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Structural formulas (I), (II), and (III)

Figure 1: Structural formulas (I), (II), and (III)

Abstract

Full Text

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STUDY OF ROUTES FOR THE SYNTHESIS OF THE PEPTIDE PORTION OF ERGOALKALOIDS*

Despite the large number of investigations devoted to the study of the chemistry of ergoalkaloids, the structure of the peptide portion of these compounds cannot be regarded as proven. It has been established that the peptide portion of ergoalkaloid molecules is a tripeptide in which the C-terminal amino acid is always proline, while the N-terminal amino acid is one of two α-hydroxy-α-amino acids, namely α-hydroxyalanine or α-hydroxyvaline. This tripeptide is closed into a ring, for which two structures have been proposed: the cyclodepsipeptide structure (I) ([1]) and the cyclol structure (II) ([2]). In addition, in our opinion, structure (III) may also be considered equally probable, in which diketopiperazine is acylated by a hydroxyamino-acid residue. It should be acknowledged, however, that none of these structures can yet fully explain all the transformations that occur with the ergoalkaloid molecule under various conditions. It is also possible that these three forms are capable of interconversion. In particular, the possibility of transition of the cyclodepsipeptide form (I) into the cyclol form (II) through transannular interaction of the ester and amide groups was pointed out as early as 1955 by Korn and Witkop ([3]). At the same time, Grob and Meier ([4]) consider structure (III) as an intermediate arising from the cyclol structure (II) during the thermal cleavage of ergoalkaloid molecules.

One route toward solving the question of the structure of the peptide portion of ergoalkaloids may be the synthesis of their peptide fragment, as well as the study, on model compounds, of the possibility of tautomeric or isomeric transformations of the type (I II III). In this connection we synthesized a tripeptide in which the sequence of amino-acid residues of ergotamine is reproduced—N-benzoyl-α-benzyloxyalaninphenylalanylproline—and also its analogue, N-benzoyl-α-benzyloxyglycylphenylalanylproline.

The fundamental possibility of synthesizing peptides containing, as N-terminal amino acids, residues of N-acyl-α-hydroxy- (or α-alkoxy)-α-amino acids had

been established by us earlier ([5]), the oxazolone method and the activated-ester method being used to create the peptide bonds. Subsequently we found that such peptides can also be obtained successfully by the carbodiimide method; moreover, the use of the more stable α-alkoxy-N-acylamino acids gives higher yields of peptide esters, and conversion of the latter into acids, in contrast to peptide esters with a free α-hydroxy group, can be effected by alkaline hydrolysis.

These data prompted us to choose, as starting substances in the present work, N-benzoyl-α-benzyloxyamino acids, which creates the possibility

* Preliminary results of these investigations were reported in 1958 at the 1st Peptide Symposium in Prague and in 1960 at the 3rd Peptide Symposium in Basel.

Table 1

Properties and analytical data of the peptides obtained

Compd.	R	R′	Mp, °C	Yield, %	Crystallization	$[\alpha]_D^{20}$, in EtOH	Calculated, % C	Calculated, % H	Calculated, % N	Found, % C	Found, % H	Found, % N	M.w. (titration), calc.	M.w. (titration), found

Dipeptides of the type

$$C_6H_5CH_2OC(R)CONHCHCOOR'$$

C_6H_5CONH $C_6H_5CH_2$

Compd.	R	R′	Mp, °C	Yield, %	Crystallization	$[\alpha]_D^{20}$, in EtOH	Calculated, % C	Calculated, % H	Calculated, % N	Found, % C	Found, % H	Found, % N	M.w. (titration), calc.	M.w. (titration), found
VIII	H	C_2H_5	88–91	79	Needles from EtOH				6.08			6.27		
X	H	H	141–144	84	Needles from AcOEt		69.43	5.59	6.47	69.50	5.62	6.42	432	447
IX	CH_3	CH_3	154–156	91	Needles from EtOH		70.42	6.10	6.08	70.23	6.18	8.14		

Compd.	R	R′	Mp, °C	Crystallization	Yield, %	$[\alpha]_D^{20}$, in EtOH	Calculated, % C	Calculated, % H	Calculated, % N	Found, % C	Found, % H	Found, % N	M.w. (titration), calc.	M.w. (titration), found
XI	CH_3	H		Amorphous	76				6.27			6.43	446	435
VIIIA	H	CH_3	143–144	Needles from EtOH	45	−35	69.93	5.87	6.27	70.21	6.07	6.14		
XA	H	H	173–175	Plates from EtOH	93	−9	69.43	5.59	6.47	69.45	5.71	6.42	432	446
VIIIB	H	CH_3		Amorphous	13	−29	69.93	5.87	6.27	70.15	6.20	6.32		
XB	H	H		Amorphous	80	−36			6.47			6.77	432	437
IXA	CH_3	CH_3	140–141*	Needles from EtOH	46	−6**	70.42	6.10	6.08	70.36	6.18	6.10		
XIA	CH_3	H		Amorphous	95	−5.5	69.93	5.87	6.27	69.66	6.17	6.64	446	450
IXB	CH_3	CH_3	140–141*	Prisms from EtOH	34	+88**	70.42	6.10	6.08	70.76	6.11	5.92		
XIB	CH_3	H	95–97	Prisms from 30% EtOH	80	+147	69.93	5.87	6.27	69.49	5.92	6.22	446	449

Tripeptides of the type

$$C_6H_5CH_2OC(R)CONHCHCON$$

$$C_6H_5CONH \quad C_6H_5CH_2 \quad COOR'$$

Compd.	R	R′	Mp, °C	Crystallization	Yield, %	$[\alpha]_D^{20}$, in EtOH	Calculated, % C	Calculated, % H	Calculated, % N	Found, % C	Found, % H	Found, % N	M.w. (titration), calc.	M.w. (titration), found
XIV	H	CH_3		Oil										
XVI	H	H	176–180	Needles from AcOEt + EtOH	13***		68.03	5.90	7.93	67.94	5.98	7.91	529	532

Compd.	R	R′	Mp, °C	Crystallization	Yield, %	$[\alpha]_D^{20}$, in EtOH	Calculated, % C	Calculated, % H	Calculated, % N	Found, % C	Found, % H	Found, % N	M.w. (titration), calc.	M.w. (titration), found
XV	CH_3	CH_3	157–159	Prisms from EtOH	87		68.92	6.33	7.54	69.16	6.42	7.59		
XVII	CH_3	H		Amorphous	41				7.73			7.92	543	536
XIVA	H	CH_3		Amorphous	90									
XVIA	H	H		Amorphous	49	−58	68.03	5.90	7.93	68.00	6.18	7.75	529	540
XVA	CH_3	CH_3	143–145	Needles from EtOH	51	−36**	68.92	6.33	7.54	69.10	6.35	7.67		
XVB	CH_3	CH_3		Amorphous	85	+26			7.54			7.46		

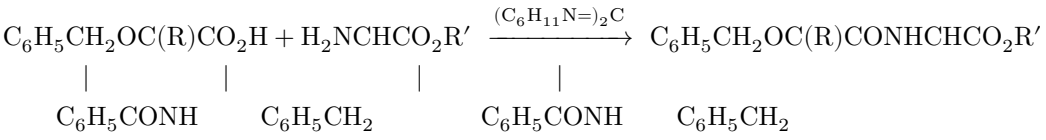
* A mixed sample of (IXA) and (IXB) melts at 121–122°.

** In $CHCl_3$.

*** Yield over two stages, calculated from (X).

the conversion by hydrogenolysis from peptides containing residues of α-alkoxy-α-amino acids to peptides with a free α-hydroxy group. The conditions for cleavage of the benzyl group were first studied with benzyl esters of *N*-benzoyl-α-benzyloxyglycine and *N*-benzoyl-α-benzyloxyalanine, and complete debenzylation was achieved at 20–25° and normal pressure in the presence of 10% Pd on charcoal. Under the same conditions, *N*-benzoyl-α-hydroxyglycyl-*L*-phenylalanine was obtained from the dipeptide (XA).

By condensation of racemic *N*-benzoyl-α-benzyloxy-α-amino acids (IV) and (V) with esters of *DL*-phenylalanine (VI) in the presence of dicyclohexylcarbodiimide, the corresponding racemic dipeptides (VIII) and (IX) were obtained; these were then saponified to the acids (X) and (XI). The latter, by reaction with the ester of *DL*-proline (XII), were converted into tripeptide esters (XIV) and (XV), and further into the acids (XVI) and (XVII).



(IV) : $R = H$ (dl) (VI) : $R' = CH_3,\ C_2H_5 (DL)$ (VIII) : $R = H,\ R' = C_2H_5$ $(dl - DL)$
(V) : $R = CH_3 (dl)$ (VII) : $R' = CH_3$ (L) (VIIIA) : $R = H,\ R' = CH_3$ $(l - L)$
(VIIIB) : $R = H,\ R' = CH_3$ $(d - L)$
(IX) : $R = R' = CH_3$ $(dl - DL)$
(IXA) : $R = R' = CH_3$ $(l - L)$
(IXB) : $R = R' = CH_3$ $(d - L)$

$$\xrightarrow{OH^-} C_6H_5CH_2OC(R)CONHCHCO_2H + HN\left(\begin{array}{c}\square\\|\\COOCH_3\end{array}\right) \xrightarrow{(C_6H_{11}N=)_2C}$$

$$\begin{array}{cc}| & |\\ C_6H_5CONH & C_6H_5CH_2\end{array}$$

(X) : $R = H$ $(dl - DL)$ (XI) : $R = CH_3 (dl - DL)$ (XII) : (DL)
(XA) : $R = H$ $(l - L)$ (XIA) : $R = CH_3 (l - L)$ (XIII) : (L)
(XB) : $R = H$ $(d - L)$ (XIB) : $R = CH_3 (d - L)$

$$\longrightarrow C_6H_5CH_2OC(R)CONHCHCON\left(\begin{array}{c}\square\\|\\COOCH_3\end{array}\right) \xrightarrow{OH^-}$$

$$\begin{array}{cc}| & |\\ C_6H_5CONH & C_6H_5CH_2\end{array}$$

(XIV) : $R = H$ $(dl - DL - DL)$ (XV) : $R = CH_3$ $(dl - DL - DL)$
(XIVA) : $R = H$ $(l - L - L)$ (XVA) : $R = CH_3$ $(l - L - L)$
(XVB) : $R = CH_3$ $(d - L - L)$

$$\longrightarrow C_6H_5CH_2OC(R)CONHCHCON\left(\begin{array}{c}\square\\|\\COOH\end{array}\right)$$

$$\begin{array}{cc}| & |\\ C_6H_5CONH & C_6H_5CH_2\end{array}$$

(XVI) : $R = H$ $(dl - DL - DL)$ (XVII) : $R = CH_3$ $(dl - DL - DL)$
(XVIA) : $R = H$ $(l - L - L)$

The problem of obtaining peptides containing residues of optically active α-substituted α-amino acids was successfully solved by condensing racemic N-acyl-α-benzyloxy-α-amino acids with the ester of an optically active amino

acid and subsequently separating the diastereoisomers formed thereby. Thus, condensation of racemic *N*-benzoyl-α-benzyloxyglycine (IV) with methyl *L*-phenylalaninate (VII) gave a mixture of diastereoisomers, separated by crystallization from alcohol into two stereoisomeric dipeptide esters (VIIIA) and (VIIIB), and saponification of these compounds gave the corresponding acids (XA) and (XB). In the same way, from racemic *N*-benzoyl-α-benzyloxyalanine (V) and the ester (VII), compounds (IXA) and (IXB) were synthesized, and then (XIA) and (XIB)*. Condensation of one of the isomeric dipeptides, namely (XA), with methyl–

* Since, on comparing compounds (VIIIA) and (IXA) with compounds (VIIIB) and (IXB), a positive shift of optical activity is observed, it may be considered that in compounds (VIIIB) and (IXB) the asymmetric carbon atom of the carbohydrate residue, denoted by an asterisk, has (+)-activity, and therefore we conventionally designate its configuration as *d*. Hence compounds (VIIIB), (IXB), etc., are designated by us as $d-L$, and compounds (VIIIA), (IXA), etc., as $l-L$ (see the scheme, and also Table 1).

with the methyl ester of *L*-proline (XIII), the ester (XIVA) was obtained, and from it—the corresponding acid (XVIA). In the same way, from two stereoisomeric dipeptides (XIA) and (XIB), the corresponding tripeptide esters (XVA) and (XVB) were obtained.

Preparation of peptide esters and their saponification

Condensation. To a suspension of 0.01 mole of an N-benzoyl-α-benzyloxy-α-amino acid or dipeptide in CH_2Cl_2 (in the preparation of (VIII), (IX), (XIV), (XVI)) or in tetrahydrofuran (in the preparation of (VIIIA), (VIIIB), (IXA), (IXB), (XIVA), (XVA), (XVB)) there is first added a solution of the amino-acid ester (VI), (VII), (XII), or (XIII)* (0.01–0.012 mole), and then dicyclohexylcarbodiimide (0.01 mole) in the same solvent. After 20–25 hr, several drops of glacial acetic acid are added, the mixture is kept for 1–1.5 hr at 0–5°, and the precipitated dicyclohexylurea is filtered off. The solvent is distilled off in vacuo; the residue is dissolved in ethyl acetate or ether; the solution is washed successively with 4% $NaHCO_3$, water, 5% HCl, and water, and dried over Na_2SO_4. After removal of the solvent in vacuo, the residue is either subjected directly to saponification or crystallized from the appropriate solvent (see Table 1). A mixture of diastereoisomeric dipeptides is separated by repeated (4–5 times) crystallization from alcohol. The isomers of series A dissolve in alcohol with greater difficulty than the isomers of series B.

Saponification. 0.01 mole of the ester of a di- or tripeptide is dissolved or suspended in 25 ml of methanol, 0.015–0.018 mole of 2*N* NaOH is added, and the mixture is stirred for 0.5–1 hr at 20–25° ((VIII), (VIIIA), (VIIIB), (XIV), (XIVA)) or at 35–37° ((IX), (IXA), (IXB), (XV)). Then 15–20 ml of water is added, the mixture is filtered, the methanol is distilled off in vacuo, and the unsaponified starting material is extracted with ether or ethyl acetate. The

aqueous solution is acidified with 10% HCl, and the resulting peptides are purified by crystallization (see Table 1) or reprecipitated from bicarbonate solution with hydrochloric acid.

Reductive debenzylation

The substance is hydrogenated in alcoholic solution at 20-25° and normal pressure in the presence of an equal weight amount of 10% Pd/C; the solvent is distilled off in vacuo, and the residue is triturated with ether and filtered.

From 0.5 g of the benzyl ester of N-benzoyl-α-benzyloxyglycine, 0.22 g (84%) of N-benzoyl-α-hydroxyglycine was obtained ([7]); from 0.5 g of the benzyl ester of N-benzoyl-α-benzyloxyalanine—0.17 g (71%) of N-benzoyl-α-hydroxyalanine ([7]); and from 0.27 g of N-benzoyl-α-benzyloxyglycyl-*L*-phenylalanine (XA)—0.19 g (90%) of N-benzoyl-α-hydroxyglycyl-*L*-phenylalanine; mp 156-158° with decomposition (from alcohol). Molecular weight: found (titration) 343, calculated 342.

$C_{18}H_{18}O_5N_2$
Found, %: C 63.33; H 5.41
Calculated, %: C 63.12; H 5.29

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* The methyl esters of *DL*-proline, *L*-proline, and *L*-phenylalanine were obtained as hydrochlorides in 75-80% yield by the action of $SOCl_2$ on a solution of the

amino acid in methanol (cf. ([6])).

Note: Figure translations are in progress. See original paper for figures.