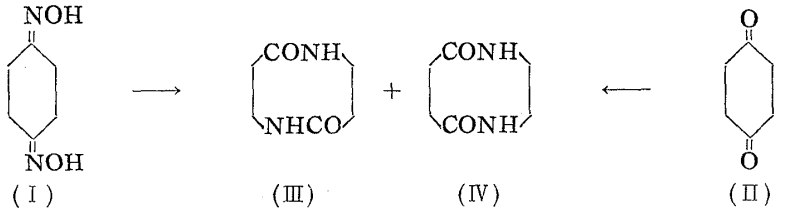
Communications to the Editor

UDC 547.594.3.07

Preparation of Cyclo-di-β-alanyl by the Schmidt Reaction of 1,4-Cyclohexadione

Cyclo-di-β-alanyl (Ⅲ), one of the most simple cyclic peptides, has not been reported to date. In general, cyclic peptides are prepared by cyclization of the corresponding linear ones, but they may also be prepared by other synthetic routes starting from the compound already having a cyclic structure. In the hope of finding such a new method, both the Beckmann rearrangement of 1,4-cyclohexadione-dioxime (I) and the Schmidt reaction of the dione (Ⅱ) itself were attempted.



(Ⅰ) (Ⅲ) (Ⅳ) (Ⅱ)

Knunyants and Fabrichnyi[1] reported the Beckmann rearrangement of (I) to (Ⅲ) and (Ⅳ), using di-*p*-tosylate of (I), but they isolated neither of the product. Later, Mamlok[2] has also tried the rearrangement of the hydrochloride of (I) by polyphosphoric acid, but he only obtained unexpected 1,4-diamino-2-chlorobenzene as the reaction product.

This Knunyants' experiment was followed by heating di-*p*-tosylate of (I) in methanol at 100° for 90 minutes in an autoclave, but no compound corresponding to (Ⅲ) or (Ⅳ) was isolated. Attempt to effect rearrangement of (I) itself to (Ⅲ) by polyphosphoric acid, perchloric acid, or trifluoroacetic acid was all unsuccessful, and *p*-phenylenediamine was obtained in each case.

When dry hydrogen chloride was passed through a chloroform solution of (Ⅱ) and hydrazoic acid at 0° to 5°, a viscous matter formed which was insoluble in chloroform but soluble in water. The substance could not be crystallized from aqueous solution even after the removal of chloride ion, but it seemed certain that this consists mainly of a mixture of rearranged products (Ⅲ) and (Ⅳ), because β-alanine, ethylenediamine, and succinic acid were identified from the hydrolysis products of this material with hydrochloric acid. When this viscous material was dissolved in hot ethanol and allowed to stand at room temperature, fine hexagonal plates of m.p. 295~300° were obtained. The elementary analyses and molecular weight (measured by Schwyzer's method[3]) of this crystal showed good agreement with theoretical values for $C_6H_{10}O_2N_2$ (*Anal.* Calcd. : C, 50.69; H, 7.09; N, 19.71; mol. wt., 142. Found : C, 50.91; H, 7.37; N, 19.68; mol. wt., 127). Furthermore, the paper chromatogram of the hydrolysate of this compound showed only one spot, which agreed completely with that of β-alanine itself. From these evidences, it is concluded that the compound obtained here by the Schmidt reaction of 1,4-cyclohexadione is the expected cyclo-di-β-alanyl (Ⅲ).

The details of the experiment will be reported in the near future.

1) I. L. Knunyants, B. P. Fabrichnyi : Doklady Akad. Nauk S. S. S. R., **68**, 701 (1949) (C. A., **44**, 1918 (1950)).
2) L. Mamlok : Bull. soc. chim. France, **1956**, 1182.
3) R. Schwyzer, *et al.* : Helv. Chim. Acta, **39**, 872 (1956).

Laboratory of Chemistry,
Faculty of Science,
Konan University,
Okamoto, Higashinada-ku, Kobe.

Hiroshi Watanabe (渡辺 熙)
Shigeru Kuwata (桑田 蕃)
Sugao Koyama* (小山清雄)

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* Present address : Sanken Ka-ko, Ltd., Todasuji, Nakano, Motoyama, Higashinada-ku, Kobe.

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Gas Chromatography of C_{27}, C_{28}, and C_{29} Sterols*[1]

Since the gas chromatographic separation of steroids was reported by Horning and his co-workers,[1] this method has become an important technique in the study of steroids. A recent report on the gas-liquid chromatography of sterol methyl ethers[2] prompted publication of the present communication.

In order to obtain a correlation between the structure of steroids and retention time, and to identify and separate the naturally occuring steroids, especially algae sterols, which we have been studying in our laboratory for some time,[3,4] gas chromatography of a number

Table I. Relative Retention Time of Sterols[a]

Compound	Time	Position of double bond
C_{27}-Sterols		
Cholestane (I)[b]	1	
Cholesterol (II)	1.69	5
$\Delta^{8(14)}$-Cholestenol (III)	1.73	8(14)
Δ^{14}-Cholestenol (IV)	1.83	14
Δ^{7}-Cholestenol (V)	1.93	7
20-Iso-22-dehydrocholesterol (VI)[5]	1.35	5, 22
22-Dehydrocholesterol (VII)[3]	1.57	5, 22
$\Delta^{7,22}$-Cholestadien-3β-ol (VIII)[6]	1.87	7, 22
C_{28}-Sterols		
Δ^{22}-24β-Methylcholesterol (brassicasterol) (IX)[8]	1.91	5, 22
Δ^{22}-24-Methylcholesterol (24α,β (1:1) mixture) (X)[8]	1.91	5, 22
Δ^{22}-24-Methylcholesterol (24α,β (3:1) mixture) (XI)[8]	1.92	5, 22
24-Methylencholesterol (XII)[9]	2.16	5, 24(28)
$\Delta^{8(14)}$-Ergosten-3β-ol (XIII)	2.17	8(14)
5,6-Dihydroergosterol (XIV)	2.21	7, 22
Δ^{24}-24-Methylcholesterol (XV)[7]	2.47	5, 24
C_{29}-Sterols		
Stigmasterol (XVI)	2.38	5, 22
Δ^{22}-24-Ethylcholesterol (24α,β (1:1) mixture) (XVII)[8]	2.38	5, 22
Fucosterol (XVIII)[4]	2.76	5, 24(28)
Δ^{24}-24-Ethylcholesterol (XIX)[7]	2.98	5, 24
Fucostadienone (XX)	3.79	4, 24(28)
Δ^{24}-24-Ethylcholestenone (XXI)[7]	4.06	4, 24

a) Barber-Colman Model-10. Argon ionization detector, 9 ft. × 8 mm. i. d. column, pressure, 35 lb./in^2, temp. 220°, 1% SE-30 phase on Chromosorb W, 60~80 mesh. Flash temp. 290°. Cell temp. 170°.
b) Retention Time, 9.1 min.

*1 Steroid Studies, Part XXXIII.

1) W. J. A. VandenHeuvel, C. C. Sweeley, E. C. Horning : J. Am. Chem. Soc., **82**, 3481 (1960), *et seq.*
2) R. B. Clayton : Nature, **190**, 1071 (1961).