12). The course of the reaction depends basically on the following factors: the electrophilicity and steric accessibility of the acylamide carbonyls, the nucleophilicity of the attacking group XH and the energetic preference for the peptide or depsipeptide structure (12) as compared with the initial acylamide (10) or intermediate cyclol (11). In some cases, for reasons that will be discussed below, the incorporation reaction may stop at the stage of cyclol formation (11); in the case of azacyclols (11, X = NH), the latter, spontaneously eliminating water, may undergo conversion to the more stable acylamidines (13).

We have investigated the hydroxy- and aminoacyl incorporation reaction in a large number of linear and cyclic amides, peptides and depsipeptides, N-acylated with various amino and hydroxy acid residues. The initial N-acylamides may be readily prepared by acylation of the amides with acyl chlorides containing protected hydroxy or amino groups (compds 14–23). The reaction is carried out either by heating in an inert solvent (compds 14–16, 17a–c, 20–23) or in the cold in the presence of triethylamine (compds 17d,e, 18,19). When incorporating α- and β-hydroxyacyl residues the hydroxyl is protected by a benzyl group, whereas on incorporating a β-aminoacyl residue benzyloxycarbonyl protection is employed. For incorporating α-aminoacyl residues it is feasible to employ α-azidoacyl chlorides the latter being sufficiently stable under the acylating conditions and yet readily transformable into hydrobromides of the aminoacyl derivatives by reaction with hydrogen bromide in glacial acetic acid.

* Regarding the possibility of mercapto acid incorporation, see Ref. 21.

[15] Th. Wieland, H. U. Lang, E. Bokelman, H. Lan and L. Bauer, *Liebigs Ann.* **583**, 129 (1953).
[16] M. M. Botvinik and L. M. Koksharova, *Zh. Obshch. Khim.* **31**, 2078 (1961).
[17] M. M. Botvinik, A. P. Andreeva and L. M. Koksharova, *Zh. Vsesoyuzn. Khim. Obshchestva im. Mendeleeva* **7**, 359 (1962).
[18] J. Knappe, K. Biederbick and W. Brümmer, *Angew. Chem.* **74**, 432 (1962).
[19] V. K. Antonov, M. M. Shemyakin and G. A. Ravdel, *Chimia* **14**, 374 (1960).
[20] A. Hofmann, A. J. Frey and H. Ott, *Experientia* **17**, 206 (1961).
[21] G. E. Utzinger, L. A. Strait and L. D. Tuck, *Experientia* **19**, 324 (1963).



N-Acylamides are mostly crystalline compounds, stable in inert solvents, but easily hydrolysable in the presence of acids and bases.

A study of the IR spectra, of the acylamides, acylpeptides and acyldepsipeptides synthesized, showed that the N-acylamide group usually has a single absorption band in the 1725–1700 cm^{-1} region (Table 1). An exception to this rule is the N-acyl derivatives of butyrolactam (16a, 17a, d, 18a and 19a) where the non-equivalency of

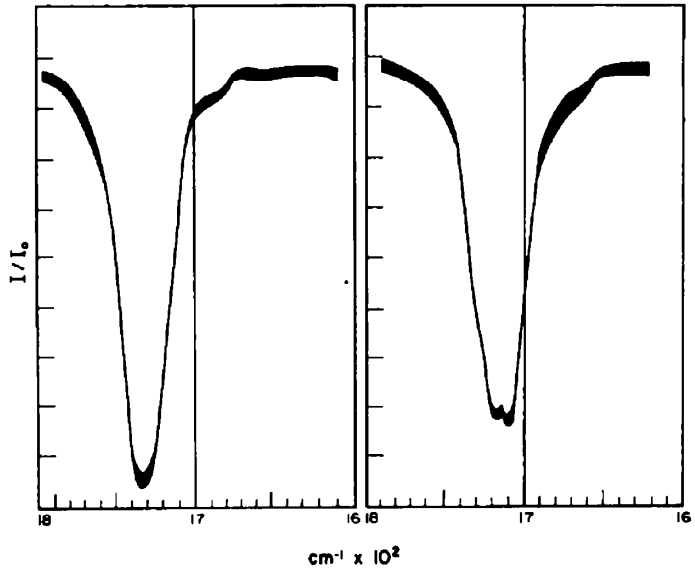


FIG. 1. CO region of the IR spectra of cyclol (88a) and N-methoxyacetylvalerolactam (89a) in tetrahydrofuran.

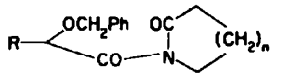
the carbonyls apparently due to the Bayer strain in the 5-membered ring causes splitting of the $(—CO)_2N$ band. When the spectra are taken under special conditions one may sometimes observe splitting of the $(—CO)_2N$ band also with other N-acylamides, as we have shown, for instance, in the case of N-methoxyacetylvalerolactam (89a) (Fig. 1). Band splitting, also observed among aroyllactams (19b,c), is ascribed to conjugation of one of the carbonyls with the aromatic ring. In such cases the low frequency band corresponds to the exocyclic carbonyl, as can be seen from the bathochromic shift of this band in N-salycylylbutyrolactam (31c) as compared with its methyl ether (89b) (1647 and 1672 cm^{-1} respectively, in CCl_4 solution).

The UV spectra of acylamides with an isolated $(—CO)_2N$ chromophore display

$PhCH_2O(CH_2)_nCON(R^1)COR^2$

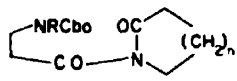
(14)

a: $R^1 = R^2 = CH_3$, $n = 1$; *b*: $R^1 = CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$, $n = 1$; *c*: $R^1 = CH_2CO_2CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$, $n = 1$; *d*: $R^1 = CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$, $n = 2$; *e*: $R^1 = CH_2CO_2CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$, $n = 2$



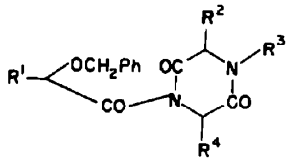
(16)

a: R = H, n = 1; *b*: R = H, n = 2; *c*: R = H, n = 3; *d*: R = H, n = 9; *e*: R = CH_3, n = 2



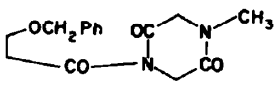
(18)

a: R = H, n = 1; *b*: R = H, n = 2; *c*: R = H, n = 3; *d*: R = CH_3, n = 3



(20)*

a: $R^1 = R^2 = R^4 = H$, $R^3 = CH_3$; *b*: $R^1 = R^3 = CH_3$, $R^2 = R^4 = H$; *c*: $R^1 = R^4 = H$, $R^2 + R^3 = (CH_2)_3(L)$; *d*: $R^1 = H$, $R^2 + R^3 = (CH_2)_3(L)$, $R^4 = CH_2CH(CH_3)_2(L)$; *e*: $R^1 = CH_3(L)$, $R^2 + R^3 = (CH_2)_3(L)$, $R^4 = CH_2Ph(L)$; *f*: $R^1 = R^2 = R^4 = H$, $R^3 = COCH_2OCH_2Ph$



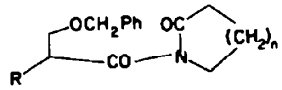
(22)

$C_6H_4(CO)_2NCH_2CONCH(R^1)COOC_2H_5$

$COCH(R^2)N_3$

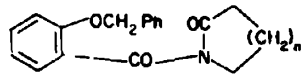
(15)*

a: $R^1 = R^2 = H$; *b*: $R^1 = CH_2CH(CH_3)_2(L)$, $R^2 = H$; *c*: $R^1 = H$, $R^2 = CH_3$



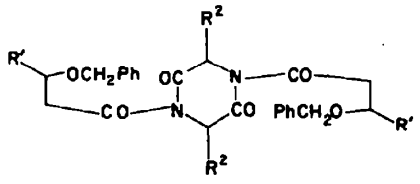
(17)

a: R = H, n = 1; *b*: R = H, n = 2; *c*: R = H, n = 3; *d*: R = $N(CO)_2C_6H_4$, n = 1; *e*: R = $N(CO)_2C_6H_4$, n = 3



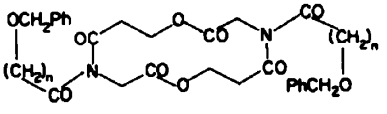
(19)

a: n = 1; *b*: n = 2; *c*: n = 3



(21)*

a: $R^1 = R^2 = H$; *b*: $R^1 = CH_3$, $R^2 = H$; *c*: $R^1 = H$, $R^2 = CH_3(L)$; *d*: $R^1 = H$, $R^2 = CH_2OAc(L)$; *e*: $R^1 = (CH_2)_6CH_2(D)$, $R^2 = CH_2OAc(L)$; *f*: $R^1 = H$, $R^2 = CH(CH_3)_2$(L and D); *g*: $R^1 = H$, $R^2 = CH_2OCHO(L)$; *h*: $R^1 = H$, $R^2 = CH_2OCH_2Ph(L)$; *i*: $R^1 = (CH_2)_6CH_2(D)$, $R^2 = CH_2OCH_2Ph(L)$



(23)

a: n = 1; *b*: n = 2

* The configuration given here refers to the asymmetric C atom which is attached to the corresponding substituent R; in all other cases the racemates were used.

TABLE 1. PROTECTED HYDROXY- AND AMINOACYLAMIDES (14)–(24)

Compound	Method of synthesis*	Yield %	Solv. used for cryst.	M.p. or b.p. °C/mm	n_D^{20}	$[\alpha]_D^{20}$ (c1, THF)	IR band position (cm^{-1}) (nujol) (—CO)$_2$N†	IR band position (cm^{-1}) (nujol) CO-amide	$\lambda_{max}^{dioxane}$ (mμ)	ε
14a	1-A	38	Et_2O	71			1710			
14b	1-B	69	C_6H_6	146			1775, 1708			
14c	1-B	40	CCl_4	120			1772, 1725, 1702			
14d	1-B	64	C_6H_6	109			1780, 1719			
14e	1-B	68	EtOH	117			1774, 1725			
15a	1-E	46	EtOH	116			1780, 1730			
15b	1-E	36	EtOH	121		−22°	1770, 1720			
15c	1-E	58	EtOH	104			1780, 1735, 1725			
16a	1-A	85	AcOEt	108			1728, 1713		215	15800
16b	1-A	72	Et_2O	41			1698		216	11300
16c	1-A	64		165/0·1	1·5407		1708 (film)		221	11700
16d	1-A	60	C_6H_{14}	55			1698		219	9700
16e	1-A	63		150/0·06	1.5327		1696 (film)		219	10700
17a	1-A	57		167/0·08	1·5391		1752, 1698 (film)		216	13400
17b	1-A	60		165/0·06	1·5387		1698 (film)		214	11100
17c	1-A	52		176/0·04	1·5334		1701 (film)		221	11700
17d	1-D	41	$CHCl_3$–pet. eth.	125			1780, 1750, 1720, 1700			
17e	1-D	50	Me_2CO–pet. eth.–Et_2O	130			1785, 1730, 1685			
18a	1-F	58	AcOEt	95			1740, 1715	1678		
18b	1-F	68	Et_2O	60			1720	1680		
18c	1-F	50	Et_2O	61			1720	1685		
18d	1-F	71		oil			1715	1695		
19a	1-D	73	AcOEt	107			1755, 1685		212, 294	31100, 2200
19b	1-D	33	AcOEt	117			1691, 1667		212, 300	28000, 2450

19c	1-D	74	AcOEt–C_6H_{12}	87		1718, 1675		209, 283 inf.	28000, 2250
20a	1-A	85	AcOEt	133		1711	1668	213	10800
20b	1-A	78	C_6H_{12}	100		1697	1661		
20c	1-A	83	AcOEt	150	−43·6°	1725	1647	214	11500
20d	1-A	74	C_6H_6	132	+103°	1718	1666		
20e	1-A	40	Et_2O	135	−145°	1720	1665		
20f	1-C	75	$C_6H_5CH_3$	176		1709			
21a‡	1-C	69	C_6H_{12}–AcOEt	106		1710			
22	1-A	96	Et_2O	74		1725	1680		
23a‡	1-C	90	CCl_4–C_6H_6	149		1720§			
24a	2	40	AcOEt	132			1630§	218, 285	37300, 7500
24b	2	96	AcOEt	141			1646§	228, 260 inf.	22100, 7550

* The number given here and in the following Tables refers to the number of the synthesis described in the experimental part.

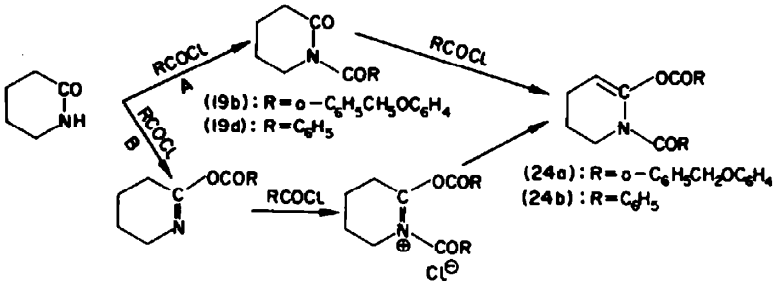
† With compounds containing the phthalyl group the low frequency band of the phthalyl CO usually coincides with the acylamide $(-CO)_2N$ band.

‡ Other compounds of this type are non-crystallizing oils and have not been specially purified.

§ Other bands: (23a)–1756(CO-est.); (24a)–1715(CO-est.), 1685(C=C); (24b)–1712(CO-est.), 1686(C=C).

a characteristic maximum in the region of 215–220 mμ, whereas N-aroyllactams (19) give a more involved picture because of conjugation of the exocyclic carbonyl with the aromatic ring.

It is noteworthy that on acylating valerolactam with excess O-benzyloxybenzoyl chloride we found besides the normal N-acylation product (Compd. 19b) also the N,O-bisacyl derivative (24a).



An analogous compound (24b) is formed in the benzoylation of valerolactam under similar conditions.* The structure of these compounds follows from their IR and NMR spectra (Table 1 and Fig. 2) as well as from their behaviour towards acidolysis, amidolysis and hydrogenolysis.

The formation of (24) apparently proceeds *via* the enol form of the acyllactam (route A), although one cannot exclude the possibility of another mechanism for this reaction (route B).

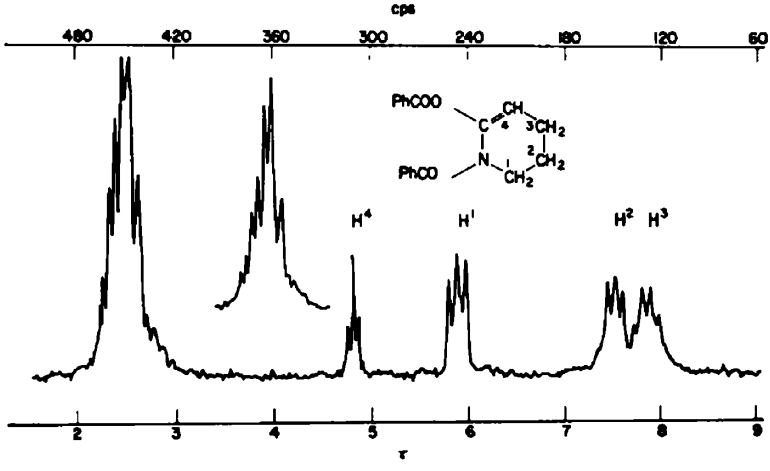


FIG. 2. NMR spectrum of N,O-dibenzoylvalerolactam (24b) in $CDCl_3$.

Returning to the hydroxy- and aminoacyl incorporation reaction, removal of the O- or N-protective groups can be conveniently accomplished by hydrogenolysis over Pd in tetrahydrofuran or alcohol solution. Sometimes aminoacylamides can be

* Compound (24b) was apparently isolated earlier by Hall *et al.*,[23] but erroneously taken to be N-benzoylvalerolactam (19d) (See Ref. 23).

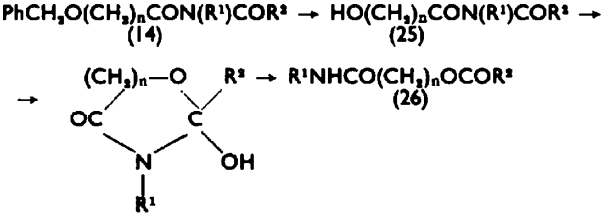
[23] H. K. Hall, M. K. Brandt and R. M. Mason, *J. Amer. Chem. Soc.* **80**, 6420 (1958).
[23] C. M. Lee and W. D. Kumler, *J. Amer. Chem. Soc.* **84**, 565 (1962).

obtained by treating the initial compounds with hydrogen bromide in glacial acetic acid and subsequent decomposition of the resulting hydrobromide with triethylamine in tetrahydrofuran.

The incorporation of hydroxy- and aminoacyl residues, in most cases occurs spontaneously and only sometimes requires the use of heat or the presence of a base.

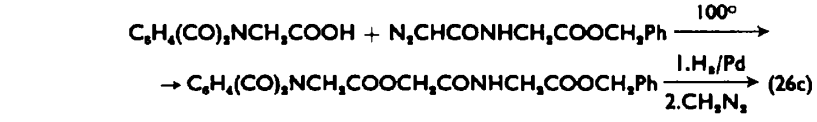
The structure of the linear or cyclic depsipeptides and peptides resulting from this reaction was proved by means of their IR, UV, NMR and mass spectra, and in some cases by counter synthesis. Since the incorporation reaction leads to the formation of an ester and an amide bond or of two amide bonds, IR spectroscopy provides a convenient structural proof as it gives the means for demonstrating the formation of both ester and amide groups, the latter by means of the amide I and amide II bands, of which the second band may be reliably identified by deuterio-exchange. In some cases it may prove highly advantageous to determine the number of amide groups in the compound by means of the integral intensity of the amide carbonyl band.

Linear hydroxyacylamides of type (25) isomerize to the corresponding depsipeptides (26) with varying ease, depending on the nature of the acyl residue in the initial amide or peptide (14). Thus N-glycolyl-N-methylacetamide (25a) spontaneously isomerizes to the amidoester (26a) immediately following hydrogenolysis of the initial benzyloxy derivative (14a), whereas formation of the depsipeptides (26b–e) containing a phthalyl residue takes place only on heating the alcoholic solutions or in the presence of a base. This difference is apparently due to the electrophilicity of the phthalyl residue, hindering the incorporation reaction (Table 2).



a: $R^1 = R^2 = CH_3$, $n = 1$; *b*: $R^1 = CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$, $n = 1$;
c: $R^1 = CH_2CO_2CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$, $n = 1$;
d: $R^1 = CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$, $n = 2$;
e: $R^1 = CH_2CO_2CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$, $n = 2$

The structure of the linear depsipeptides (26) synthesized follows from their IR spectra (Table 2) and in the case of compounds (26a) and (26c) this is supported by synthesis, which, for instance for compound (26c) was carried out according to the following scheme:



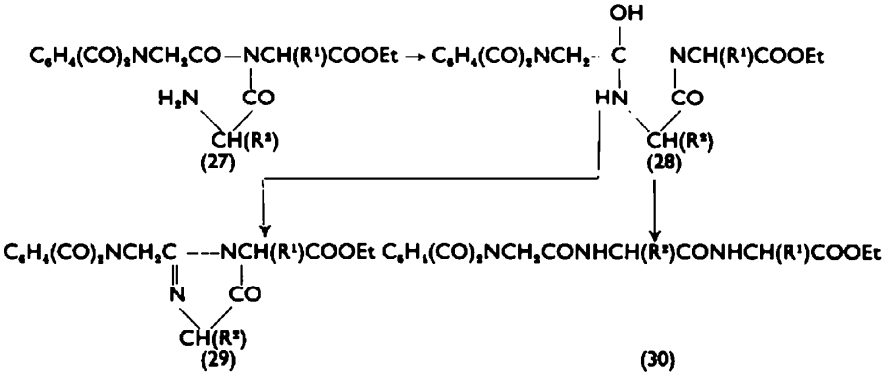
Aminoacyl incorporation into the peptide chain was undertaken with the N-α-aminoacylphthalyldipeptide esters (27) isolated as the hydrobromides. Here besides normal peptidic incorporation products (30), simultaneous formation of imidazolinones of type (29) took place (Table 3). The latter owe their origin to dehydration of the intermediate azacyclols (28). It was shown that the direction of splitting of the

TABLE 2. LINEAR HYDROXYACYLAMIDES (25) AND THEIR REARRANGEMENT PRODUCTS (26)

Compound	Method of synthesis	Yield %	Solv. used for cryst.	M.p. °C	IR band position (cm^{-1}) (nujol)				
					(—CO)$_2$N*	CO-ester	Amide I	Amide II	OH or NH
25b	3-B	90	C_6H_6	131	1778, 1731, 1692				3100
25c	3-B	100	C_6H_6–AcOEt	118	1775, 1727, 1717	1745			3450
25d	3-B	85	C_6H_6	116	1769, 1709				3540, 3580
25e	3-B	95		oil†					
26a	3-A	30		39		1755	1675	1560	3100, 3300
26b	3-B	65	Me_2CO–Et_2O	150	1778, 1725	1751	1652	1569	3100, 3330
26c	3-B	80	AcOEt	131	1778 inf., 1725	1762	1702	1547	3390
26d	3-B	70	EtOH	194	1778, 1722	1749	1665	1575	3120, 3320
26e	3-B	70	AcOEt	172	1777, 1727	1743	1647	1566	3100, 3320

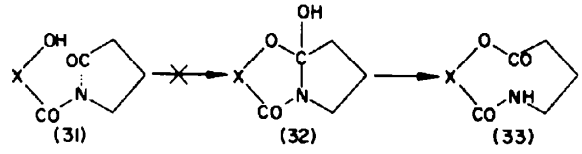
* Values refer to the absorption bands of both the phthalyl and acylamide groups.
† This compound could not be purified because of its isomerization to (26e).

latter greatly depends on the conditions. If the hydrobromide of aminoacyldipeptide (27) is treated with triethylamine in tetrahydrofuran, the tripeptide (30) largely forms. However, if the same hydrobromide is dissolved in water, instead of the tripeptide the insoluble imidazolinone (29) forms spontaneously, the solution acquiring a strongly acid reaction (pH $\sim$ 1)*.



a: $R^1 = R^2 = H$; *b*: $R^1 = CH_2CH(CH_3)_2$(L), $R^2 = H$; *c*: $R^1 = H$, $R^2 = CH_3$

These factors which are responsible for the incorporation reaction (see p. 3539) are especially important when the incorporation occurs in cyclic amides: lactams, diketopiperazines and cyclodepsipeptides. Thus the reduced electrophilicity of the carbonyl characteristic of five-membered rings is demonstrated here by the fact that no spontaneous isomerization of the hydroxyacylbutyrolactams (31) (Table 4) to the corresponding cyclols (32) or macrocycles (33) occurs and only compound (31c) can under certain conditions be converted into the cyclol derivative (95a) (see p. 43).



a: $X = CH_2$; *b*: $X = (CH_2)_2$; *c*: $X = o\text{—}C_6H_4$; *d*: $X = C_6H_4(CO)_2NCHCH_2$—

However, incorporation in a five-membered ring readily takes place in the case of N-(β-alanyl)-butyrolactam (53, n = 1), which contains a more nucleophilic amino group (see p. 3554).

Further, it was observed that hydroxy- and aminoacyl incorporation into cyclic amides takes place with sufficient ease only when the size of the resultant ring for each type of compound exceeds a certain critical value. If the ring size is below this critical value transannular interaction of the amide group with an ester or another amide group takes place, so that the macrocyclic structure becomes energetically less preferable than the corresponding cyclolic structure. Indeed, hydroxy acid incorporation into lactams, diketopiperazines and cyclodepsipeptides occurs without exception only when the resultant cyclodepsipeptides contain 11 or more atoms in the ring; in

* Similar formation of imidazolinones has been observed before.[34]

[34] M. Brenner, J. P. Zimmermann, J. Wehrmüller, P. Quitt, A. Hartmann, W. Schneider and U. Beglinger, *Helv. Chim. Acta* **40**, 1497 (1957).

TABLE 3. IMIDAZOLINONES (29) AND TRIPEPTIDES (30)

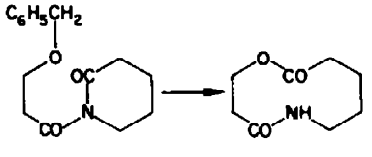
Compound	Method of synthesis	Yield %	Solv. used for cryst.	M.p. °C	$[\alpha]_D^{24}$	IR band position (cm^{-1}) (nujol)					
						$(—CO)_2N$ phthalyl	CO-est.	Amide I	Amide II	C=N	NH
29a	4-B	73	C_6H_6–pet. eth.	154		1776, 1730	1755	1720		1650	
29b	4-B	63	C_6H_6	118	−6·3° *c*1, C_6H_6	1780, 1730	1745	1720		1643	
30a	4-A	69	EtOH	229		1775, 1730	1730	1667, 1645	1560		3300, 3100
30b	4-A	70	EtOH	156	+7·1° *c*3, $CHCl_3$	1778, 1729	1729	1670, 1650	1550		3320, 3080
30c	4-A	56	EtOH	231		1775, 1725	1725	1648	1555		3300, 3080

TABLE 4. HYDROXYACYLBUTYROLACTAMS (31)

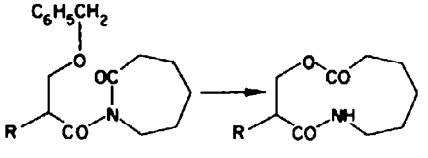
Compound	Method of synthesis	Yield %	Solv. used for cryst.	M.p. °C	IR band position (cm^{-1}) (nujol)		$\lambda_{max}^{dioxane}$ (mμ)	ε
					$(—CO)_2N$	OH		
31a	5	93	AcOEt	69	1726, 1689	3480	220	7250
31b	5	50		oil	1740, 1695	3420	219	8900
31c	5	80	AcOEt	121	1765, 1722, 1670, 1640	3300	210, 225, 302*	18700, 12500, 2300
	11	18						
31d	5	57	$CHCl_3$–Et_2O	172	1780, 1735, 1715	3510		

* In C_2H_5OH.

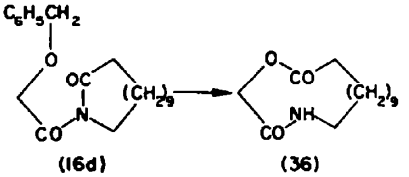
certain cases 10-membered cyclodepsipeptides can also form, but the 9-membered compounds of this series do not form.[25]



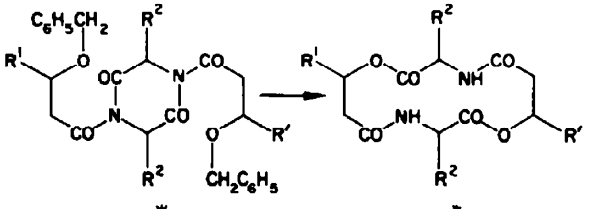
(17b) (34)



(17c): R = H (35a): R = H
(17e): R = N(CO)$_2$C$_6$H$_4$ (35b): R = N(CO)$_2$C$_6$H$_4$



(16d) (36)



(21)* (37)*

a: $R^1 = R^2 = H$; *b*: $R^1 = CH_3$, $R^2 = H$;
c: $R^1 = H$, $R^2 = CH_3$(L);
d: $R^1 = H$, $R^2 = CH_2OAc$(L);
e: $R^1 = (CH_2)_6CH_2$(D), $R^2 = CH_2OAc$(L);
f: $R^1 = H$, $R^2 = CH(CH_3)_2$(L and D);
g: $R^1 = H$, $R^2 = CH_2OCHO$(L)

a: $R^1 = R^2 = H$; *b*: $R^1 = CH_3$, $R^2 = H$;
c: $R^1 = H$, $R^2 = CH_3$(L);
d: $R^1 = H$, $R^2 = CH_2OAc$(L);
e: $R^1 = (CH_2)_6CH_2$(D), $R^2 = CH_2OAc$(L);
f: $R^1 = H$, $R^2 = CH(CH_3)_2$(L and D);
g: $R^1 = H$, $R^2 = CH_2OCHO$(L);
h: $R^1 = (CH_2)_6CH_2$(D), $R^2 = CH_2OH$(L);
i: $R^1 = H$, $R^2 = CH_2OH$(L)

For instance, after hydrogenolysis of N-benzyloxyacyl derivatives of lactams (17b,c,e) and (16d) the corresponding 10-, 11- and 16-membered cyclodepsipeptides (34), (35a,b) and (36)† form spontaneously (Table 5), and hydrogenolysis of bis-(β-benzyloxyacyl)-derivatives of diketopiperazines (21a–g) leads to the formation of the 14-membered cyclodepsipeptides (37a–g) built up of two β-hydroxy acid and two α-amino acid residues.[25–27]

The above described two stage synthesis of cyclotetradepsipeptides (37) which starts with the corresponding diketopiperazines and proceeds through their bis-(β-benzyloxyacyl) derivatives (21) was found to be very convenient because of the small

* See footnote on p. 3541.

† This compound possesses a faint musk odour characteristic of macrocyclic carbonyl compounds of such ring size. The existence of an odour and the volatility is in accord with the absence of hydrogen bonding in the crystals of this compound following from the position of the NH band (3400 cm^{-1}).

[25] V. K. Antonov, A. M. Shkrob, V. I. Shchelokov, M. M. Shemyakin, *Tetrahedron Letters* No. 21, 1353 (1963).
[26] V. K. Antonov, M. M. Shemyakin and A. M. Shkrob, *Tetrahedron Letters* No. 7, 439 (1963).
[27] V. K. Antonov, V. I. Shchelokov and M. M. Shemyakin, *Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk* 1145 (1963).

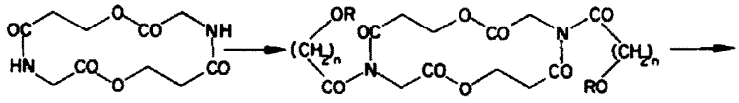
TABLE 5. CYCLODEPSIPEPTIDES (34)–(36)

Compound	Method of synthesis	Yield %	Solv. used for cryst.	M.p. °C	IR band position (cm^{-1})				
					Conditions	CO–ester	Amide I	Amide II	NH
34	6-A	98	CCl_4	110	nujol	1726	1658 w, 1635		3020, 3300
					THF(c1)	1744	1690	1546	3350
					CCl_4(c0·1)	1729	1692, 1672 w	Solv. abs.	3080, 3340 inf., 3400 w, 3450
					CCl_4(c1)	1730	1690, 1670	1525	3080, 3340, 3400 w, 3450 w
35a	6-A	97	EtOH	142	nujol	1729	1645	1568	3100, 3300
					THF(c0·3)	1747	1685	1556	3360
					CCl_4(c0·1)	1748	1695	Solv. abs.	3370 w, 3450
35b	6-B	30	$C_2H_4Cl_2$	226	nujol	1730 br.	1670	1565	3100, 3320
36	6-A	100	C_6H_{14}–Et_2O	98	nujol	1730	1680, 1665	1540	3400
					THF(c0·5)	1751	1690	1537	3350
					CCl_4(c0·3)	1755	1695	1540	3450

* ν_{max}(—CO)$_2$N phthalyl: 1780, 1730 cm^{-1}.

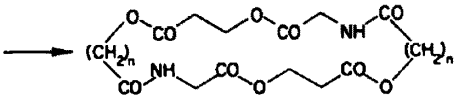
number of stages, the absence of racemization of the amino and hydroxy acid residues and the relatively high yields (Table 6). For example in this way we were able to prepare easily the diacetyl derivative of serratamolide (37e)[28] which by mild hydrolysis was transformed into the antibiotic itself (37h),[29] which was recently isolated from a *Serratia marcescens* culture.[30,31] Hydrolysis of (37g) afforded the serratamolide analogue (37i).

A second bis-acylation of the 14-membered cyclodepsipeptide (37a), followed by hydrogenolysis of the products (23a, b) allowed the bis-(N-hydroxyacyl)-derivatives (38a, b) to be isolated. The latter on heating or prolonged standing in dimethylformamide are isomerized to 20- and 22-membered cyclodepsipeptides (39a, b) (Table 6).



(37a)

(23a): R=CH_2Ph, n=1
(23b): R=CH_2Ph, n=2
(38a): R=H, n=1
(38b): R=H, n=2



(39a): n=1
(39b): n=2

The rings can be enlarged still more by further incorporation of hydroxy- and amino acid residues.

It also turned out that incorporation can take place in N-β-hydroxyalkylimides of the type (40b) providing the imide ring is sufficiently large. Such incorporation leads to the formation of lactonolactams of the type (41).*

It should be pointed out that the reaction may take a quite different course when the N-hydroxyacylamide molecule possesses additional nucleophilic groups. Thus in the hydrogenolysis of the tetrabenzyl derivatives of bis-(β-hydroxyacyl)-serylseryllactams (21h, i) the acyl residue is transferred to the hydroxy group of serine (21) → (42) → (43). A similar reaction takes place in the case of N,N-bisacetyldiketopiperazine (44) to give compound (45).

* According to Griot and Frey[28] and our own data the derivative of glutarimide (40c) does not undergo the incorporation reaction. However, Glover and Rapoport,[28] found that incorporation takes place in the case of β-amino-ethylglutarimide. It is sufficiently clear that possibility of hydroxy- and amino-alkyl incorporation depends on both the nucleophilicity of the OH and NH_2 groups and the ring size, determining the configuration of the $(—CO)_2N$ group.

[28] M. M. Shemyakin, Yu. A. Ovchinnikov, V. K. Antonov, A. A. Kiryushkin, V. T. Ivanov, V. I. Shchelokov and A. M. Shkrob, *Izv. Akad. Nauk SSSR, Otdel. Khim. Nauk* 2233 (1963); *Tetrahedron Letters* No. 1, 47 (1964).

[29] V. K. Antonov, V. I. Shchelokov, M. M. Shemyakin, I. I. Tovarova and O. A. Kiseleva, *Antibiotiki* No. 5, 387 (1965).

[30] A. J. Castro, A. H. Corwin, F. J. Waxham and A. L. Beilby, *J. Org. Chem.* **24**, 455 (1959).

[31] H. H. Wasserman, J. J. Keggi and J. E. McKeon, *J. Amer. Chem. Soc.* **84**, 2978 (1962).

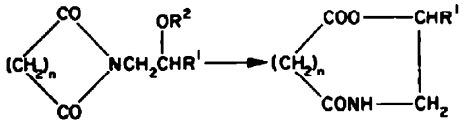
[32] R. G. Griot and A. J. Frey, *Tetrahedron* **19**, 1661 (1963).

[33] G. I. Glover and H. Rapoport, *J. Amer. Chem. Soc.* **86**, 3398 (1964).

TABLE 6. CYCLODEPSIPEPTIDES (37)–(39), LACTONO-LACTAM (41) AND DIACYLDIKETOPIPERAZINES (43) AND (45)

Compound	Method of synthesis	Yield %	Solv. used for cryst.	M.p. °C	$[\alpha]_D^{20}$	IR band position (cm^{-1}) (nujol)			
						CO–ester	Amide I	Amide II	OH and NH
37a	6-C	99	H_2O	258		1743	1657	1562	3295, 3090
37b	6-C	24	DMF	232		1740	1650	1558	3325, 3090
37c	6-C	80	80%–MeOH	251	−120°(*c*1 in 85% HCOOH)	1738	1657	1548	3315, 3285, 3087
37d	6-C	31	EtOH	242	−47°(*c*0·14 in 85% HCOOH)	1746	1661	1559	3300, 3100
37e	6-C	27	EtOH	223	+8°(*c*1, $CHCl_3$)	1739	1659	1562	3330, 3095
37f	6-C	10	MeOH	266		1741	1648	1562	3285, 3090
37g	6-C	6	DMF–EtOH	232		1738, 1725	1655	1561	3300, 3100
37i	6-D	96		237		1737	1656	1560	3615, 3560, 3295 3100
38a	6-E	97	DMF–MeOH	151		1725*			3490
38b	6-E	70	DMF	174		1739*			3455
39a	6-E	91	MeOH	189		1742	1693	1535	3435
39b	6-E	100	MeOH	229		1735	1646	1554	3320, 3080
41	6-G	65	CCl_4–pet. eth.	65		1726, 1712†	1658	1566	3300, 3100
43a	6-F	45	EtOH	165		1755	1687		3330, 3200, 3090
43b	6-F	50	EtOH	172	−22°(*c*0·8, AcOH)	1750	1685		3320, 3210, 3100
45	6-F	86	H_2O	230	−70°(*c*0·9, AcOH)	1760	1685		3300, 3200, 3080

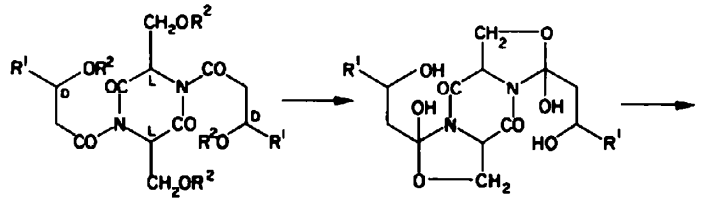
* ν_{max}(—CO)$_2$N group of compd. (38a): 1722 and for compd. (38b): 1715 cm^{-1}.
† In CCl_4 (c = 1) ν_{max}: 1742, 1690, 1515 cm^{-1}.



(40a): $R^1 = H$, $R^2 = CH_2Ph$, $n = 17$
(40b): $R^1 = R^2 = H$, $n = 17$
(40c): $R^1 = CH_3$, $R^2 = H$, $n = 3$

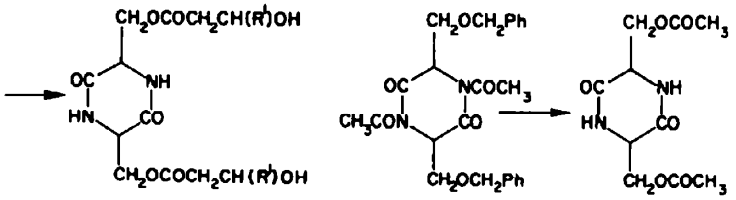
(41): $R^1 = H$, $n = 17$

Moderate sized (9- and 10-membered) cyclopeptides and cyclodepsipeptides deserve special consideration because of their specific behaviour, due to transannular interaction of two amide groups or an ester and amide group. Such interaction in the 10-membered cyclodepsipeptide (34) is manifested for instance in a bathochromic shift of the ester band in the IR spectra of the carbon tetrachloride solution, relative to its position in the spectrum of the 11-membered cyclodepsipeptide (35a) (Table 5).



(21h): $R^1 = H$, $R^2 = CH_2Ph$
(21i): $R^1 = (CH_2)_6CH_3$

(42)



(43a): $R^1 = H$
(43b): $R^1 = (CH_2)_6CH_3$

(44) (45)

Since according to IR data the cyclodepsipeptide (34) tends to associate in solution it might have been inferred that the ester band shift is due to intermolecular hydrogen bonding. However, it turned out that such bonding occurs with participation of the amide rather than ester carbonyl, as can be seen from the splitting of the amide I band of compound (34) in CCl_4 solution with increase in concentration, and a parallel reduction in intensity of the free-NH band and increase in intensity of the bound-NH band. Moreover, the ester band undergoes a shift in polar solvents regardless of whether they are proton donors or acceptors. All this shows that in compound (34) the bathochromic shift must be ascribed to transannular interaction of the amide and ester groups. As a result the electron density on the nitrogen atom must be reduced and hence the amide proton should be endowed with greater mobility. In fact, according to IR data, deuteroexchange of the cyclodepsipeptide (34) in C_2H_5OD proceeds much faster than, say, with the dodecalactam or the 16-membered cyclodepsipeptide (36).

Finally, the occurrence of transannular interaction in the 10-membered compound

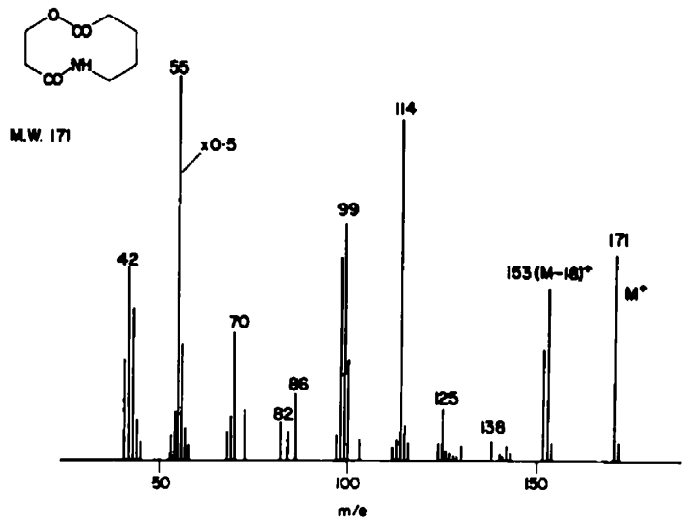
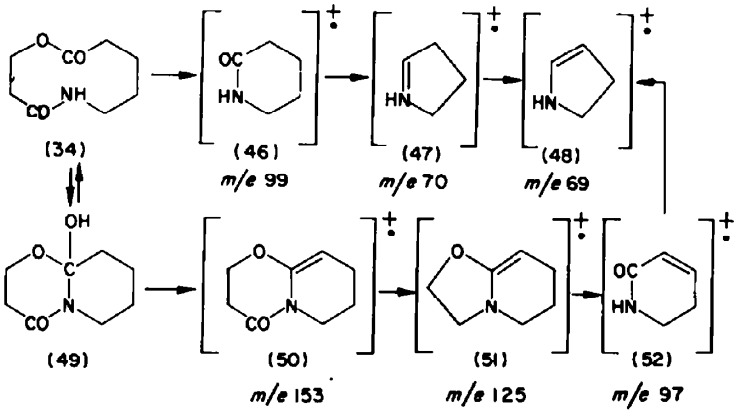


FIG. 3. Mass spectrum of the cyclodepsipeptide (34).

(34) is particularly well expressed in the mass spectrometric fragmentation of this compound, which sharply differs from that of the 11-membered cyclodepsipeptide (35a) under the same conditions. Here besides fragmentation beginning with the lactam ion (46), a route which is common to both compounds, there is also fragmentation along the route (50) → (51) → (52) → (48) which has its origin in the ion (50) formed as a result of dehydration of the cyclol (49)[84] (Fig. 3).



We have observed transannular interaction with 9-, 10- and 11-membered cyclopeptides (54; n = 1, 2, 3) obtained by incorporation of a β-alanine residue into the corresponding lactam ring according to the scheme (18) → (53) → (57) → (54).[85] Indeed, their mass spectrometric behaviour shows that under these conditions the azacyclol structure of type (57) forms, since one of the principal fragmentation routes begins with elimination of the elements of water following which it coincides with

[84] N. S. Wulfson, V. I. Zaretzkii, V. A. Puchkov, V. G. Zaikin, A. M. Shkrob, V. K. Antonov and M. M. Shemyakin, *Dokl. Akad. Nauk. SSSR* **153**, 336 (1963).

[85] V. K. Antonov, Z. E. Agadzhanian, T. R. Telesnina, M. M. Shemyakin, G. G. Dvoriantzeva and Yu. N. Sheinker, *Tetrahedron Letters* No. 13, 727 (1964).

fragmentation of the corresponding bicyclic acylamidines (56) (Fig. 4).* Formation of the acylamidines (56; n = 1, 2) is also observed on heating the corresponding cyclopeptides (54; n = 1, 2) in xylene, but the 11-membered cyclopeptide (54; n = 3) remains unchanged under the prevailing conditions. On the other hand in aqueous solution the 9-membered cyclopeptide (54; n = 1) slowly undergoes conversion at 20° into the amidino acid (55; n = 1), whereas compounds (54; n = 2, 3) remain unchanged.†

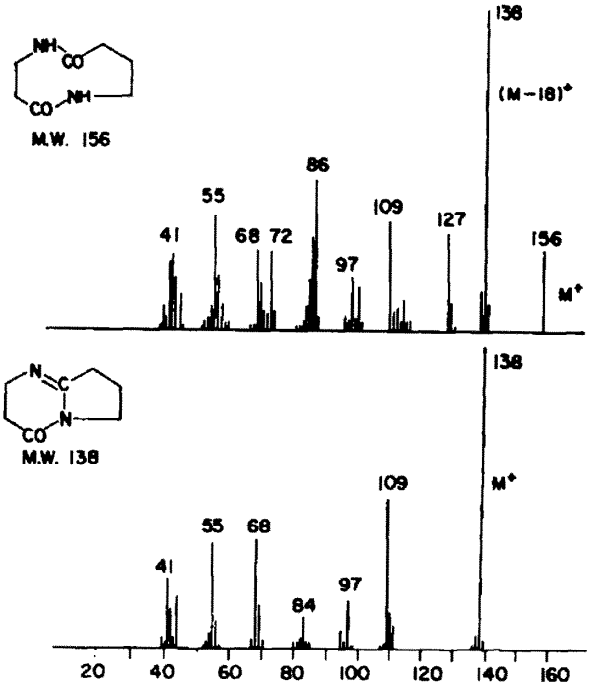


FIG. 4. Mass spectra of the cyclodipeptide (54; n = 1) and acylamidine (56; n = 1).

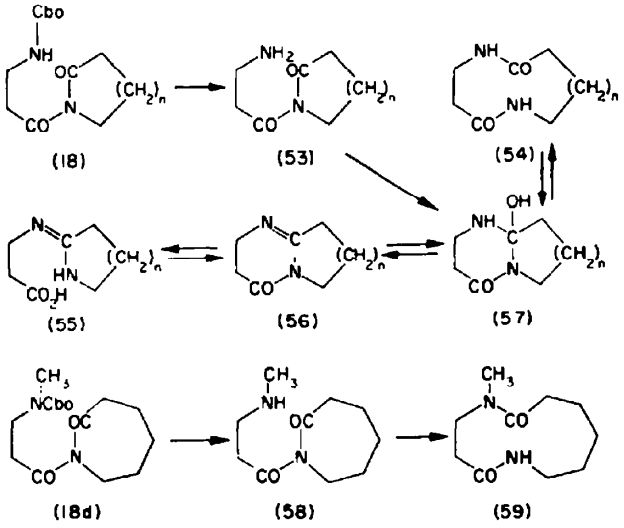
The conversion of the cyclopeptides (54) into azacyclols (57) and acylamidines (56) is also reversible. For instance, in aqueous solutions at 20° the acylamidines (56) undergo hydration, yielding besides the amidinoacids (55) the cyclopeptides (54) among which the most easily formed is the 11-membered ring (54; n = 3), the 10-membered cyclopeptide (54; n = 2) forms less readily and the 9-membered cyclopeptide (54; n = 1) forms only on hydration of the corresponding acylamidine in the presence of bases. All these reactions, which are quantitatively characterized in Table 7, show the possible conversions of the azacyclols (57) formed in the process of aminoacyl incorporation, as well as the relative stability of the cyclopeptides of medium ring size‡ (Table 8).

* It should be pointed out that the N-methylated cyclodipeptide (59) we prepared from the acyllactam (18d) according to the scheme (18d) → (58) → (59) undergoes fragmentation according to an entirely different route.

† The structures of the acylamidines (56) and the amidinoacids (55) have been proved by their counter-synthesis (see Experimental, 7-B(a)).

‡ Soon after our paper read at the Kyoto Symposium[1] there appeared a report[18] on similar observations of transannular amide–amide interaction in the case of 6,10-dioxo-1,5-diazacyclodecane.

The stability of macrocyclic systems depends not only on the size of the ring, but, at least for 9- to 11-membered rings, also on the mutual arrangement of the amide or amide and ester groups. Thus, earlier[36,37] it was shown that the only product of the interaction between the amino group and the endocyclic carbonyl in N-(α-aminoacyl)-lactams of type (60) is the corresponding acylamidines (62). We have observed that N-(glycolyl)-caprolactam (64) is incapable of conversion into the 10-membered



cyclodepsipeptide (66) and in solution exists in tautomeric equilibrium with the cyclol form (65).[38,*] On the other hand, as has been noted above, N-(β-hydroxypropionyl)-valerolactam readily isomerizes into the 10-membered cyclodepsipeptide (34). A comparison of the molecular models of compounds (34) and (66) shows that the ring is much more strained in the latter, which apparently is the reason for this difference.

TABLE 7. RELATIVE STABILITY OF CYCLOPEPTIDES (54)

Number of atoms in ring	Yield %		
	(54) → (56)	(54) → (55)	(56) → (54)
9	68	80	0†
10	45	0	45
11	0	0	50

† In absence of base.

The strain becomes still greater on passing to 9-membered cyclodepsipeptides, so that the N-(α-hydroxyacyl)-valerolactams (67) and the N-(α-hydroxyacyl)-diketopiperazines (70) exist either completely as cyclols (68 or 71), or in tautomeric equilibrium.[39] Moreover, it has recently been shown that even when the 9-membered ring

* The same phenomenon has been observed by Griot and Frey.[23]

[36] G. Reinisch, *Faserforsch. Textiltech.* **13**, 43 (1962).

[37] M. Rothe, *Angew. Chem.* **74**, 725 (1963).

[38] M. M. Shemyakin, V. K. Antonov and A. M. Shkrob, *Peptides. Proc.* VI-*th European Symposium* p. 321. Athens 1963, Pergamon Press, (1964).

TABLE 8. CYCLOPEPTIDES (54) and (59), AMIDINOACIDS (55) AND ACYLAMIDINES (56)

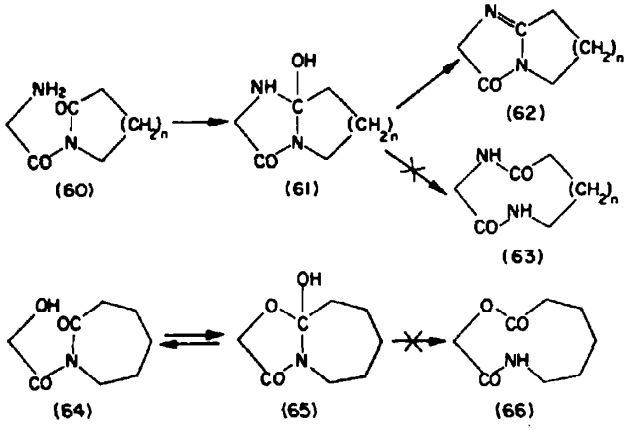
Compound	Method of synthesis	Yield %	Solv. used for cryst.	M.p. °C or B.p. °C/mm	IR band position (cm^{-1}) (nujol)					Integral intensity of the amide I band*
					Amide I	Amide II	CO–acid	C=N	NH or OH	
54; n = 1	7-A(a)	38	EtOH	173	1665, 1620	1570†			3200, 3080	$10{\cdot}8 \times 10^4$
	7-A(b)	60								
	7-A(c)	54								
54; n = 2	7-A(a)	20	EtOH	187	1660	1560			3300, 3080	
	7-A(c)	45								
54; n = 3	7-A(a)	61	EtOH	259	1655	1560			3300, 3080	$8{\cdot}2 \times 10^4$
	7-A(b)	70								
	7-A(c)	50								
55; n = 1	7-B(a)	97	EtOH	187			1570	1685	2750 br.	
	7-A(d)	80								
55; n = 2	7-B(a)	95	EtOH	186			1580, 1515	1660, 1620	2720 br.	
55; n = 3	7-B(a)	95	EtOH	200			1595, 1550	1670, 1630	2720 br.	
56; n = 1	7-B(a)	81		152/12	1700‡			1700‡		
	7-B(b)	68								
56; n = 2	7-B(a)	91		160/12	1700‡			1665‡		
	7-B(b)	45								
56; n = 3	7-B(a)	80		185/10	1710‡			1660‡		
59	7-A(a)	74	EtOH–Et_2O	136	1645, 1624	1568			3320, 3120	

* 1 $mole^{-1}\,cm^{-2}$; in C_2H_5OD. For compound (34), containing one amide bond, the value of the integral intensity is $4{\cdot}2 \times 10^4$.

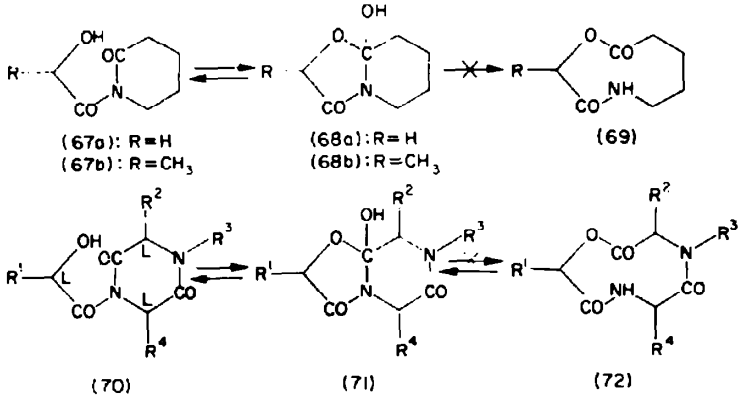
† In alcohol; in nujol this band is absent.

‡ As film.

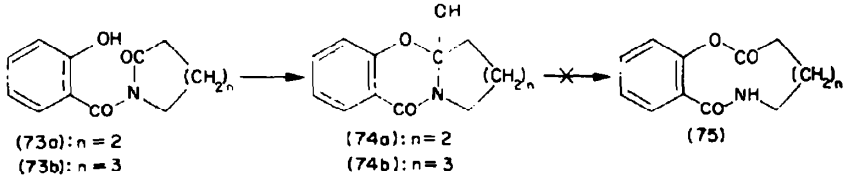
(72d) can be obtained in some round-about manner it easily and irreversibly isomerizes into the cyclol (71d).[40]



Stable cyclols can form from N-salycylyllactams (73), but, in contrast to the cases discussed, cyclols with both 10 (74a) and 11 (74b) atoms in a bicyclic system can be



a: $R^1 = R^3 = R^4 = H$, $R^2 = CH_3$; *b*: $R^2 = R^4 = H$, $R^1 = R^3 = CH_3$*; c: $R^1 = R^4 = H$, $R^2 + R^3 = (CH_2)_3$†; *d*: $R^1 = H$, $R^3 + R^3 = (CH_2)_3$, $R^4 = CH_2CH(CH_3)_2$; *e*: $R^1 = CH_3$, $R^2 + R^3 = (CH_2)_3$, $R^4 = CH_2Ph$



obtained due to the presence of a benzene ring, stabilizing the cyclol grouping by flattening the molecule.

* Racemate.

† Racemization occurs on conversion of 70c to 71c. (See p. 3565.)

[39] M. M. Shemyakin, V. K. Antonov, A. M. Shkrob, L. B. Senyavina and Yu. N. Sheinker, *Tetrahedron Letters* No. 16, 701 (1962).

[40] R. C. Sheppard, *Experientia* **19**, 125 (1963).

The tautomeric equilibrium between the cyclol and N-hydroxyacyl forms depends on structural factors. Thus, in tetrahydrofuran solution N-glycolylvalerolactam (67a) is in equilibrium with the cyclol form (68a). This cyclol can be isolated as crystals which on dissolution will again establish equilibrium with the N-hydroxyacylamide form. N-Glycolylglycylsarcosyllactam (70a) also partially isomerizes in tetrahydrofuran solution to the cyclol (71a). However, here the equilibrium is shifted largely in favour of the hydroxyacylamide which is the only form that can be isolated from the solution. Tautomeric equilibrium between the hydroxyacylamide and cyclol forms is also observed in the case of N-glycolylglycylprolyllactam (70c). The cyclols (68b) and (71b, d, e) obtained from the N-lactyl derivatives of the lactam (67b) and the diketopiperazines (70b, e) and also from the N-glycolyldiketopiperazine (70d) are so stable that no tautomeric interconversions can be observed with these compounds (Table 9). The bis-glycolyldiketopiperazine (76) formed by hydrogenolysis of the bis-benzyl derivative (20f) transforms into the bis-cyclol (77) in methanol solution at 20°. If the methanol solution is heated the bismethyl ether (78) of the cyclol is produced.

Although cyclolization of β-hydroxypropionyldiketopiperazine (79) could not be detected spectroscopically, its treatment with alcohol in the presence of dry HCl leads to the formation of the ethyl ether of the cyclol (80) and a small amount of ethyl β-hydroxypropionylglycylsarcosinate (82). Similar products (81 and 83) are obtained on methylation of the hydroxy derivative (79) with CH_3I in the presence of Ag_2O.*

The cyclol–N-hydroxyacylamide interconversions can be followed by means of the extinction in the 210–220 mμ region of the UV spectrum, characteristic of absorption by the $(—CO)_2N$ chromophore and where there is practically no absorption by the cyclols (Fig. 5).

Such transformations in the case of the hydroxyacyl derivatives of diketopiperazines may also be detected by means of IR spectroscopy since the spectra of the

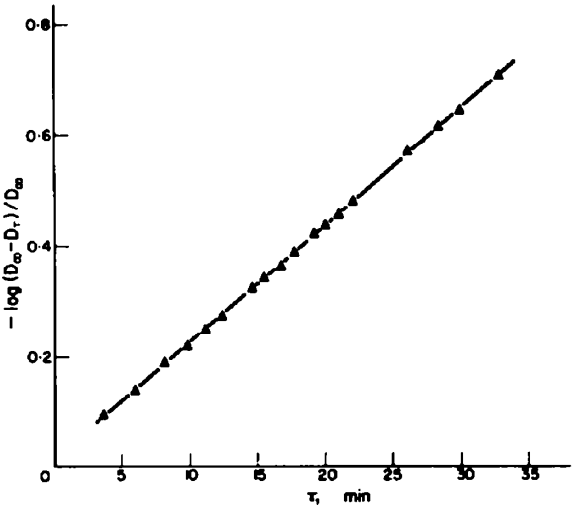


FIG. 5. Change in the optical density of dioxan solution of the cyclol (68a) at 220 mμ ($\varepsilon_{\text{fin.}}$ 1600).

* The constants of the O-alkyl derivatives of the cyclols are given in Table 10.

Table 9. Hydroxyacylamides (64), (70) and cyclols (65), (68), (71), (74) and (77)

Compound	Method of synthesis	Yield %	Solv. used for cryst.	M.P. or b.p. °C (mm)	IR band position (cm^{-1}) (in THF)				$\lambda_{max}^{dioxane}$ (mμ)	ε
					$(—CO)_2N$	CO–oxazolidinone	CO–oxazinone	CO–piperazinone		
64 ⇄ 65	8	90		110–115 (0·05)*	1718	1718			214	1700
68a	8	86	CCl_4	97		1725			221	300 (1600**)
68b	8	73	AcOEt	92		1724				
70a	8	92	AcOEt	119	1730			1693	216	7500 (5650**)
70c	8	95		oil***	1730			1695	214	7500 (4210**)
71b	8	95	Me_2CO–Et_2O	150		1733		1674		
71c	8	35	Me_2CO–Et_2O	148§		1730		1672		
71d	8	20	AcOEt	117		1730		1662		
71e	8	45	AcOEt	197§§		1734		1666		
74a	9	93	EtOH	164			1677		208, 238, 298§§§	33700, 9400, 2900
	11	74								
74b	9	84	Me_2CO	132			1676		207, 236, 295§§§	32600, 9950, 2400
	11	42 (60‡)								
77‡‡	8	100	DMF	186 (dec.)		1708				

* n_D^{20} 1·5003. ** Solution in dioxan of an equilibrium mixture of isomers. *** $[\alpha]_D^{20}$ −52° (*c* 0·5, THF). § Optically inactive. §§ $[\alpha]_D^{20}$ −22° (*c* 1, THF). §§§ In C_2H_5OH. ‡ From the boron fluoride of (95d). ‡‡ The structure was proved mass-spectrometrically.

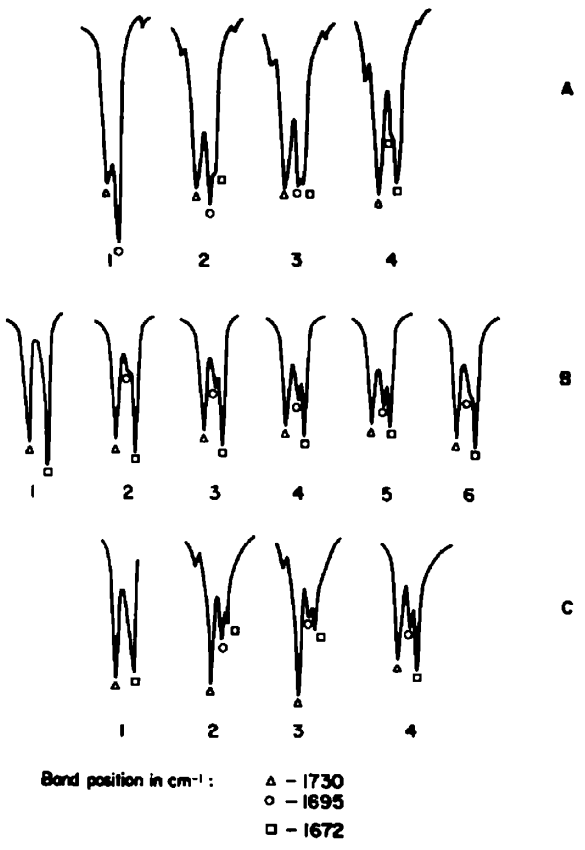
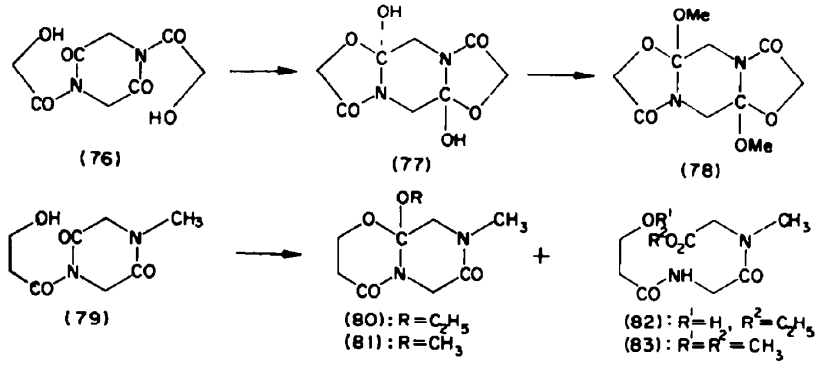


FIG. 6. Carbonyl region of the IR spectra of the N-hydroxyacyldiketopiperazine (70c) and cyclol (71c) in tetrahydrofuran. A: 1. Solution of (70c) following hydrogenolysis; 2, 3 and 4. After 5, 10 and 15 days (20°); B: 1. Fresh solution of (71c); 2, 3, 4 and 5. After 30, 60, 90 and 120 min. (35°); 6. Control (240 min at 20°); C: 1. Fresh solution of (71c); 2. After heating to 100° (15 min); 3. After cooling and keeping for 12 hr at 35°; 4. Control (12 hr at 35°).

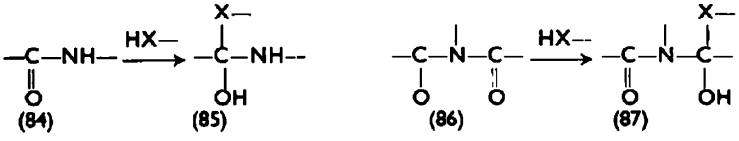


acylamide form (70) differ from those of the cyclols (71) in the position of the piperazinone amide carbonyl band. Indeed, as can be seen from Fig. 6, the equilibrium mixture of the tautomeric forms established in the case of both the N-hydroxyacylamide (70c) and the cyclol (71c) displays bands characteristic of both forms (1695 cm^{-1} and 1672 cm^{-1}). At the same time with N-hydroxyacyldiketopiperazines of type (70) the position of the $(—CO)_2N$ band practically coincides with that of the carbonyl in the 5-membered oxazolidinone ring of cyclol (71). Therefore the tautomeric conversion (70) $\rightleftarrows$ (71) is not accompanied by appreciable changes in the band in the region of 1730 cm^{-1}. However if the cyclolization leads to the 6-membered 1,3-oxazinonic ring, as for instance in the conversion of the salycylyllactams (73) into the cyclols (74), then instead of the $(—CO)_2N$ band there appears the usual amide carbonyl band.[38,39,41]

The spectral relationship between the acylamides and cyclols were very useful in affording final proof of the structures of some naturally occurring and synthetic compounds of a cyclolic nature. Thus, it was our results[38,39,41] and the data subsequently published by the Swiss workers[32,42] on the spectral identification of cyclols that made the assignment of a cyclolic structure to the peptide moiety of the ergot alkaloids possible.

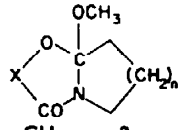
The stable cyclols we have investigated are unique examples of ortho acid amides, which on the basis of indirect evidence have time and again been cited as hypothetical intermediates in the reaction of carboxylic acid derivatives with various nucleophilic reactants.[43] Brenner[44] made use of the possible formation of cyclolic structures to explain the rearrangement of the O-amino acid acylated salycylylamides. Bernhard[45] used the structures in an attempt to elucidate the mechanism of the enzymatic action of esterases, whereas Wrinch[46] even regarded the cyclol grouping as the basic structural element of proteins. Similar cyclic ortho acid amide structures have been ascribed to the alkaloid rhetsinine,[47] the antibiotics griseolutein B[48] and blasticidin S.[49]

Ordinarily cyclols are extremely unstable, since their formation is accompanied by disappearance of the resonance-stabilized amide group (84) → (85), but the retention of one of the carbonyl groups of the initial N-acylamide (86) → (87) may be one of the reasons for the stability. We have now accumulated a number of facts concerning the chemical properties of these unusual compounds.

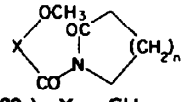


[41] V. K. Antonov, A. M. Shkrob and M. M. Shemyakin, *Peptides. Proc. V-th European Symposium* p. 221. Oxford (1962), Pergamon Press (1963).
[42] K. Stich and H. G. Leemann, *Helv. Chim. Acta* **46**, 1151 (1963).
[43] M. L. Bender, *Chem. Rev.* **60**, 54 (1960).
[44] M. Brenner, *J. Cellular Comp. Physiol.* **54**, Suppl. 1, 221 (1959).
[45] S. Bernhard, *J. Cellular Comp. Physiol.* **54**, Suppl. 1, 252 (1959).
[46] D. Wrinch, *Chemical Aspects of the Structure of Small Peptides.* Munksgaard, Copenhagen (1960).
[47] A. Chatterjee, S. Bose and C. Ghosh, *Tetrahedron* **7**, 257 (1959).
[48] S. Nakamura, K. Maeda and H. Umezawa, *J. Antibiotics, Japan*, **17**, 33 (1964).
[49] H. Yonehara, *I.A.M. Symposia on Microbiology*, No. 6, *Chemistry of Microbial Products* p. 31. Tokyo (1964). *Added in proof:* Recently N. Otake, S. Takeuchi, T. Endo and H. Yonehara have proved that Clasticidin S is not the cyclol (*Tetrahedron Letters* No 19, 1411 (1965)).

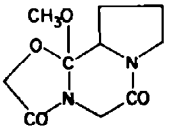
It was shown that the hydroxy group in the cyclols (68, 71 and 74) is easily methylated by methyl iodide in the presence of silver oxide and that the methyl ethers of the cyclols (88 and 90) are spectrally similar to the cyclols, but markedly different from their isomeric N-(methoxyacyl)-amides (89 and 91). The latter or their degradation products are often formed on methylation of cyclols, which substantiates the tautomeric transformations in the course of the reaction. On the other hand isocyanates may be fruitfully utilized to characterize N-hydroxyacylamides, which give crystalline urethanes (92a–c and 93a, b). These derivatives can be prepared not only from the pure hydroxyacylamides, but also from the equilibrium mixtures of the cyclol and hydroxyacylamide forms (Table 10).



(88a): X = CH_2, n = 2
(88b): X = $CHCH_3$, n = 2
(88c): X = CH_2, n = 3
(88d): X = o—C_6H_4, n = 3

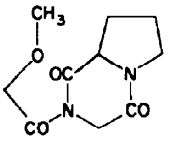


(89a): X = CH_2, n = 2
(89b): X = o—C_6H_4, n = 1
(89c): X = o—C_6H_4, n = 3

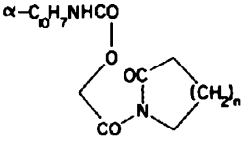


(90)

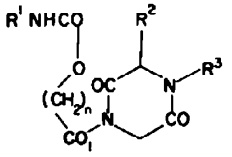
The hydrogenolysis of the hydroxy group of cyclols, using Pd in the presence of traces of acid was investigated taking as example the aromatic cyclols (74a, b). The products obtained were the desoxy compounds (94a, b) whose structure was established on the basis of IR and mass spectrometric data. These compounds are also formed



(91)

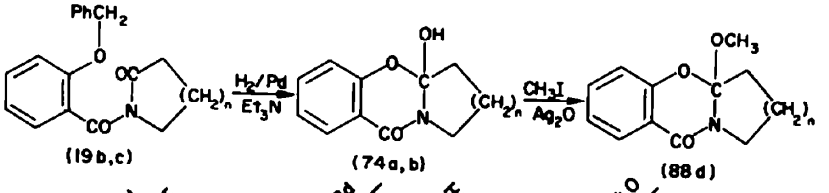


(92a): n = 1
(92b): n = 2
(92c): n = 3

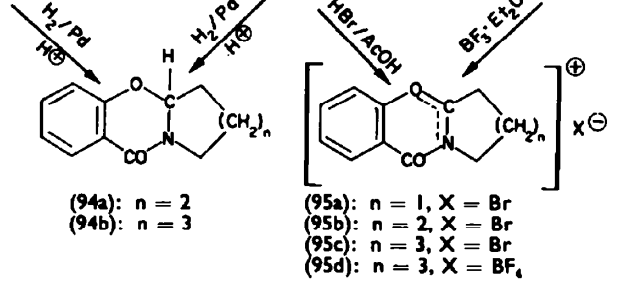


(93a): R^1 = α—$C_{10}H_7$, $R^2 + R^3 = (CH_2)_3$, n = 1
(93b): $R^1 = C_6H_5$, R^2 = H, $R^3 = CH_3$, n = 2

in the hydrogenolysis of N-(*o*-benzyloxybenzoyl)-lactams (19b, c) in the presence of acids. Similar behaviour has been observed by other workers[32] in the case of cyclols with aliphatic hydroxy acid residues.



(19b,c) (74a,b) (88d)



(94a): n = 2
(94b): n = 3

(95a): n = 1, X = Br
(95b): n = 2, X = Br
(95c): n = 3, X = Br
(95d): n = 3, X = BF_4

TABLE 10. FUNCTIONAL DERIVATIVES OF CYCLOLS (78), (80), (81), (88), (90) AND HYDROXYACYLAMIDES (89), (91)–(93)

Compound	Method of synthesis	Yield %	Solv. used for cryst.	M.p. or B.p. °C/mm	IR band position (cm^{-1}) (nujol)			$\lambda_{max}^{ethanol}$ (mμ)	
					$(—CO)_2N$	CO–amide	CO–ureth.		
78	10-A (b)	100	DMF–MeOH	222		1722			
80	10-A (b)	64	EtOH	148		1680, 1668			
81	10-A (a)	57	EtOH	118		1682, 1653			
88a	10-A (a)	48		37*		1724		221§	160
88b	10-A (a)	100§§	Isopentan	112		1720			
88c	10-A (a)	40		85/0·05		1725 br.			
88d**	10-A (a)		AcOEt	117		1668		209, 236, 294	35200, 9950, 2650
89a	10-B	73	C_6H_{14}	28	1710 br.			220§	9500
89b	10-B	68	AcOEt	82	1750, 1660			214, 290	15300, 1200
89c**	10-A (a)		AcOEt	115	1707, 1661			212, 223, 290	16700, 15200, 2250
	10-B	49							
90	10-A (a)	50	AcOEt–Et_2O	174	1720	1655		220§	1150
91	10-B	oil			1722 (THF)	1693 (THF)			
92a	10-C	79	AcOEt	170	1738, 1710		1738		
92b	10-C	13	CCl_4	166	1712, 1682		1736		
92c	10-C	60	AcOEt	133	1715, 1692		1725		
93a	10-C	55	CH_3CN	218	1718	1656			
93b	10-C	72	MeOH	190	1716	1662	1712		

* B.p. 45–48/0·02 mm. ** Isolated from a mixture of (88d) and (89c) by thin layer chromatography on Al_2O_3 (Activity II) in the system AcOEt–C_6H_{14} (1:4), R_f (88d): 0·55, R_f (89c): 0·3. § In dioxane. §§ Crude product.

It is natural to assume that the necessity of an acid catalyst in the hydrogenolysis of the cyclol hydroxyl is due to the formation of the corresponding intermediate cation of the type (95). In fact, it was found that salts of these cations readily form on acidolysis of the cyclols (74a, b). For instance, treatment of the latter with hydrogen bromide in glacial acetic acid yields the bromides (95b, c). It is noteworthy that such a bromide (95a) is also formed from salycylylbutyrolactam (31c) which like the other N-hydroxyacylbutyrolactams do not transform into cyclols or desoxycyclols. Obviously, bromides of the type (95) can be formed by acidolysis of benzyloxybenzoyllactams (19a–c). Treatment of the methyl ether of the cyclol (88d) with $BF_3 \cdot Et_2O$ leads to formation of the fluoborate (95d). The structures of the bromides and the fluoborate was confirmed by their IR spectra, the existence of bands in the 1565–1580 cm^{-1} region characteristic of the $>C=\overset{\oplus}{N}<$ group indicating that the positive charge of the cation is apparently predominantly localized on the nitrogen atom (Table 11)*. It is to be mentioned that on treatment of the salts (95b, c) with water they are transformed into the corresponding cyclols (74a, b), whereas the bromide (95a) yields the salycylylbutyrolactam (31c). It is also of interest that according to the NMR spectra of solutions of the bromide (95c) in CH_3COOD, the hydrogen atoms of one of the methylene groups of this compound readily undergoes exchange with deuterium (Fig. 7).

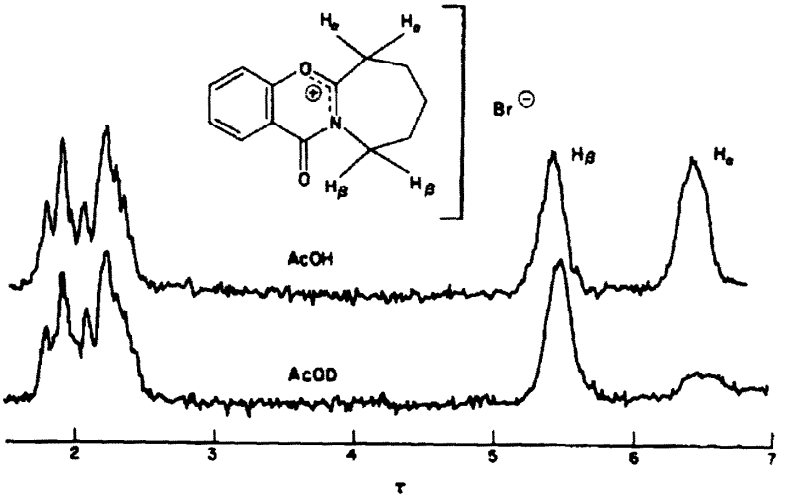


FIG. 7. NMR spectra of the bromide (95c) in acetic and deuteroacetic acids.

In the light of the above facts, another interesting property of the cyclols is their optical lability. It has been found that the optical activity of N-glycolyl-L-propylglycyllactam (70c) diminishes at a rate commensurate with the formation of the cyclol (71c) (Fig. 8). The latter was found to be optically inactive when isolated from solutions in which equilibrium was reached.[59] This gives grounds for assuming that the optical lability of the asymmetric centre α to the cyclolic grouping is due to the strong tendency of the latter to cation formation, which should considerably enhance the mobility of the α-hydrogen atom. It is possible that similar effects may play some

* A similar type of ambidentate cations has recently been described in a review by Hünig.[60]

[60] S. Hünig, *Angew. Chem.* (Internat. Ed.), 3, 548 (1964).

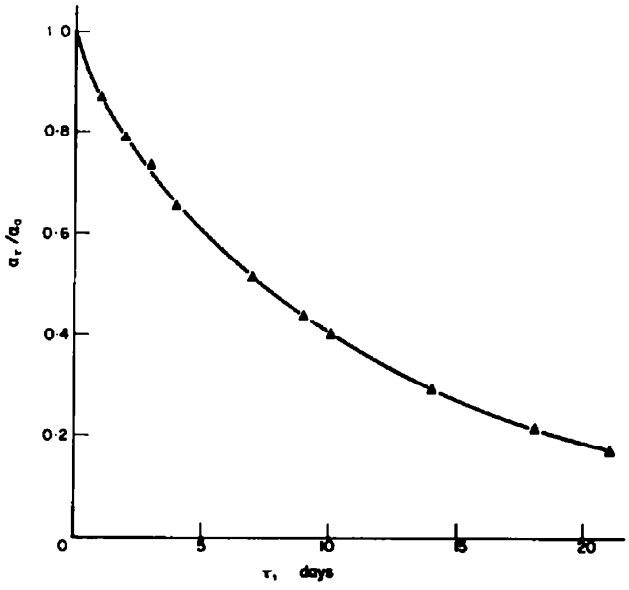


FIG. 8. Change in optical activity of the dioxan solution of N-glycolyl-L-prolylglycyl-lactam (70c). (Cf. Fig. 6-A, 2–4).

part in the racemization often observed with C-terminal amino acid residues (96) in the course of peptide synthesis where the degree of racemization can be determined by the lifetime of the intermediate ortho acid form (97).

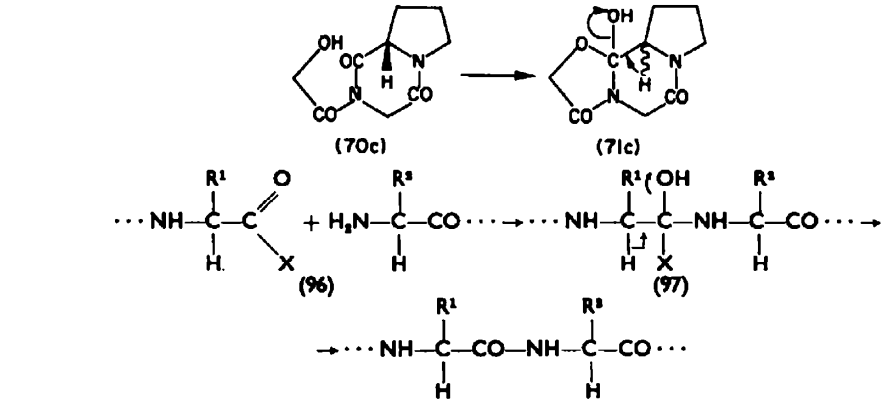
In conclusion some consideration should be given to the role of hydroxy- and amino acid incorporation into peptide systems in biochemical reactions. In this connection the following facts are deemed worthy of mention.

Firstly, the incorporation reaction may prove to be one of the biosynthetic pathways to some naturally occurring depsipeptides, including depsipeptide antibiotics. In the case of the cyclotetradepsipeptide antibiotic serratamolide (37h), the biosynthesis could take place by acylation of the diketopiperazine (98) according to the scheme (98) → (99) → (37h). The validity of such a scheme is supported by our synthesis of diacetylserratamolide (37e) (see p. 22).

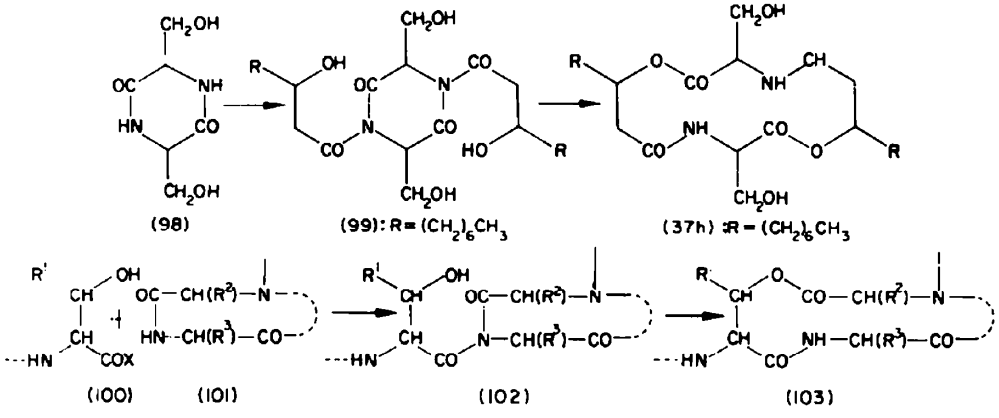
TABLE 11. DESOXYCYCLOLS (94) AND SALTS (95)

Compound	Method of synthesis	Yield %	Solv. used for cryst.	M.p. °C	IR band position (cm^{-1}) (nujol)		
					CO–oxazinone	C=N+	C=C(arom.)
94a	10-D(a)	100	Et_2O–C_6H_{14}	68	1675		1615, 1593
	10-D(b)	95					
94b	10-D(a)	97	AcOEt–C_6H_{14}	79	1670, 1663		1615, 1585
	10-D(b)	93					
95a	10-E(a)	60		198*	1753	1576	1625, 1602
	10-E(b)	55					
95b	10-E(a)	54		215*	1750	1578	1630, 1597
	10-E(b)	50					
95c	10-E(a)	43		202*	1747	1566	1630, 1597
	10-E(b)	35					
95d	10-F	60	AcOH	202*	1749	1570	1632, 1599

* In sealed capillary at heating rate of 4 deg/min.



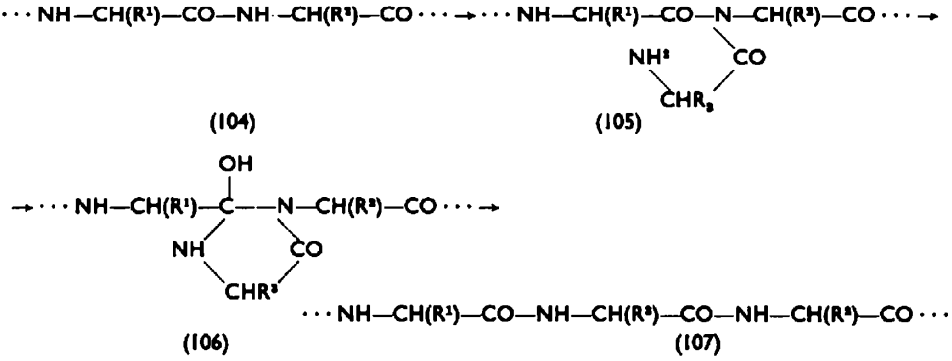
The above biogenetic pathway is also a quite probable one for a large group of depsipeptide antibiotics of similar structure containing one or two α-amino-β-hydroxy acid residues (serine or threonine) in their molecules. This group includes etamycin, staphylomycin S, ostreogrycin B and related antibiotics as well as echinomycin, destruxin B, telomycin, etc.[51–54] The biosynthesis of these antibiotics possibly occurs *via* N-acylation of the corresponding cyclopeptides (101) by serine or threonine, with activated carboxyl group (100) followed by rearrangement of the resultant N-acyl-cyclopeptide (102) to the ultimate cyclodepsipeptide (103). The last stage in this biogenetic scheme we demonstrated by the model incorporation of a serine residue into a cyclic amide. The correspondingly protected N-serylcaprolactam (17e) after removal of the blocking group was found capable of readily isomerizing to the cyclodepsipeptide (35b) (see p. 3549). We are at present in the progress of further experimental proof of the aforementioned biogenetic schemes.



In addition it is possible that for antibiotics, hormones and other metabolites of a peptide nature whose biosynthesis probably takes place along pathways differing

[51] M. M. Shemyakin, *Uspekhi Khimii* **31**, 269 (1962).
[52] M. M. Shemyakin, *I.A.M. Symposia on Microbiology*, No. 6, *Chemistry of Microbial Products* p. 97. Tokyo (1964).
[53] S. Tamura, *I.A.M. Symposia on Microbiology*, No. 6, *Chemistry of Microbial Products* p. 127. Tokyo (1964).
[54] J. S. Sheehan, *Angew. Chem.* **76**, 793 (1964).

from the usual biosynthesis of proteins, aminoacyl incorporation may prove to be one of the means for construction of the peptide chain (104) → (105) → (106) → (107).



Finally, the hydroxy- and aminoacyl incorporation reaction may be one of the ways by which rearrangement occurs in peptide and protein molecules. In the presence of a carboxyl group and a nucleophilic group suitably located with respect to an amide bond, rearrangement of this part of the chain may occur with the formation of a fragment containing a different sequence of amino acid residues.

These conjectures while needing further proof, should however be borne in mind in studies of chemical and biochemical transformations of peptides and proteins.

EXPERIMENTAL

The following is a description of the general methods used in the synthesis of compounds of the types investigated. The constants of the compounds are given in Tables; in all cases the analytical, and calculated data are in agreement. The mol. wts have been determined mass spectrometrically, and in some cases thermoelectrically or cryoscopically. The IR spectra have been taken on the Zeiss UR-10 spectrometer, the UV spectra on the Zeiss VSU-1 and Hitachi EPS-2 spectrometers, the NMR spectra on the 60 Mc/s spectrometer JNM-C-60 and the mass spectra on the spectrometer MX 1303*. The m.ps are uncorrected.

I. *Synthesis of protected hydroxy- and aminoacylamides* (*Table* 1)

A. *Compounds* (14a), (16a–e), (17a–c), (20a–e) *and* (22). A solution of 0·05 moles amide and 0·06 moles benzyloxyacyl chloride in 30 ml abs. toluene was refluxed until evolution of HCl ceased (6–8 hr). The toluene was distilled off and the residue crystallized by adding hexane or ether, or distilled in vacuum.

B. *Compounds* (14b–e) were prepared from methyl N-benzyloxyacylglycinates (0·1 mole) and phthalylglycyl chloride (0·12 mole) using the conditions of experiment I–A.

C. *Compounds* (20f), (21a–i) *and* (23a,b). Finely ground diketopiperazine (or cyclodepsipeptide; 0·1 mole) in toluene containing 0·22 moles benzyloxyacyl chloride was refluxed for 10–15 hr. The mixture was then filtered, the solvent distilled off and the residue triturated with pet. ether. In the case of non-crystallizing compounds the pet. ether was decanted off and the trituration repeated twice, following which the oil obtained was utilized without further purification.

D. *Compounds* (17d,e) *and* (19a–c). An ether solution of benzyloxyacyl chloride (0·13 mole) was slowly added at 5–7° to a solution of 0·1 mole lactam in 50 ml abs. ether containing 0·15 moles triethylamine. The mixture was stirred for 1·5–2 hr at 20°, 200 ml water added and the ether layer washed with 5% H_2SO_4 and water. After drying the ether was distilled off and the residue recrystallized.

* We express our thanks to G. Yu. Peck for the NMR spectra and his assistance in their interpretation.

The mass spectrometric study of the compounds described here was carried out by N. S. Wulfson, V. I. Zaretzkii and V. A. Puchkov and will be the subject of a separate communication.

E. *Compounds* (15a–c). A mixture of 0·1 mole phthalyldipeptide ethyl ester and 0·2 mole azidoacyl chloride was refluxed in abs. toluene for 10 hr. The solution was filtered, cooled and the precipitated dipeptide filtered off by suction. The filtrate was evaporated and the residue recrystallized.

F. *Compounds* (18a–d). The lactams were acylated with carbobenzoxy-β-alanyl chloride or carbobenzoxy-β-N-methyl-alanyl chloride using the conditions of experiment 1-D. In the case of (18a) the precipitate was filtered off by suction, washed with water on the funnel and recrystallized. In the other cases the acylated product was isolated by evaporating the ether solution.

2. *Synthesis and properties of 1-aroyl-2-aroyloxy-valerolactams* (*Table* 1)*

Synthesis of compound (24a). Valerolactam (0·1 mole) was acylated with 0·13 moles *o*-benzyloxybenzoyl chloride using the conditions of experiment 1-D. After dilution of the mixture with water a crystalline product was precipitated, and after separation was washed with 5% H_2SO_4 and water, dried and recrystallized.

Synthesis of compounds (24b). Valerolactam (0·1 mole) was acylated with 0·35 mole benzoyl chloride in the presence of 0·5 mole triethylamine using the conditions of experiment 1-D.

Acidolysis of (24b). N,O-dibenzoyllactam (24b, 250 mg) was dissolved in 10 ml dry dichloroethane saturated with HCl. After 10 min, the mixture was evaporated, the residue triturated with abs. ether and then recrystallized. A 99% yield of N-benzoylvalerolactam was obtained, m.p. 110–111°; cf.[55]

Hydrogenolysis of (24b). The compound (24b; 0·6 g) was hydrogenated in abs. tetrahydrofuran over Pd–black, 3 moles of H_2 being taken up. The mixture was filtered, distilled and the residue crystallized from ethyl acetate, yielding 89% of N-benzoylvalerolactam. Toluene was detected in the distillate by means of gas-liquid chromatography.

Aminolysis of (24a). Valerolactam (1 g) was heated at 160–170° for 1 hr with 5·24 g of compound (24a), yielding 93% of N-(O-benzyloxybenzoyl)-valerolactam (19b).

3. *Hydroxyacyl incorporation into linear amides and peptides* (*Table* 2)

A. N-*Methyl acetoxyacetamide* (26a). (a) N-benzyloxyacetylamide (14a; 0·02 mole) was hydrogenated in 15 ml abs. tetrahydrofuran in the presence of Pd-black. After filtration and evaporation a colourless oil was obtained which was distilled and crystallized on seeding. (b) Methylamine hydrochloride (0·1 mole) and acetoxyacetyl chloride (0·11 mole) were refluxed in abs. toluene for 3 hr. The toluene was distilled off and the residue distilled in vacuum.

B. *Phthalyldepsipeptides* (26b–e). Hydrogenation of compounds (14b–e) in tetrahydrofuran over Pd–black afforded the crystalline hydroxyacylpeptides (25b–d) or the oily product (25e). Compounds (25b–d) were heated to their m.p. and then kept at that temp for 1–2 min. On cooling, compounds (26b–d) were obtained which were crystallized by triturating with benzene or ether. The substance (25e) was mixed with 5% $NaHCO_3$aq and within a few minutes the oil hardened, yielding (26e).

C. *Counter synthesis of* (26c). Benzyl diazoacetylglycinate[56] (16 mmoles) and 12 mmoles N-phthalylglycine in 80 ml dioxane were refluxed for 7 hr. The dioxane was distilled off and the oily residue crystallized from a tetrahydrofuran–hexane mixture. The product was benzyl O-(N-phthalylglycyl)-glycolylglycinate, m.p. 121–122°; yield 64%. The ester was hydrogenated in tetrahydrofuran over Pd–black giving the corresponding acid with m.p. 203° (from acetonitrile); yield 93%. The acid was treated with diazomethane in a dioxan–ether mixture; yielding 85% of (26c).

4. *Aminoacyl incorporation into linear peptides* (*Table* 3)†

A. *Tripeptides* (30a–c) *from azidoacyldipeptides* (15a–c). Compound (15a–c) was mixed with excess of 28% HBr solution in glacial acetic acid. The mixture was kept for 6 hr at 20° and overnight at +5°. Abs. ether was then added; the precipitate filtered off by suction and carefully washed with ethyl acetate and ether. The hydrobromide of N-(α-aminoacyl) dipeptide (27a–c) was obtained (50% yield) which without further purification was suspended in abs. tetrahydrofuran and after adding an equimolar amount of dry triethylamine the precipitate was filtered off, washed with water

* With the participation of Yu. I. Krylova.

† With the participation of T. R. Telesnina.

[55] C. Schotten, *Ber. Dtsch. Chem. Ges.* **21**, 2239 (1888).

[56] V. K. Antonov, *Izv. Akad. Nauk. SSSR, Otdel. Khim. Nauk* 1135 (1963).

and crystallized. The tripeptides (30a, b) were also prepared by counter synthesis according to Ref. 57.

B. *Imidazolinones* (29a, b). The hydrobromide of (27a, b), prepared in experiment 4-A was dissolved in a small volume of water and stirred for 5 min. The precipitate was filtered off, washed with water, dried and crystallized.

5. *Synthesis of hydroxyacylbutyrolactams* (31a–d) (*Table* 4)

N-Benzyloxyacylbutyrolactams (16a; 17a, d) and (19a) were hydrogenated in tetrahydrofuran over Pd-black. The catalyst was separated and the solvent evaporated in vacuum. The residue was crystallized by triturating with ethyl acetate–hexane mixture, while in the case of (31b) it was subjected to chromatography on Al_2O_3 (activity II) with gradient elution by ether–hexane (1:1) → ethyl acetate.

6. *Hydroxyacyl incorporation into cyclic amides* (*Tables* 5 *and* 6)

A. *Cyclodepsipeptides* (34), (35a) *and* (36). These compounds were formed in almost quantitative yield by hydrogenation of solutions of N-benzyloxyacyllactams (17b, c) and (16d) in tetrahydrofuran over Pd-black.

B. *Cyclodepsipeptide* (35b)*. On hydrogenolysis of compound (17e) using the conditions of the previous experiment a mixture was formed from which the cyclodepsipeptide (35b) could be isolated in only 15% yield after multifold crystallization from dichloroethane. Evaporation of the combined mother liquors and recrystallization from alcohol gave N-phthalylserylcaprolactam in 50% yield, m.p. 131° (from toluene); ν_{max}^{nujol} (cm^{-1}): 1780, 1725, 1710, 3420. This compound when refluxed in toluene with 1 mole of triethylamine for 1 hr was converted into the cyclodepsipeptide (35b) in 30% yield.

C. *Cyclodepsipeptides* (37a–g). Bis-benzyloxyacyldiketopiperazines (21a–g) were hydrogenated as above, filtered, evaporated and the residue dissolved in acetone. After keeping for 3–7 days at 20–25°, the precipitated cyclodepsipeptide was recrystallized.

D. *Serratamolide* (37h) *and its analogue* (37i). O,O'-Diacetylserratamolide (37e; 25 mg) was dissolved in a mixture of 5 ml $CHCl_3$, 1 ml 30% HCl in CH_3OH and 0·05 ml H_2O. The homogeneous solution was allowed to stand for 2 days at 20°, evaporated and the residue dissolved in MeOH, following which it was subjected to thin layer chromatography with aqueous silicic acid in the systems $CHCl_3$–AcOEt (1:3) and Me_2CO–$CHCl_3$–AcOEt (1:2:6) using biosynthetic serratamolide as reference substance. The R_f values of the reference substance and the hydrolysis products coincided for both systems. The cyclodepsipeptide (37i) was prepared similarly by hydrolysis of compound (37g).

E. *Cyclodepsipeptides* (39a, b). By hydrogenation of bis-(benzyloxyacyl)-cyclodepsipeptides (23a, b) under the usual conditions bis-(hydroxyacyl)-cyclodepsipeptides (38a, b) were obtained, which were heated in dimethylformamide for 10–15 min or are left to stand in this solvent for 2 days at 20°, following which on evaporation the cyclodepsipeptides (39a, b) were obtained.

F. N → O *Acyl migration in bis-(hydroxyacyl)-diketopiperazines.* Compounds (43a, b) and (45) were formed on hydrogenolysis of the corresponding benzyl derivatives (21h, i) and (44) over Pd-black in tetrahydrofuran. After filtration and evaporation the residue was dissolved in abs. alcohol, allowed to stand for 24 hr at 0° and the precipitate is filtered off and recrystallized.

G. *Lactonolactam* (41)†. Nonadecane-1,17-dicarboxylyl chloride (7·3 g; prepared from 8 g of the acid and $SOCl_2$) was dissolved in 3 l. abs. C_6H_6 and a solution of 3·32 g O-benzylethanolamine and 12·2 ml triethylamine in 300 ml abs. C_6H_6 added at 80° in the course of 30 hr. (with the aid of a linear dosing device). The mixture was filtered, the filtrate evaporated and the residue chromatographed on neutral Al_2O_3 (activity II) with gradient elution by $CHCl_3$–hexane (1:20) → $CHCl_3$. From the first fractions 0·8 g (8%) of the imide (40a) was obtained in the form of an oil; ν_{max}^{film} (cm^{-1}): 1705 (CONCO). Hydrogenation of the latter over Pd-black in tetrahydrofuran solution yielded (after the usual treatment) 0·4 g (65%) of lactonolactam (41)

7. *Aminoacyl incorporation into cyclic amides* (*Table* 8)

A. *Cyclodipeptides* (54; $n = 1, 2, 3$) *and* (59). (a) The acyllactams (18a–d; 2 g) were hydrogenated in 100 ml abs. alcohol over Pd-black. After the usual treatment the oily residue was crystallized by

* With the participation of L. I. Andreeva.

† With the participation of V. S. Morgulyan.

[87] F. E. King, J. W. Clark-Lewis, R. Wade and W. A. Swindin, *J. Chem. Soc.* 873 (1957).

trituration with a small amount of alcohol and then recrystallized. (b) The acyllactams (18a, c) were treated with a 27% solution of HBr in AcOH. The mixture was allowed to stand for 45 min, the solvent distilled off in vacuum and abs. ether added to the residue. The resultant β-alanyllactam hydrobromide was carefully washed with ether and without further purification treated with Ag_2O in aqueous solution. After filtration the solution was evaporated *in vacuo* and the residual cyclodipeptide recrystallized. (c) A solution of 0·2 g of the acylamidine (56; n = 1, 2, 3) in 2 ml water was allowed to stand for 2 days at 20°, following which a 20-fold amount of acetone was added and the precipitated acid (55; n = 1, 2, 3) filtered off. The cyclodipeptide (54; n = 2, 3) was obtained by evaporating the filtrate. Under the above conditions the compound (56; n = 1) yielded only the acid (55; n = 1). In order to obtain the cyclodipeptide (54; n = 1) from (56; n = 1) the aqueous solution of the latter was stirred with Ag_2O for 5 min and after filtration, addition of acetone and removal of the acid (55; n = 1) the filtrate was evaporated and the residual cyclodipeptide recrystallized. (d) The cyclodipeptide (54; n = 1) was allowed to stand in aqueous solution for 48 hr, following which it was subjected to the usual treatment yielding (55; n = 1). Under the above conditions compounds (54; n = 2, 3) remain unchanged.

B. *Acylamidines* (56; *n* = 1, 2, 3). (a) To a solution of β-alanine in abs. MeOH an equimolar amount of the corresponding O-methyllactim was added dropwise during 20 min; the mixture was boiled for 10 min., filtered and diluted with abs. ether. The precipitated amidino acid (55; n = 1, 2, 3) was heated in toluene or 1,3-dichlorobenzene with azeotropic distillation of water. After removal of the theoretical amount of water the solvent was distilled off and the acylamidine purified by distillation. (b) The cyclopeptide (54; n = 1, 2, 3; 0·5 g) was heated in xylene for 1 hr with azeotropic distillation of water. This resulted in the formation of (56; n = 1, 2) from (54; n = 1, 2), whereas the cyclopeptide (54; n = 3) remained unchanged under the prevailing conditions.

8. *Preparation of the hydroxyacylamides* (64) *and* (70) *and cyclols* (65), (68), (71) *and* (78) (*Table* 9)

These compounds were prepared by hydrogenolysis of the α-benzyloxyacyllactams (16b, c, e), α-benzyloxyacyldiketopiperazines (20a–e) and bis-(benzyloxyacetyl)-diketopiperazine (20f) in abs. tetrahydrofuran over Pd–black. After filtering off the catalyst, the solvent was evaporated *in vacuo* at a bath temp of 3–5°. In the case of the compound (16b) the solid cyclol (68a) is rapidly crystallized. In the case of the substance (16c) the resultant oily product (64) ⇄ (65) was distilled. The cyclols (68b) and (71b, d, e) and the hydroxyacyldiketopiperazine (70a) obtained by hydrogenolysis of compounds (16e) and (20a, d, e) are crystalline solids. Hydrogenolysis of compound (20c) afforded the hydroxyacyldiketopiperazine (70c), as an oil which on standing for 10–15 days over P_2O_5 was converted into a glassy product. The latter was triturated with ether and the crystalline cyclol (71c) which resulted was recrystallized. The oil obtained on hydrogenolysis of (20f) was dissolved in MeOH and after 1 hr the cyclol (77) was filtered off.

9. *Preparation of the cyclols* (74a, b) (*Table* 9)

To a solution of 17 mmoles acyllactam (19b, c) in 15 ml abs. tetrahydrofuran, 16 mmoles triethylamine was added and the mixture hydrogenated in the presence of 100 mg PdO, following which 50 ml warm tetrahydrofuran was added. The catalyst was filtered off and the filtrate distilled off in vacuum. The residue was washed with 2% HClaq, water, and ether and then recrystallized.

10. *Reactions of hydroxyacylamides and cyclols* (*Tables* 10 *and* 11)

A. O-*Alkylation of cyclols.* (a) The cyclol (0·05 moles) was added with stirring to a mixture of 25 ml MeI and 25 g finely ground Ag_2O. The mixture was stirred for another 3 hr and filtered, the precipitate being washed with methylene chloride. The filtrates were distilled and the residue crystallized. In this way the O-methylcyclols (88a–c) were prepared. In the methylation of the cyclol (68a) methyl 4-methoxyacetylaminovalerate (yield 33%, b.p. 114–115°/4·10⁻³ mm, n_D^{20} 1·4610, ν_{max}^{film} 1745 (CO-est.), 1680 (amide I), 1549 (amide II), 3350 (NH) cm^{-1}) was obtained besides the O-methylcyclol (88a). In the case of the cyclol (74b) a mixture of the compounds (88d) and (89c) was formed, which was separated by thin layer chromatography on Al_2O_3 in the system ethyl acetate–hexane (1:4). Methylation of the cyclol (71c) to the compound (90) was carried out in dimethylformamide solution at 20° for 16 hr. (b) The compound obtained by hydrogenolysis of (20 f) was refluxed for 10 min in MeOH and on cooling the methyl ether (78) was obtained. The compound (79) prepared by hydrogenolysis of (22) was left to stand for 24 hr in EtOH containing traces of HCl. After distilling

off the solvent the residue was treated with a small amount of cold alcohol and the crystalline ethyl ether (80) was then removed. From the filtrate after evaporation, ethyl β-hydroxypropionylglycyl-sarcosinate (82) was obtained as an oil, ν_{max}^{film} (cm^{-1}): 1757 (CO-est.), 1659 (amide I), 1560 (amide II), 3350 (NH and OH). Compound (82) is identical with a specimen prepared by counter synthesis.

Methylation of (79) using the conditions of experiment 10-A (a) yielded the methyl ether (81) and the linear peptide (83), also obtained by counter synthesis; oil, ν_{max}^{film} (cm^{-1}): 1756 (CO-est.), 1670 (amide I), 1539 (amide II), 3355 (NH).

B. *Methoxyacyllactams* (89a–c), (91). These compounds were formed from methoxyacyl chlorides and lactams using the conditions of experiment 1-D.

C. *Hydroxyacylamideurethanes* (92a–c) *and* (93a, b). α-Naphthylisocyanate (1 mmole) was added to a methylene chloride solution of 1 mmole of an equilibrium mixture of the cyclol and hydroxyacyllactam forms (64 ⇄ 65) or (67a ⇄ 68a). The reaction was carried out at 20° in the presence of 1 or 2 drops of triethylamine. After allowing the mixture to stand for 48 hr it was filtered, the filtrate evaporated and the residue triturated with hexane or ether. In the case of N-glycolyl-butyrolactam (31a) the corresponding naphthylurethane (92a) was obtained by heating with α-naph-thylisocyanate at 40° for 1 hr. The naphthylurethane (93a) was prepared under the same conditions. The phenylurethane (93b) was obtained from (79) and phenylisocyanate in dioxane (2 days, 20°).

D. *Desoxycyclols* (94a, b). (a) The cyclol (74a, b) was suspended in 10 ml tetrahydrofuran and after addition of 1–2 drops conc. HClaq the mixture was hydrogenated over Pd–black. The initial cyclol completely dissolved on absorption of 1 mole of H_2. The mixture was filtered, the filtrate evaporated and the residual oil crystallized by trituration with hexane. (b) The acyllactam (19b, c) was dissolved in warm tetrahydrofuran and hydrogenated over Pd–black in the presence of 1 or 2 drops of conc. HClaq, 2 moles of H_2 being taken up. The mixture was treated as in the foregoing experiment.

E. *Bromides* (95a–c). (a) Acyllactam (19a–c; 3 mmoles) and 8 ml 20% solution of HBr in glacial acetic acid were heated in a sealed tube for 2·5 hr at 75°. The solution was then evaporated *in vacuo* and the residue triturated with hexane and abs. ether, following which the crystallized product was washed with abs. acetone and abs. ether. (b) The cyclols (74a, b) or salicylylbutyrolactam (31c) were dissolved in a 10-fold amount of 20% HBr in glacial AcOH and left to stand for 1 hr at 20°. The mixture was then evaporated and the residue treated as described in the previous experiment.

F. *Fluoborate* (95d). The methyl ether (88d; 1 mmole) was dissolved in 1·5 ml freshly distilled $BF_3 \cdot Et_2O$ and the solution kept for 15 min at 35°, following which another 1·5 ml $BF_3 \cdot Et_2O$ was added and the mixture kept at the above temp for another 1·5 hr. It was then diluted with 40 ml abs. ether and the oil produced crystallized by trituration with abs. ether.

11. *Reaction of compounds* (95a–d) *with water*

The salt (95a–d; 0·3 g) was shaken for 15 min with 2 ml water and 3 ml ether, the crystals which had initially deliquesced, again solidifying. They were filtered off, washed with water and then with ether. The salts (95b, c, d) yielded the cyclols (74a, b), whereas the salt (95a) yielded the salicylyl-butyrolactam (31c) (Tables 4 and 9).