

THE "GAS-SOLID-PHASE" 2,5-DIOXOPIPERAZINE SYNTHESIS. CYCLIZATION OF VAPOROUS DIPEPTIDES ON SILICA SURFACE

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The "gas-solid-phase" method is used for the preparation of both symmetric and asymmetric 2,5-dioxopiperazines via cyclization of vaporous linear dipeptides in the presence of silica.

Recently we have reported on the "gas-solid-phase" synthesis of 2,5-dioxopiperazines (DOPs) via cyclodimerization of vaporous amino acids on silica surface¹. The prerequisite for realization of this method is the ability of amino acids to sublime in vacuum without decomposition, intermolecular condensation and loss of optical activity². Besides amino acids themselves, this property is characteristic also for some simple dipeptides, i.e. dipeptides derived from aliphatic bifunctional amino acids². Although DOPs can be detected in dipeptide sublimates, their yields, according to Gross and Grodsky², do not exceed 7%.

Taking into account that in the course of amino acid cyclodimerization on silica surface dipeptides must be formed as intermediates^{1,3}, we supposed that dipeptides themselves can be used as starting materials for the "gas-solid-phase" DOP synthesis. By this method it would be possible to obtain both symmetric and asymmetric DOPs.

In the present communication we report on the synthesis of several DOPs, including optically active ones, via cyclization of vaporous linear dipeptides in the presence of silica.

EXPERIMENTAL

HPLC analyses were performed on Milikhrom 1A microcolumn chromatograph (Nauchpribor, Orel, Russia) with UV detection at 220 – 240 nm. Stainless steel microcolumns, 64 × 2 mm, were packed with Silasorb 600 silica gel (4 μm) and reversed phase Separon C18 (5 μm), both from Chemapol (Prague, The Czech Republic). Ligand-exchange chromatography of amino acid enantiomers was performed on a reversed phase coated with *N*-octyl-L-proline (see ref.⁴ and literature cited herein). Acetate buffer solution (pH 5.21)–acetonitrile, 4 : 6 (v/v), and 10⁻⁴ M Cu(NO₃)₂ in acetate buffer solution of pH 5.21 were used as mobile phases. Optical rotations were determined on Perkin-Elmer 141 spectropolarimeter. Fast-atom-bombardment mass spectra were obtained on MX 1310 mass spec-

trometer (Nauchpribor, Orel, Russia); DOP samples were in the form of suspensions in glycerol. Energy of bombarding argon atoms was 3.5 keV; intensity of bombarding beam, about 1 μ A; angle of incidence to the target, 70° to the normal.

Dipeptides H-Gly-Gly-OH, H-Gly-DL-Ala-OH, H-Gly-DL-Leu-OH, H-Gly-L-Leu-OH, H-Gly-L-Val-OH, DL-H-Ala-DL-Ala-OH, L-H-Ala-L-Ala-OH and L-H-Val-L-Val-OH were purchased from Reanal (Budapest, Hungary). Silokhrom C-120 macroporous silica from Reakhim (Russia), specific surface area about 120 m²/g, was preliminarily heated at 400 °C in the air for 3 h to remove adsorbed contaminations.

The "gas-solid-phase" DOP synthesis was performed in the simple vacuum reactor used earlier^{1,4}.

General Procedure

Dipeptide (0.5 – 2.0 g) and silica (2 – 5 g) are placed into the reactor connected to a vacuum source and pumped out to vacuum of about 10 Pa. The bottom zone of the reactor is heated to 180 – 210 °C for 1 – 3 h. Sublimate, containing DOP and unreacted dipeptide, condenses in the reactor cold zone as a crust. To increase the conversion of linear dipeptide to DOP, the sublimate is returned to the reaction zone, and the procedure is repeated; duration of the second sublimation cycle is 1 – 2 h. Crude product is extracted from the reactor and recrystallized from water or water-ethanol.

For the ligand-exchange HPLC analyses of enantiomeric composition, DOP samples are hydrolyzed with 6 M HCl for 24 h at 100 °C in evacuated ampoules.

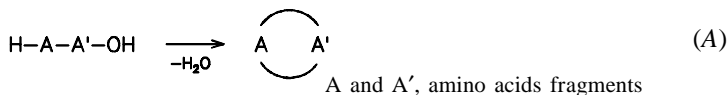
RESULTS AND DISCUSSION

As starting materials only dipeptides derived from aliphatic amino acids containing no other functional groups can be used (otherwise the synthesis can be complicated by the formation of by-products in appreciable amounts due to increasing a number of reaction channels on the hot silica surface). The dipeptides were sublimed in the presence of macroporous silica at reduced pressure and temperatures of 180 – 210 °C. After the first sublimation cycle, DOPs condensed contain small amount of unreacted dipeptides. The process should be repeated once more to increase the conversion of dipeptides and to obtain pure DOPs. Indeed, HPLC analyses of crude products did not reveal appreciable amounts of the starting dipeptides.

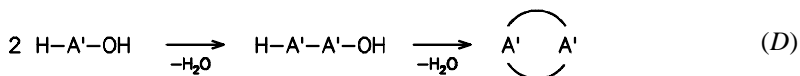
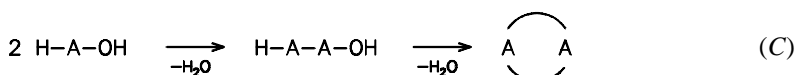
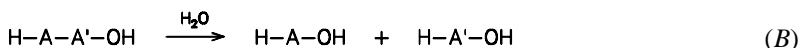
Mechanism of the cyclocondensation, similarly as for amino acids³, includes transient formation of a surface ester of orthosilicic acid and may be represented by Scheme 1.

The method proposed can be considered as a rough analogue to the solid-phase DOP synthesis^{5–8}, from which any solvent and protection-deprotection steps are excluded.

The system, which is considered in the present case, has the following important feature. At temperatures about 200 °C the silica surface still contains appreciable amount of adsorbed water and catalyzes not only condensation of amino acids and peptides, but also hydrolysis of the latter³. If the main process may be represented by Eq. (A)

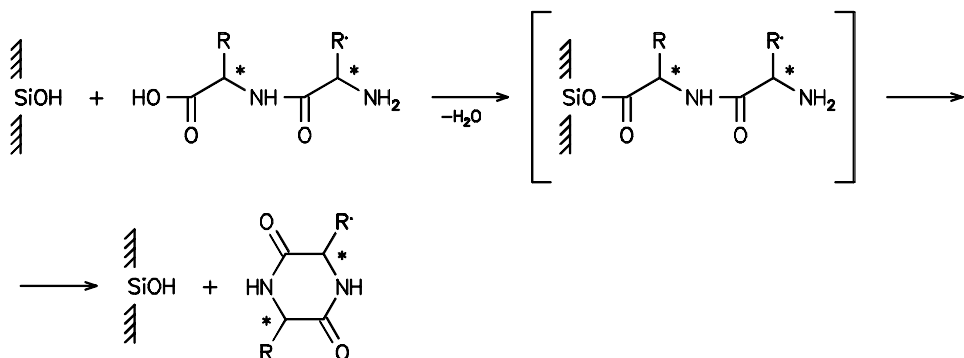


the following undesirable reactions leading to two symmetric DOPs are possible in principle (Eqs (B) – (D)).



We have tested such possibility by means of HPLC and have found that the corresponding by-products are formed in negligible amounts. For cyclo(-Gly-L-Leu-) the content of cyclo(-L-Leu-L-Leu-) and cyclo(-Gly-Gly-) made up less than 1% even in the crude sublimate (Fig. 1). Similar results were obtained also for other asymmetric DOPs specified in Table I.

Crude products were recrystallized from water or water-ethanol mixtures. Yields of purified DOPs, melting points, optical rotation values in comparison with literature data and corresponding peaks in fast-atom-bombardment mass spectra are presented in Table I. For cyclo(-Gly-L-Val-) and cyclo(-L-Ala-L-Ala-) optical rotation values are fairly close to those reported in literature. For cyclo(-L-Val-L-Val-) and cyclo(-Gly-L-Leu-) the obtained optical rotations were found to be somewhat lower than the literature values that points to the racemization. In order to characterize this possibility in more detail, optically active DOPs prepared have been hydrolyzed to amino acids, and enantiomeric composition of the latter has been examined by the ligand-exchange chro-



SCHEME 1

TABLE I
Yields and properties of DOPs prepared

Compound	Sublimation temperature, °C	Yield, %	M.p., °C	$[\alpha]_D^{25}$, °	m/z ($M + H^+$)
cyclo(-Gly-Gly-)	180 – 200	46	308 – 309 (ref. ⁷ ; 308 – 309)		115
cyclo(-Gly-DL-Ala-)	190 – 205	48	237 – 238 (ref. ⁹ ; 240)		129
cyclo(-Gly-DL-Leu-)	190 – 210	44	240 – 243 (ref. ⁹ ; 239)		171
cyclo(-Gly-L-Leu-)	190 – 210	40	252 – 254 (ref. ¹⁰ ; 254 – 256)	–12.0 ($c = 2$, H_2O); ref. ¹⁰ ; –14.8	171
cyclo(-Gly-L-Val-)	200 – 210	82	261 – 263 (ref. ¹⁰ ; 261 – 263)	+15.0 ($c = 1.5$, H_2O); ref. ¹⁰ ; +15.1	157
cyclo(-DL-Ala-DL-Ala-)	170 – 190	43	270 – 273 (ref. ¹¹ ; 256 – 259)		143
cyclo(-L-Ala-L-Ala-)	190 – 210	80	278 – 280 (ref. ¹² ; 276 – 280)	–22.0 ($c = 0.5$, HOAc); ref. ¹² ; –22.8	143
cyclo(-L-Val-L-Val-)	190 – 210	72	270 – 272 (ref. ¹² ; 272 – 273)	–52.0 ($c = 0.5$, HOAc); ref. ¹² ; –62.0	199

matography on C18 reversed phase coated with *N*-octyl-L-proline⁴; for comparison the same procedure has been performed with the starting dipeptides. The results are presented in Fig.2. In the case of cyclo(-Gly-L-Leu-) appreciable racemization is ob-



FIG. 1

The HPLC control of cyclo(-Gly-L-Leu-) purity. Column: 64×2 mm; stationary phase: Silasorb 600, $4 \mu\text{m}$; mobile phase: acetate buffer (pH 5.21)-acetonitrile, 4 : 6 (v/v); flow rate: $100 \mu\text{l/min}$; temperature: ambient; detection: UV at 230 nm. 1 cyclo(-L-Leu-L-Leu-); 2 cyclo(-Gly-L-Leu-); 3 cyclo(-Gly-Gly-)

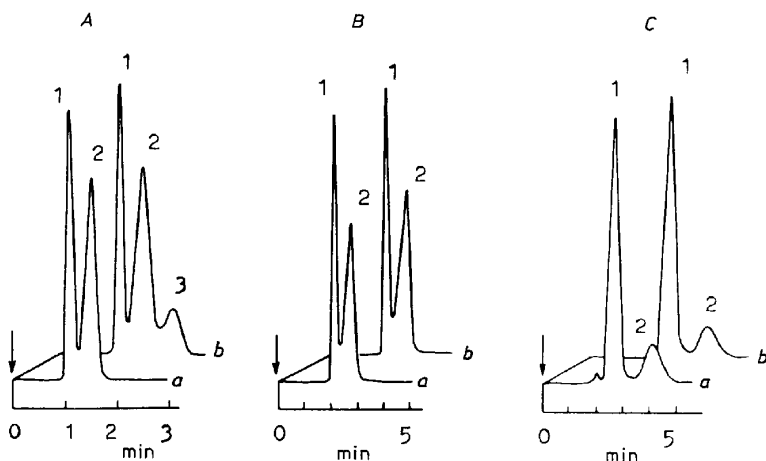


FIG. 2

Enantiomeric composition of hydrolyzates of optically active DOPs and starting linear dipeptides by the ligand-exchange HPLC. Hydrolysis: evacuated ampules, 6 M HCl, 24 h, 100°C ; column: 64×2 mm; stationary phase: Separon C18 ($5 \mu\text{m}$) coated with *N*-octyl-L-proline; mobile phase: 10^{-4} M $\text{Cu}(\text{NO}_3)_2$ in acetate buffer solution pH 5.21; flow rate: $100 \mu\text{l/min}$ (A) and $50 \mu\text{l/min}$ (B, C); temperature: ambient; detection: UV at 240 nm. Hydrolyzates: A: H-Gly-L-Leu-OH (a) and cyclo(-Gly-L-Leu-) (b); 1 H-Gly-OH; 2 L-H-Leu-OH; 3 D-H-Leu-OH. B: H-Gly-L-Val-OH (a) and cyclo(-Gly-L-Val-) (b); 1 H-Gly-OH; 2 L-H-Val-OH. C: L-H-Val-L-Val-OH (a) and cyclo(-L-Val-L-Val-) (b); 1 L-H-Val-OH; 2 D-H-Val-OH

served (about 20% of initial L-H-Leu-OH is present in the D-form); in the case of cyclo(-L-Val-L-Val-) approximately equal amounts of D-H-Val-OH are present both in the DOP and in commercially available L-H-Val-L-Val-OH, i.e., racemization is insignificant. For cyclo(-Gly-L-Val-) racemization is not observed. Alanine enantiomers (for cyclo(-L-Ala-L-Ala-)) are not resolved using chromatographic conditions described.

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