INTRAMOLECULAR REARRANGEMENTS IN PEPTIDE SYSTEMS: HYDROXY- AND AMINO-ACYL INCORPORATION INTO PEPTIDES

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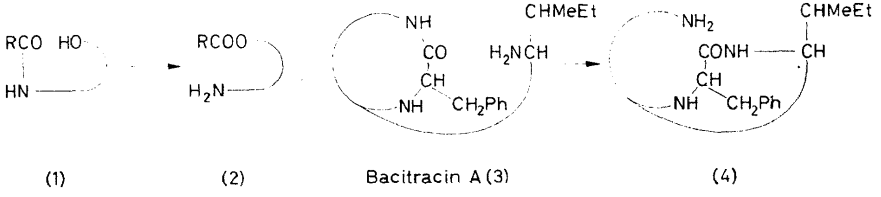
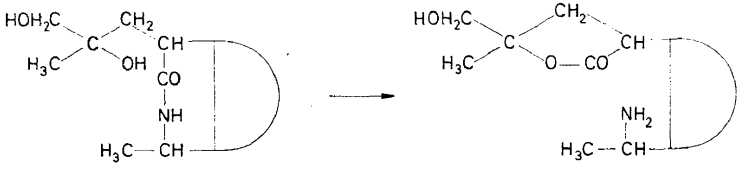
Intramolecular rearrangements in peptide systems, associated with the conversion of amide groups into labile intermediate structures that endow the molecules with peptide-atypical properties have long attracted the attention of research workers; since fundamental changes can thereby take place in the molecular structure. However, such rearrangements have as yet not been sufficiently elucidated although they may play an essential part in the chemistry and biochemistry of peptides and proteins.

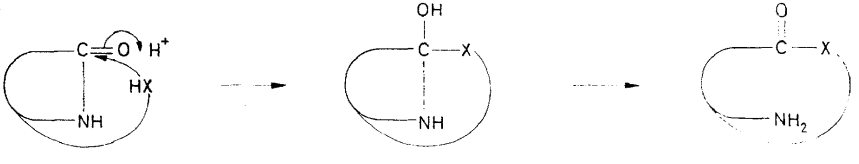
The resonance-stabilized amide group is rather inert towards nucleophilic reagents. However, the existence of such processes as enzymic hydrolysis or transpeptidation, shows that Nature possesses the means to augment greatly its reactivity.



(1) (2) Bacitracin A (3) (4)



Phalloidine (5) Secophalloidine (6)

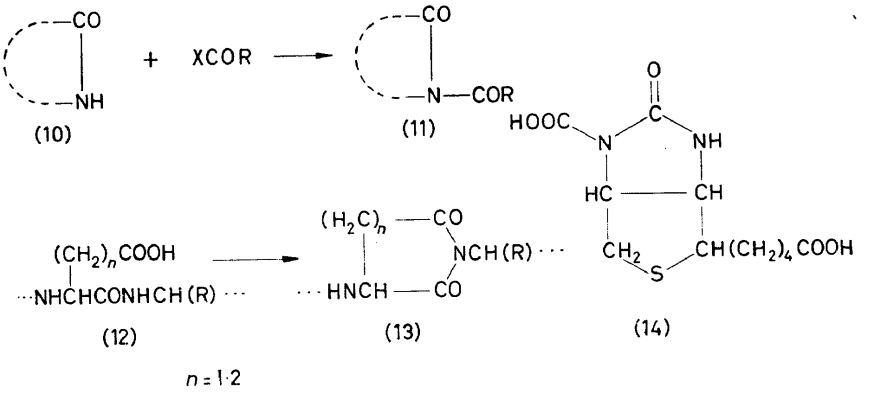


(7) (8) (9)

X = NH, O, S

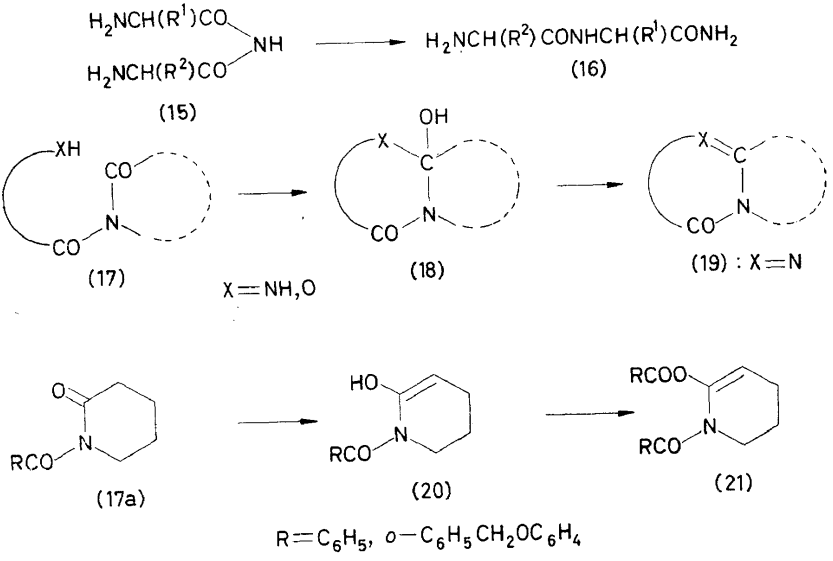
Numerous cases are also known when nucleophilic reactions of amides occur under mild conditions in the absence of enzymes. Among them one can mention, for example, the well-known $N \rightarrow O$ acyl migrations[1] in peptides and proteins (1) → (2) or the formation of anomalous cleavage products in the acid hydrolysis of such naturally occurring peptides as bacitracin A (3) → (4), phalloidine (5) → (6) and others[2]. In all such cases the amino or hydroxy group reacts with the amide grouping to form a new amino group and a new amide or ester grouping. Activation of the amide group in these cases may be explained by an increase in the positive charge on the carbonyl carbon due to *O*-protonation (7) on suitable steric location of the attacking nucleophilic group. Rearrangements of the type (7) → (9) apparently proceed through the hypothetical amides of the ortho acids (8), whose formation has been repeatedly postulated[3].

One can, however, conceive of another possible way of activating the amide group, due to the incorporation of electron acceptor substituents, for example, by *N*-acylation of the amides (10) → (11). There are at present few data regarding the formation of *N*-acylamides in biological systems; but, the ease with which *N*-acylamides are known to appear in peptides containing glutamic or aspartic acid residues (12) → (13)[4], the possibility of the enzymic synthesis of *N*-carboxybiotin (14)[5] and the existence of such active biological acylation agents as thioesters and mixed anhydrides with phosphoric acid derivatives, lends considerable support to the assumption that *N*-acylamides participate in biochemical processes. All this led us to propose a mechanism for the biosynthesis and metabolism of some peptides and depsipeptides which is presented in the following.



N-Acylamides are incomparably more active than ordinary amides towards nucleophilic reactants. For instance they very readily undergo hydrolysis and aminolysis including the intramolecular reaction (15) → (16)[6]. Our studies have shown that in respect of their electrophilicity, the carbonyl groups of *N*-acylamides are comparable with those of aldehydes and ketones. In particular, *N*-hydroxyacyl- and *N*-aminoacylamides (17) very readily afford the cyclols (18)[7], the acylamidines (19)[8] and also, *via* enolization, the esters (21)[9].

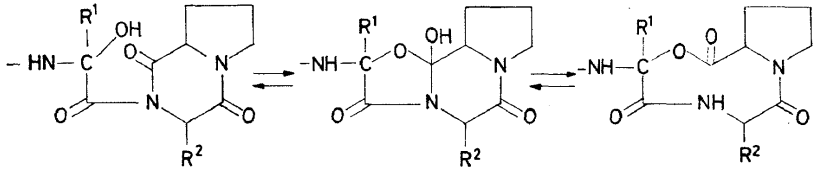
The appearance of *N*-acylamide groupings in peptides may lead to far going structural changes due to intramolecular interaction of these groupings with various nucleophiles. We discussed the possibility of such isomeric or tautomeric transformations between *N*-hydroxyacylamides, cyclols and depsipeptides (22) ⇆ (23) ⇆ (24) for the first time at the Basle Peptide



Symposium in 1960 in connection with the problem of synthesizing the peptide moiety of ergot alkaloids[10]. A similar idea was later put at the basis of the ergotamine synthesis carried out by Hofmann and collaborators[11]. In the course of our studies[7–10, 12–28] it appeared that such conversions are of a very general character, being a new universal reaction for the incorporation of hydroxy-, amino- and possibly mercapto-acids into the peptide chain or ring, with the formation of linear or cyclic peptides, depsipeptides and thiodepsipeptides (25) → (26) → (27).

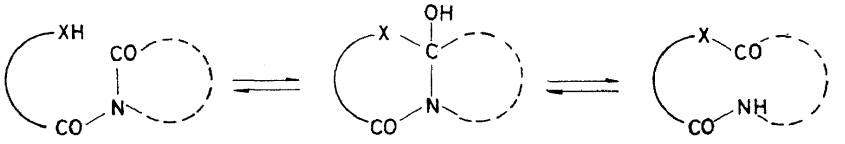
The course of this reaction depends mainly on three factors: the electrophilicity of the acylamide carbonyls, the nucleophilicity of the attacking XH group and the extent to which the peptide or depsipeptide structure (27) is energetically preferable to that of the initial acylamide (25) or intermediate cyclol (26). In certain cases, for reasons that will be dwelt on later, the reaction stops at the stage of oxa or azacyclols (26), the latter spontaneously eliminating water and transforming into the more stable acylamidines (19).

We investigated the reaction of hydroxy- and amino-acyl incorporation on a large number of linear and cyclic peptides, acylated by various hydroxy and amino-acid residues. The initial *N*-acylamides are easily prepared by acylation of the amides (29) with hydroxy- or amino-protected acyl chlorides. The reaction is carried out either by heating in an inert solvent or in the cold in the presence of triethylamine. With α- and β-hydroxyacyl residues the hydroxyl group is protected by benzyl (28) and with β-aminoacyl residues the amino group is protected by the benzyloxycarbonyl grouping (31)[7, 8, 28].

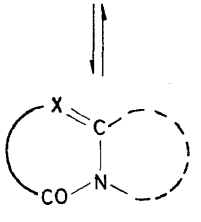


(22) (23) (24)

Ergotamine: $R^1 = CH_3$, $R^2 = CH_2C_6H_5$

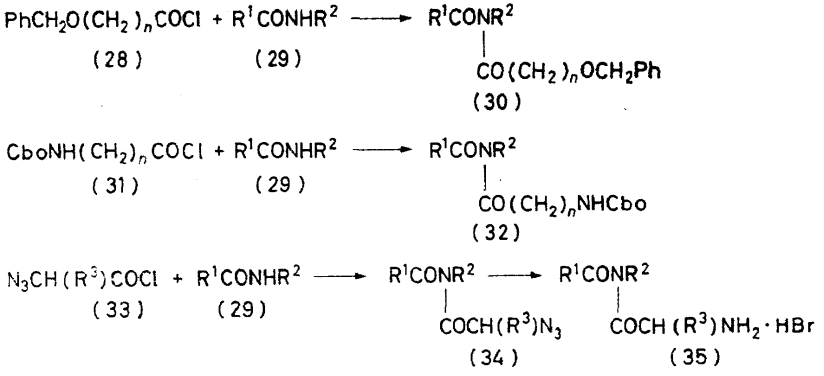


(25): X = NH, O, S (26): X = NH, O, S (27): X = NH, O, S



(19): X = N

The agents best suited for incorporating α-aminoacyl residues are the azidoacyl chlorides (33)[8, 27]. The latter are a very convenient form of *N*-protected amino-acids, being sufficiently stable under the acylating conditions and easily converted into the hydrobromides of the amino derivatives (35) by hydrogen bromide in glacial acetic acid. The *N*-acylamides are for the main part crystalline compounds, stable in inert solvents, but readily hydrolysed in solutions of acids or bases. Their infrared spectra exhibit bands in the region 1700–1740 cm^{-1}, characteristic of the CONCO group and in the ultraviolet region they absorb appreciably at 210–220 mμ[7, 12].

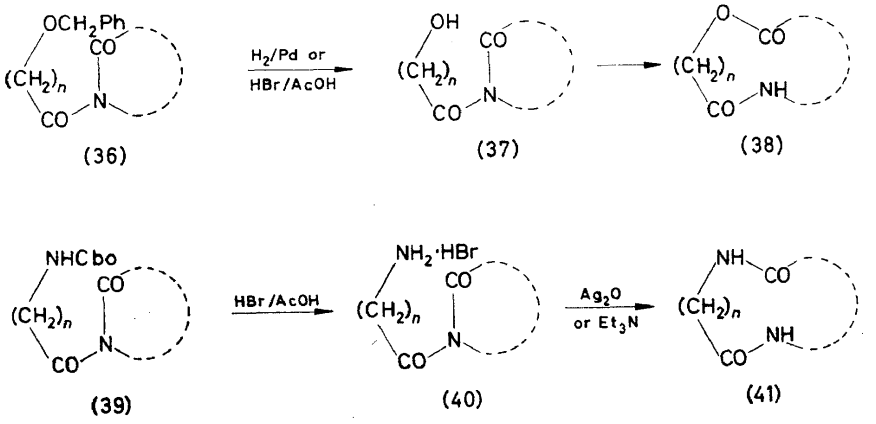


(28) (29) (30)

(31) (29) (32)

(33) (29) (34) (35)

Removal of the protective groups is achieved by hydrogenolysis of the acylated products (36) and (39) in the presence of palladium in tetrahydrofuran or alcohol solution, or by the action of hydrogen bromide in glacial acetic acid[28]. In the latter case the aminoacylamides are liberated from the hydrobromides (40) by triethylamine in tetrahydrofuran or by silver oxide in aqueous alcohol[8, 28].



(36) (37) (38)

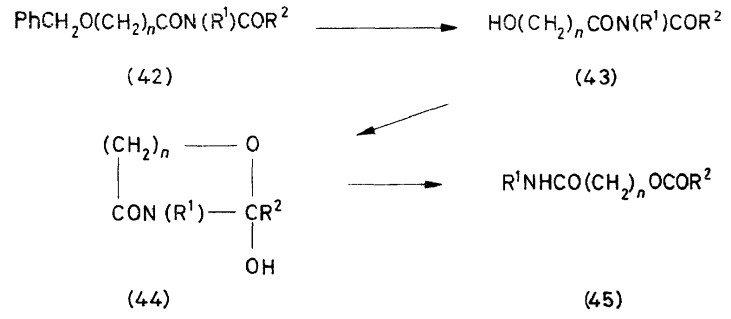
(39) (40) (41)

In the majority of cases the hydroxy and aminoacyl incorporation reaction takes place spontaneously and only in isolated cases is heat or the presence of bases required.

The structure of the resultant linear and cyclic depsipeptides (38) and peptides (41) was proved with the aid of their infrared, ultraviolet, nuclear magnetic resonance and mass spectra, and in certain cases by counter synthesis. Since the incorporation leads to the formation of an ester and amide or of two amide bonds, infrared spectroscopy provides a convenient structural proof, formation of the amide group being followed by appearance of the amide I and amide II bands of which the second band can be reliably identified through deutero-exchange. In some cases it is feasible to determine the number of amide groups in the compound from the integral intensity of the amide carbonyl bands.

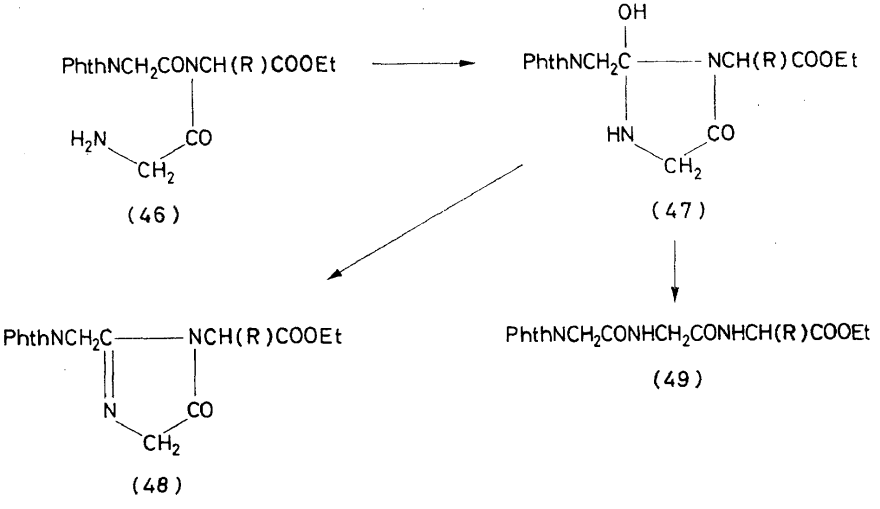
Let us look somewhat more closely into the behaviour of some types of *N*-hydroxyacyl and *N*-aminoacylamides in the incorporation reaction.

Linear *N*-hydroxyacylamides of type (43) isomerize into the respective depsipeptides (45) with varying readiness, depending on the nature of the acyl residue in the initial amide or peptide[16, 17, 25]. Thus, *N*-glycolyl-*N*-methylacetamide (43a) isomerizes spontaneously to the final product (45a) immediately after hydrogenolysis of the initial benzyloxy derivative (42a), whereas formation of the depsipeptides (45b-e) containing a phthaloyl residue takes place only on heating the alcohol solutions or in the presence of bases. This difference is apparently due to the considerable electrophilicity of the phthaloyl residue which hinders the incorporation reaction.



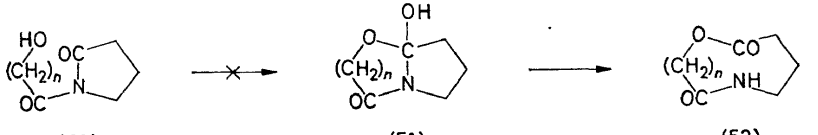
a : $n = 1$, $R^1 = R^2 = CH_3$; b : $n = 1$, $R^1 = CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$;
c : $n = 1$, $R^1 = CH_2CO_2CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$; d: $n = 2$, $R^1 = CH_3$,
$R^2 = CH_2N(CO)_2C_6H_4$; e: $n = 2$, $R^1 = CH_2CO_2CH_3$, $R^2 = CH_2N(CO)_2C_6H_4$.

Aminoacyl incorporation into the peptide chain proceeds similarly to hydroxyacyl incorporation. We studied this process on the example of phthaloyldipeptide esters acylated by α-amino acids (46), azidoacyl chlorides being utilized as the acylating agent. In this case, besides the normal incorporation products, namely the corresponding tripeptides (49), imidazolinones of the type (48) are formed, evidently due to dehydration of the intermediate azacyclols (47)[8, 27].



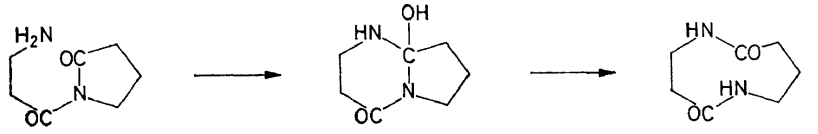
The factors which determine the course of the incorporation reaction can be distinguished particularly well in the case of hydroxy and amino-acid incorporation into cyclic amides, *viz.*, lactams, diketopiperazines and cyclodepsipeptides. Thus, the diminished electrophilicity of the carbonyl carbon characteristic of five-membered rings is manifested in the absence of

spontaneous isomerization of *N*-hydroxyacylbutyrolactams (50) to the corresponding cyclols (51) or macrocycles (52)[7]. On the other hand, *N*-(β-alanyl)-butyrolactam (53), which contains an amino group, a much stronger nucleophile than hydroxyl, readily isomerizes to the cyclodipeptide (55)[8].



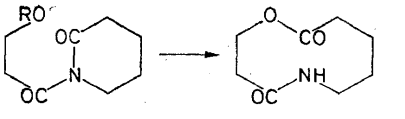
(50) (51) (52)

n = 1 or 2

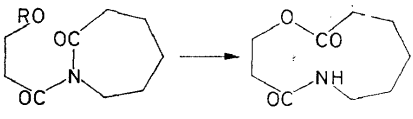


(53) (54) (55)

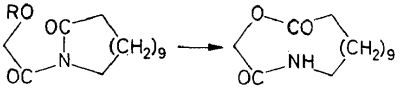
Further, it turned out that incorporation of hydroxy and amino-acids into cyclic amides proceeds with sufficient ease only when the size of the resultant ring exceeds a certain critical value characteristic for the given type of compounds. When the ring size is below the critical value, transannular interaction arises between the amide groups and the ester or other amide group, so that the macrocyclic structure becomes energetically less advantageous than the corresponding cyclol structure. In fact, incorporation of hydroxy acids into lactams, diketopiperazines and cyclodepsipeptides proceeds readily and with high yields only when the resultant cyclodepsipeptides contain 11 or more atoms in the ring. The formation of 10-membered cyclodepsipeptides goes sluggishly and sometimes not at all; 9-membered rings in general have not been observed to form in this way. As illustration of the above-mentioned hydrogenolysis of the *N*-benzyloxyacylated lactams (56), (59) and (62) results in the spontaneous formation of the corresponding 10-, 11- and 16-membered cyclodepsipeptides[13, 16], whereas isomerization of *N*-(β-hydroxypropionyl)-glycylsarcosyl-lactam (66) only slowly gives the 10-membered cyclodepsipeptide (67)[17].



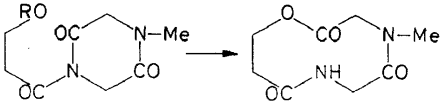
(56): R = CH_2Ph (58)
(57): R = H



(59): R = CH_2Ph (61)
(60): R = H

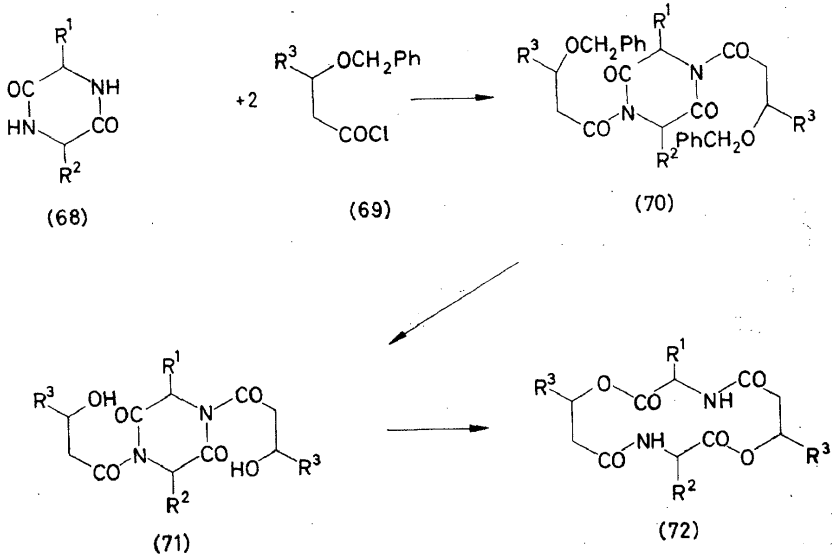


(62): R = CH_2Ph (64)
(63): R = H



(65): R = CH_2Ph (67)
(66): R = H

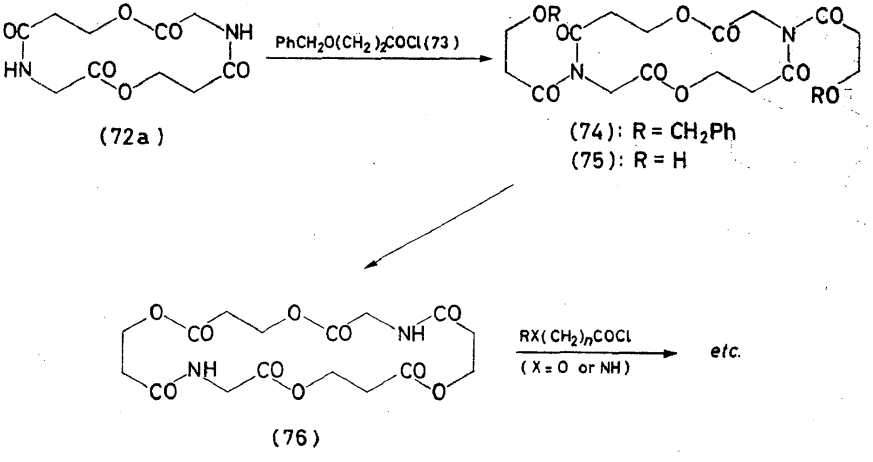
Acylation of both amide groups in the diketopiperazines (68) by β-benzyloxyacylchlorides (69) yielded the bis-(β-benzyloxyacyl) derivatives (70), whose hydrogenolysis directly afforded the 14-membered cyclodepsipeptides (72), built of two β-hydroxy acid and two α-amino-acid residues[14–17, 26]. This two-stage method for the synthesis of cyclotetradepsipeptides proved to be very convenient from a preparative aspect, not only



(68) (69) (70) (71) (72)

a: $R^1=R^2=R^3=H$; b: $R^1=R^2=CH_3$(L), $R^3=H$; c: $R^1=R^2=H$, $R^3=CH_3$;
d: $R^1=R^2=CH_2OAc$, $R^3=H$

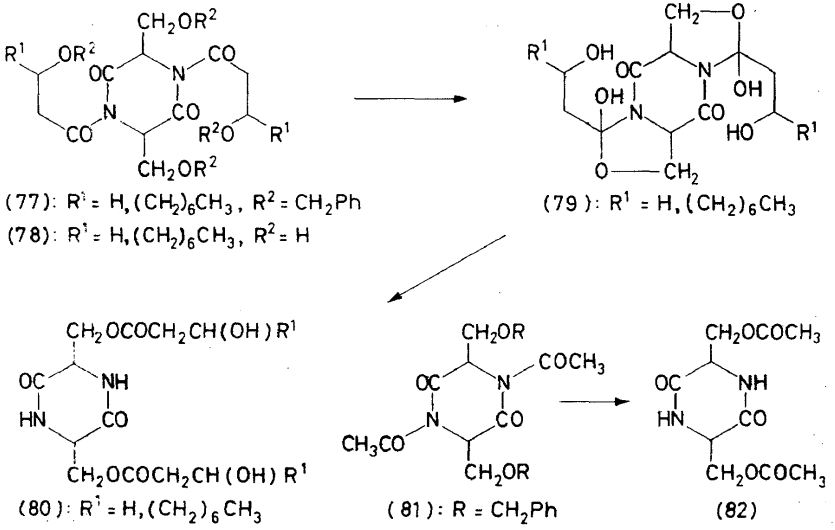
because of the small number of stages, but also because of the absence of racemization of the amino and hydroxy acid residues, as well as of the high yields, which usually amount to 50–60 per cent, but in some cases may even reach 90 per cent. The incorporation reaction could again be realized by



(72a) (74): R = CH_2Ph (75): R = H (76)

bis-acylation of the 14-membered cyclodepsipeptide (72a) and hydrogenolysis of the acylation product (74), the 22-membered cyclohexadepsipeptide (76) now being obtained. In this case, after hydrogenolysis the bis-(hydroxyacyl) derivative (75) can be isolated, which isomerizes to the cyclohexadepsipeptide on heating in alcohol for a short time. In principle one should be able in this way to enlarge the ring still further.

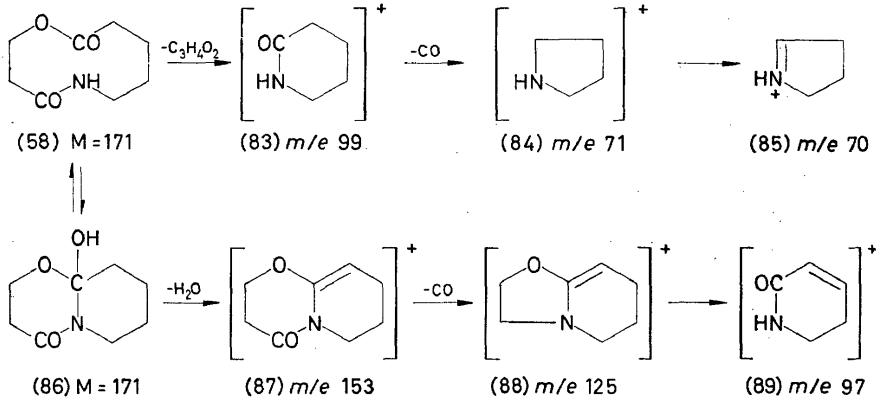
It should be noted, however, that the course of the reaction may change if the *N*-hydroxyacyllactam molecule contains other nucleophilic groupings. Thus, in the hydrogenolysis of the tetrabenzyl derivatives of bis-(β-hydroxyacyl)-serylseryllactams (77) migration of the acyl residue to the serine hydroxyl takes place (78) → (79) → (80). Similar conversions occur in the case of *N*,*N*-bisacetyldiketopiperazine (81) → (82)[20].



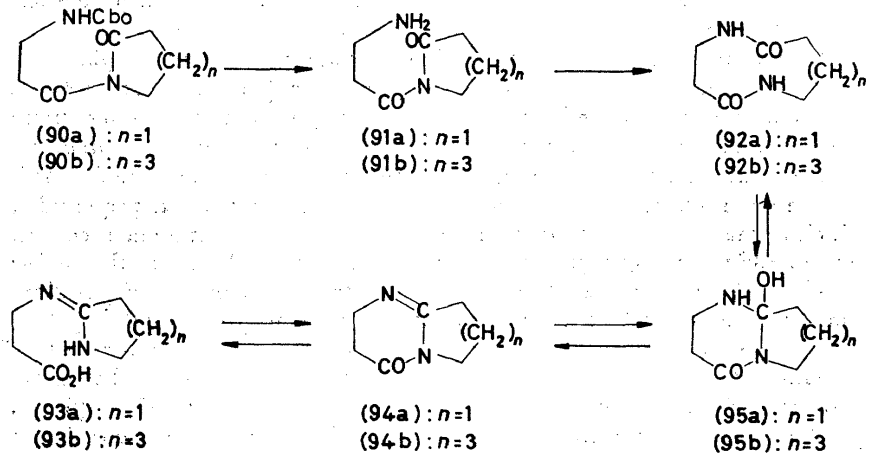
Cyclopeptides and cyclodepsipeptides with medium 9–10 membered rings deserve special consideration, because they exhibit peculiar properties, due to transannular amide–amide or ester–amide interaction.

Whereas the 11-membered cyclodidepsipeptide (61) does not display such interaction, the 10-membered cyclodidepsipeptide (58) manifests it[13] in a bathochromic shift of the infrared light absorption band of the ester group in carbon tetrachloride solution, as well as in the augmented rate of deutero-exchange and particularly in the mass spectroscopic fragmentation. With this compound fragmentation typical of the medium ring cyclodepsipeptides, which begins with the formation of the lactam ion (83), is supplemented by a considerable contribution from fragmentation typical of all the stable cyclols we have investigated, which is characterized by initial dehydration, leading to the ion (87), and the subsequent conversions (87) → (88) → (89)[18].

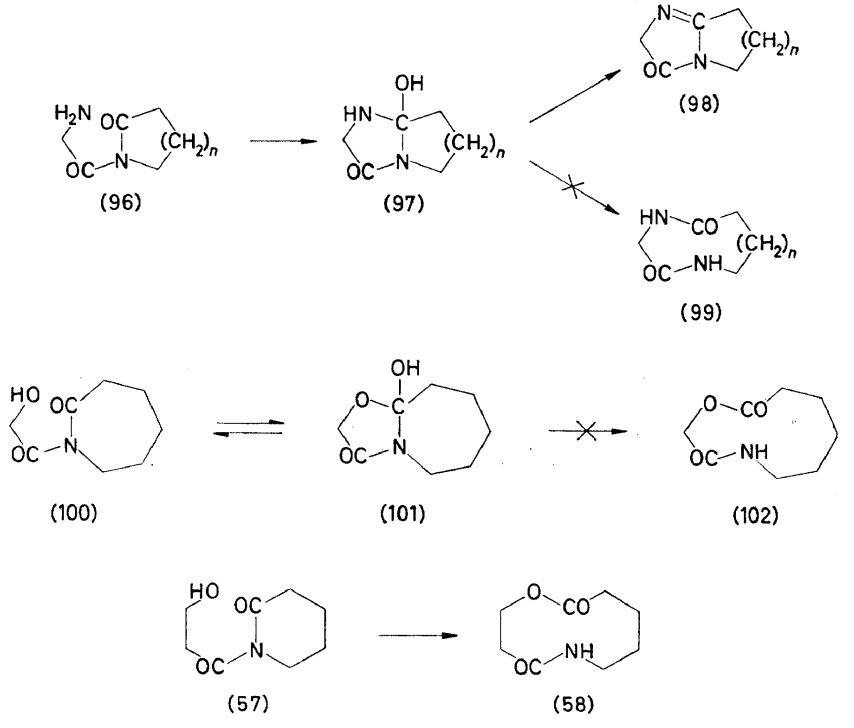
Transannular interaction has been observed by us both in the case of 9-membered and also 11-membered cyclodipeptides, obtained by incorporation of the β-alanine residue into the lactam ring (90) → (91) → (92)[8, 27]. Indeed, the mass spectrometric behaviour of the 9-membered



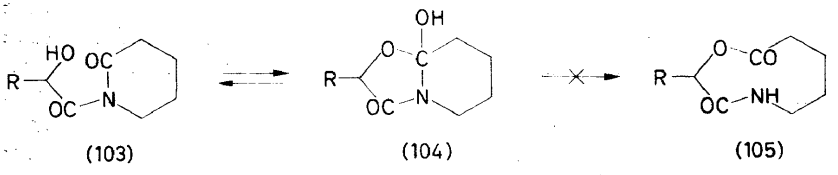
(92a) and, to a lesser degree, of the 11-membered (92b) cyclodipeptide, shows that azacyclol structures of the type (95) form under these conditions; in both cases fragmentation begins with elimination of water and then coincides to a considerable degree with the fragmentation undergone by the corresponding bicyclic acylamidines (94). Acylamidine formation is also observed simply on heating the 9-membered cyclodipeptide (92a), whereas heating it in aqueous solution causes further decomposition to the amidino-acid (93a). The 11-membered cyclodipeptide (92b) is converted into the corresponding acylamidine (94b) under less mild conditions. On the other hand this acylamidine (94b) was found capable of undergoing hydration with the formation of the initial cyclodipeptide (92b) even on standing in air, whereas the acylamidine (94a) is converted into the 9-membered cyclopeptide (92a) only under more drastic conditions, for example, by treatment with water in the presence of silver oxide. These reactions demonstrate both the possible ways for cleavage of the intermediately forming azacyclols (95) and the relative stabilities of the 9- and 11-membered cyclodipeptides (92)[8, 27].



The stability of the macrocyclic systems depends not only on the size of the ring, but, at least in the case of 9 to 11 membered rings, also on the relative positions of the amide groups or of the amide and ester groups. Thus, it was shown by Reinisch[29] and Rothe[30] that the only products of the reaction between the amino group and the endocyclic carbonyl in *N*-(α-aminoacyl)-lactams of the type (96) are the corresponding acylamidines (98). We have also observed this in the case of *N*-(α-hydroxy-acetyl)-caprolactam (100). In solution this compound exists in tautomeric equilibrium with the cyclol form (101), but it is not spontaneously transformed into the 10-membered cyclodidepsipeptide (102). On the other hand, as was mentioned above, *N*-(β-hydroxypropionyl)-valerolactam (57) quite readily isomerizes directly to the 10-membered cyclodidepsipeptide (58). A comparison of the molecular models of (58) and (102) shows that the second has a much more strained ring, which thus determines the above differences.

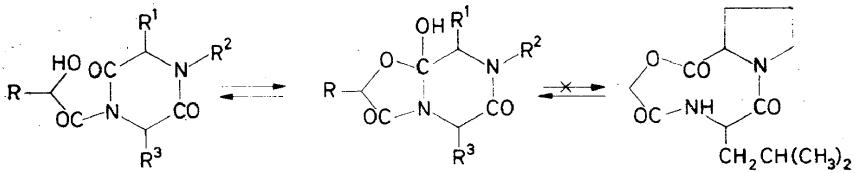


The strain becomes more pronounced on passing to 9-membered rings, so that the *N*-(α-hydroxyacyl)-valerolactams (103) and *N*-(α-hydroxyacyl)-diketopiperazines (106) we investigated exist either only as the cyclol (104) or (107) or in tautomeric equilibrium with it[7, 12, 23, 24]. Moreover, Sheppard[31] recently showed that even when the 9-membered cyclodepsipeptide (108) can be obtained by some roundabout way, it easily and irreversibly isomerizes into the cyclol (107d).



(103) (104) (105)

a: R=H; b: R=CH_3



(106) (107) (108)

a: R=R^1=R^3=H, R^2=CH_3; b: R=R^2=CH_3, R^1=R^3=H;

c: R=R^3=H, R^1+R^2=$(CH_2)_3$; d: R=H, R^1+R^2=$(CH_2)_3$,

R^3=$CH_2CH(CH_3)_2$; e: R=CH_3, R^1+R^2=$(CH_2)_3$, R^3=CH_2Ph

(109) (110)

a: n=2; b: n=3

Cyclol formation takes place also with *N*-(hydroxyaroyl)-lactams (109). In contrast to the above described examples, in this case cyclols both with ten (110a) and eleven (110b) atoms in the bicyclic system are stable. This is due to the presence of a benzene ring in the molecules which stabilizes the cyclol system by flattening the latter[17, 22].

Both the stability of the cyclols and the position of the tautomeric equilibrium between the cyclol and acylamide forms are strongly dependent on structural factors. Thus, in tetrahydrofuran solution *N*-glycolylvalerolactam (103a) is to a large extent in the form of the cyclol (104a). The latter can be isolated as crystals that on dissolution revert to the equilibrium mixture with the *N*-hydroxyacylamide form. Crystalline *N*-glycolylglycylsarcosyllactam (106a) in tetrahydrofuran solution only partially isomerizes to the cyclol (107a). Tautomeric equilibrium is also observed in the case of glycolylglycylprolyllactam (106c). On the other hand *N*-lactyl derivatives of the lactams (103b) and diketopiperazines (106b, e) completely isomerize to cyclols and no tautomeric conversions are observed. It thus follows that the presence of a methyl group at the α-carbon atom of the hydroxyacyl residue considerably promotes isomerization of the *N*-hydroxyacylamide form to the cyclol.

Interconversions of cyclol and *N*-hydroxyacylamide systems can be followed by the change in ultraviolet light absorption at 210–220 mμ. Cyclols

practically do not absorb in this region, owing to disappearance of the acylamide CONCO chromophore. In the case of hydroxyacyl derivatives of diketopiperazines, one can also make use of the infrared spectra, since the acylamide (106) spectra differ from the spectra of the cyclols (107) by the position of the amide carbonyl band of the piperazine ring. On transformation of the acylamide to the cyclol form, this band shifts by about 25 cm^{-1} to the shorter wavelength region. In fact, as one can see from *Figure 1*, the equilibrium mixture of the tautomers, obtained both by cyclolization of

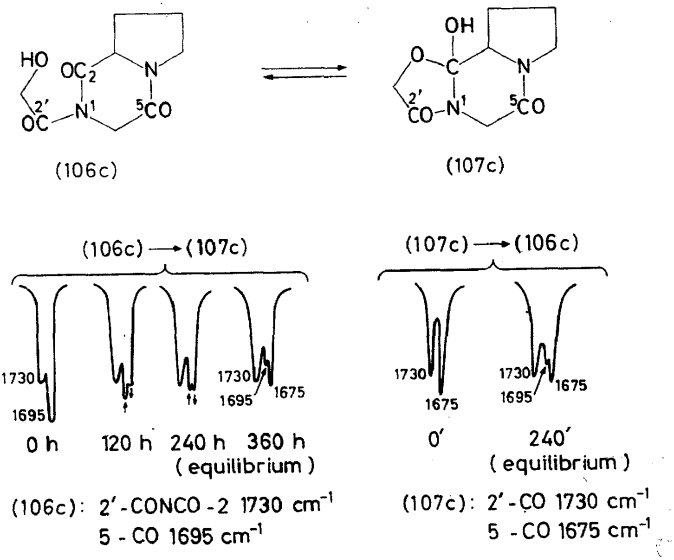
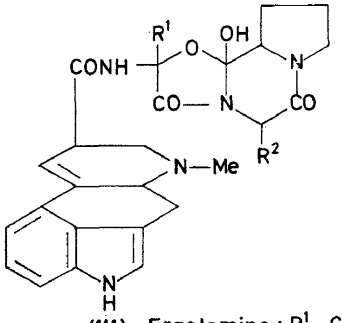


Figure 1. Tautomeric conversion between the *N*-hydroxyacylamide and cyclol forms

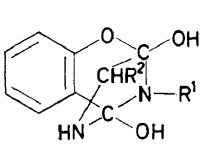
(106c), or decyclolization of (107c) exhibit bands characteristic of both forms (1695 and 1675 cm^{-1})[7, 12]. Regrettably no significant change can be observed in the position of the CONCO band when *N*-hydroxyacyldiketopiperazines of the type (106) pass over to the cyclols (107), because the position of this band practically coincides with that for the carbonyl of the 5-membered oxazolidinone ring of the cyclol. If, however, cyclolization leads to the formation of a 6-membered 1,3-oxazinone ring, as for instance in the conversion of salicyloyllactams (109) to the corresponding cyclols (110), the acylamide band disappears and a normal amide carbonyl band appears[22].

The spectral characteristics we obtained of the acylamides and cyclols proved to be very useful in structural studies of a number of naturally occurring and synthetic compounds of cyclolic nature. Thus, our data, and data published later by Stich and Leemann[32] and by Griot and Frey[33] concerning the spectral identification of cyclols, served as reliable proof for the structure of the peptide moiety of ergot alkaloids (111), which, as in the case of the penicillins, remained unresolved even after synthesis of ergotamine. The stable cyclols we have investigated are almost unique examples of the amides of ortho acids, which on the basis of indirect evidence, were

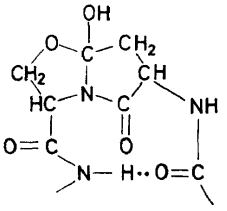
repeatedly advanced as hypothetic intermediates in the reaction of carboxylic acid derivatives with various nucleophilic reactants[34]. Thus Brenner[35] postulated the formation of cyclol structures (112) to explain the rearrangement of *O*-amino-acid-acylated salicyloyl amides he investigated; Bernhard[36] utilized it in an attempt to elucidate the enzymic mechanism of esterases (113), whereas Wrinch[37] even attempted to consider the cyclol system as the fundamental structural element of proteins. Ordinarily cyclols are highly unstable, their formation being accompanied by disappearance of the resonance-stabilized amide group (114) → (115). The stability of the cyclols obtained by us is due to their retention of one of the amide groups of the



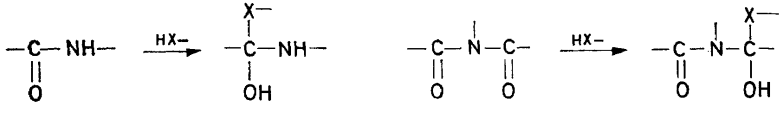
(111) Ergotamine : $R^1 = CH_3$, $R^2 = CH_2Ph$



(112)



(113)



(114) (115) (116) (117)

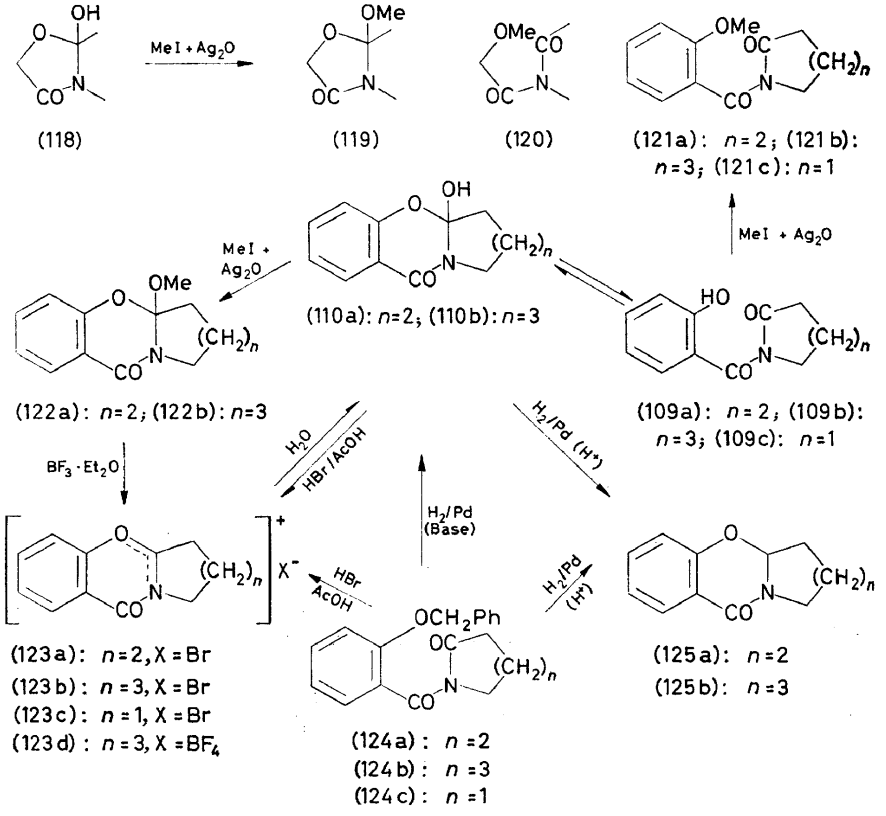
initial *N*-acylamide (116) → (117). We have now accumulated quite a number of facts regarding the chemical behaviour of these unusual compounds.

It has been shown that the hydroxyl group of cyclols (118) is readily methylated by methyl iodide in the presence of silver oxide, the *O*-methyl ethers of the cyclols (119) exhibiting spectra characteristic of cyclols and quite different from those of their isomeric *N*-(methoxyacyl)-amides (120). The latter compounds or their fission products are often formed on methylation of cyclols, for instance (110), which bears evidence of tautomeric conversions occurring in the course of the reaction[7, 12, 22].

We have investigated the hydrogenolysis of the tertiary hydroxy group in cyclols on the example of aromatic cyclols, the reaction taking place in the presence of palladium and traces of acid. Thus hydrogenolysis of the cyclols (110a, b) yielded hydroxyl-free compounds which according to infrared and nuclear magnetic resonance data proved to be desoxycyclols (125a, b)[17, 22]. These compounds are also formed in the hydrogenolysis of benzyloxybenzoyllactams (124a, b) in the presence of acids. Similar results have also been obtained by Griot and Frey[33].

It was natural to infer that the necessity of an acid catalyst for hydrogenolytic cleavage of the cyclol hydroxyl is associated with intermediate formation

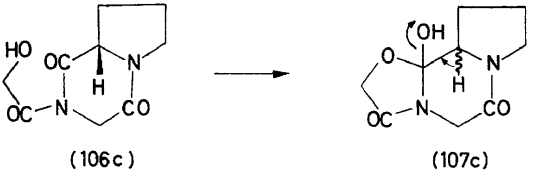
of a cation stabilized in the form of the salt (123). Support for this assumption we found in experiments on the acidolysis of the cyclols (110a, b). It was found that they reacted with hydrogen bromide in glacial acetic acid to form the bromides (123a, b). An interesting fact is that the corresponding bromide (123c) is also formed from salicyloylbutyrolactam (109c) that under



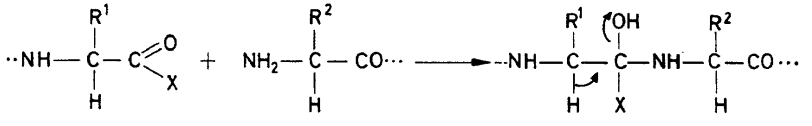
ordinary conditions does not give a cyclol or desoxycyclol. The same bromides can also be obtained directly by acidolysis of the benzyloxybenzoyllactams (124a–c). Finally, the salt (123d) was obtained by the action of boron trifluoride etherate on the *O*-methyl ether of the cyclol (122b). The structure of the bromides is supported by their infrared spectra, the presence of bands in the 1565–1580 cm^{-1} region, being apparently due to the $\rangle C{=}\overset{+}{N}\langle$ group, showing that the positive charge of the cation is localized mainly at the nitrogen atom. It is noteworthy that treatment of the bromides (123a, b) with water transforms them into the corresponding cyclols, and the bromide (123c) to salicyloylbutyrolactam (109c)[22].

Finally, another interesting property of the cyclols should be considered, namely, their optical lability. We found that the optical activity of *N*-glycolyl-L-prolylglycyllactam (106c) diminishes at a rate commensurate with that of formation of the cyclol (107c). The latter which was isolated on reaching

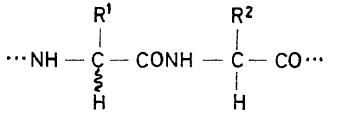
equilibrium proved to be inactive[7, 12]. This fact gives grounds for the assumption that the optical lability of the asymmetric centre in α-position to the cyclol grouping is due to the tendency of the latter for cation formation which should considerably augment the mobility of the α-hydrogen atom. It is quite possible that similar causes lie at the basis of racemization often observed with *C*-terminal amino-acid residues (126) in the course of peptide



(106c) (107c)



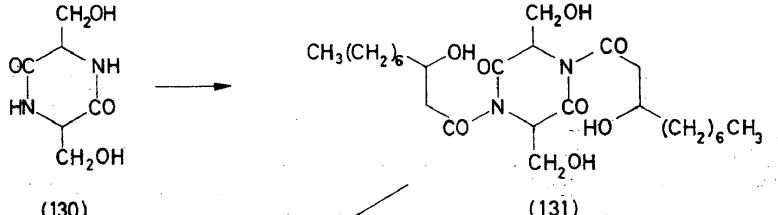
(126) (127) (128)



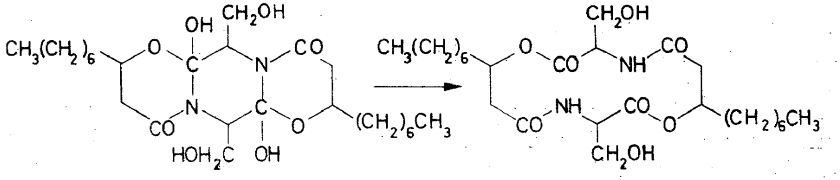
(129)

synthesis, where the degree of racemization can be determined by the lifetime of the ortho acid intermediate (128). We are at present engaged in verification of this hypothesis.

In conclusion some consideration should be allotted to the question of what part could be played by hydroxy- and amino-acyl incorporation in



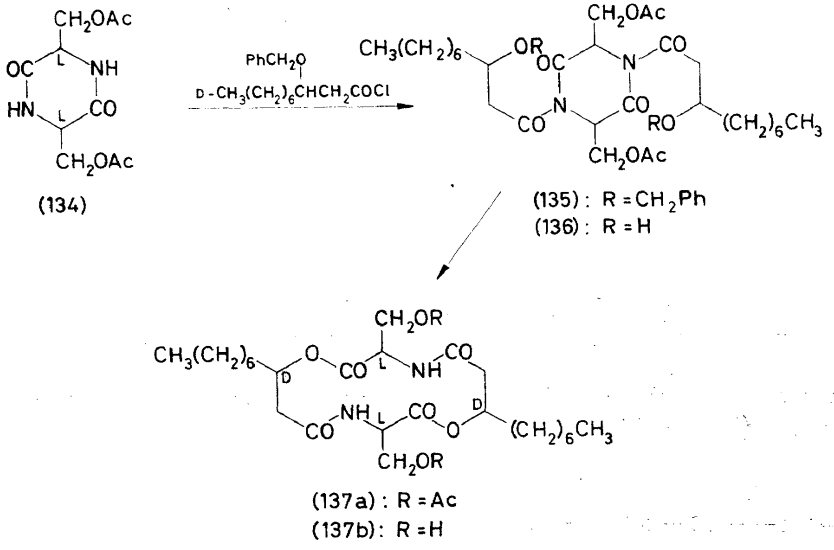
(130) (131)



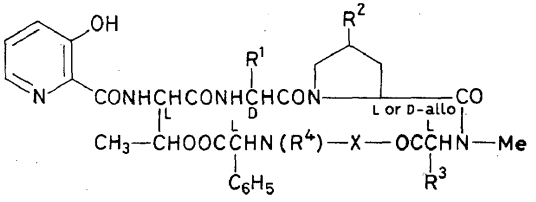
(132) (133)

peptide chain or ring in metabolitic processes. There are three features which, in our opinion, are of interest from a biochemical standpoint.

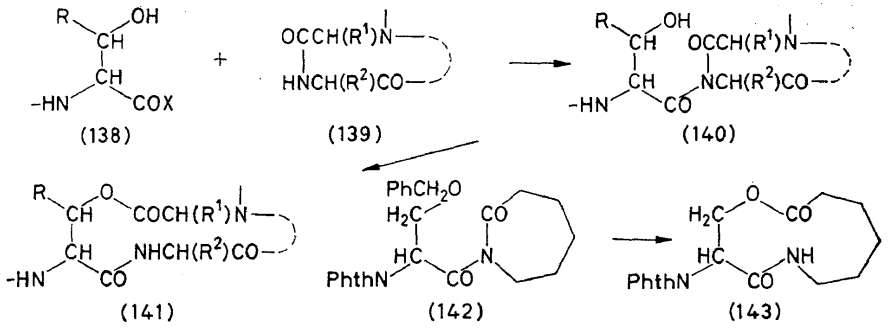
Firstly, the incorporation reaction may be one of the pathways for the biosynthesis of some depsipeptides, including antibiotics. This conclusion suggests itself the most forcefully in the case of the cyclodepsipeptide antibiotic serratamolide (133), whose biosynthesis may take place according to the scheme (130) → (131) → (132) → (133). Such pathway finds support in the synthesis of diacetylserratamolide (137a)[19, 20] which we carried out starting from *O,O'*-diacetyl-L-seryl-L-seryl-lactam (134). The latter was acylated by D-β-benzyloxydecanoyl chloride, following which the resultant diacyl derivative (135) was subjected to hydrogenolysis. This led to a 40 per cent yield of diacetylserratamolide (137a), which was recently converted to the antibiotic itself (137b)[38, 39].



The above biogenetic pathway appears to be also probable in the case of a large group of closely allied depsipeptide antibiotics containing one or two α-amino-β-hydroxy acids (serine or threonine) in the molecule. This group includes etamycin, staphylomycin S, ostreogrycin and antibiotics closely related to the latter, echinomycin *etc.* The biosynthesis of all these antibiotics is possibly carried out by the *N*-acylation of the corresponding cyclopeptides (139) with serine or threonine, activated at the carboxyl group (138), following which the resultant *N*-acylcyclopeptide (140) spontaneously rearranges to the cyclodepsipeptide (141). That at least the final stage of this biogenetic scheme (140 → 141) is easily realized was demonstrated by us on the model reaction of incorporation of a serine residue into a cyclic amide. The suitably protected N-serylcaprolactam (142) after removal of the benzyl protection was found to readily isomerize to the cyclodepsipeptide (143). At present we are continuing work on the verification of these biogenetic schemes.

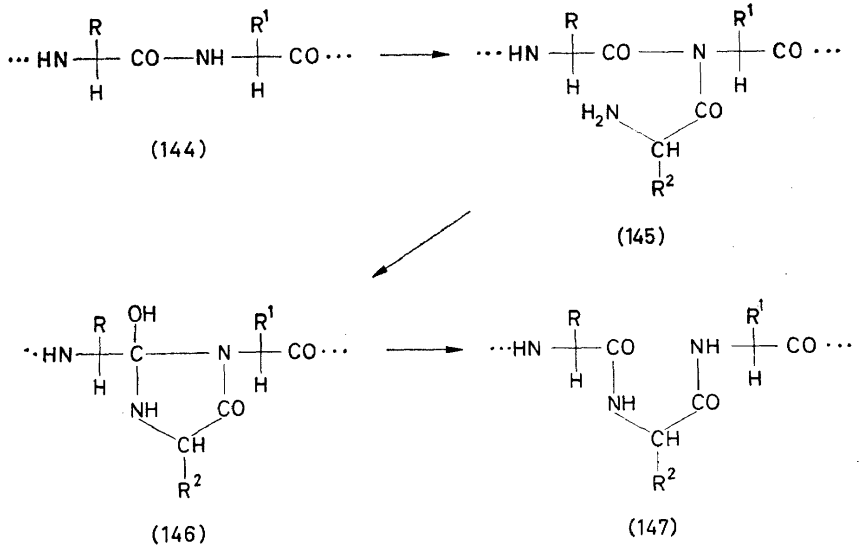


Etamycin: $R^1 = Bu^i$, $R^2 = OH$, $R^3 = H$, $R^4 = Me$, X = L-DiMeLeu. L-Ala
Staphylomycin S: $R^1 = Et$, $R^2 = H$, $R^3 = CH_2Ph$, $R^4 = H$, X = L-Oxopipec
Ostreogricin B: $R^1 = Et$, $R^2 = H$, $R^3 = CH_2C_6H_4NMe_2$-*p*, $R^4 = H$, X = L-Oxopipec



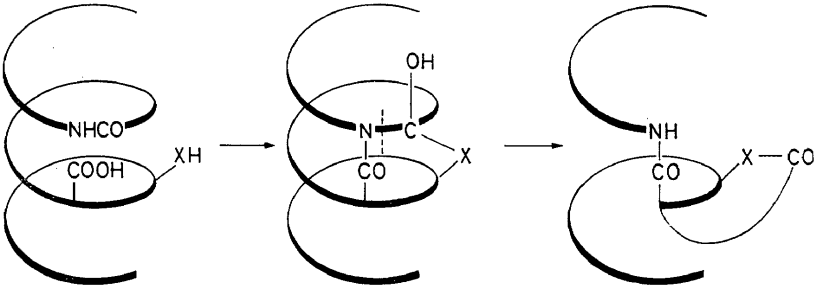
(138) (139) (140) (141) (142) (143)

Further, it is possible that for antibiotics, hormones and other metabolites of peptide nature, the biosynthesis of which may take place by routes differing from those usually accepted for proteins, amino-acyl incorporation may be one of the ways by which the peptide chain is built up (144) → (145) → (146) → (147).



(144) (145) (146) (147)

Finally hydroxy- and amino-acyl incorporation may be one of the ways by which peptide and protein molecules undergo alteration. Such reaction may follow the course outlined in the following scheme (148) → (149) → (150). When a carboxyl and nucleophilic group are suitably located with respect to an amide bond, rearrangement of the corresponding region of the chain may take place, with the formation of a fragment containing another sequence of amino-acid residues.



(148) (149) (150)

Obviously, for the present such inferences can only be regarded as hypothetical, but their possibility must be borne in mind when studying the chemical and biochemical transformations taking place in peptides and proteins.

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