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Interconversible *cis* and *trans* Rotational Isomers of 1,8-Di(1-naphthyl)naphthalene

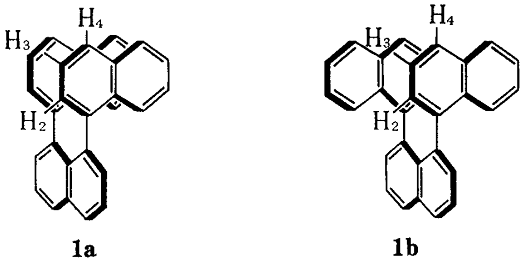
New stable *cis* and *trans* rotational isomers of 1,8-di(1-naphthyl)naphthalene were prepared by the Kharash-type Grignard cross-coupling of 1-naphthylmagnesium iodide and 1,8-diiodonaphthalene in the presence of *N*,*N'*-bis(1-methyl-3-oxobutylidene)ethylenediaminatonickel(II). Thermoanalytical and proton magnetic resonance spectral studies indicated that both rotamers are stable in the solid state as well as in a solution, but are interconversible at temperatures above their melting points. A convenient method, obtaining pure *trans* rotamer from the equilibrium mixture of both rotamers, is also presented.

Keywords——Ni-complex-catalyzed cross-coupling; 1.8-diarylnaphthalene; rotational isomer; interconversion; DSC; NMR

Concerning the geometry of *peri*-aryl naphthalenes, it has already been known that phenyl rings in 1,8-diphenylnaphthalene are constrained to the conformation in which both benzene rings are approximately parallel to one another and perpendicular to the plane of naphthalene ring.[1] This would lead to the expectation that 1,8-diphenylnaphthalenes having *o*- or *m*-substituent on each phenyl ring should exist as stable *cis* and *trans* isomers due to restricted rotation around the phenyl-naphthylene bonds. However, the attempts, proceeded by House and Bashe, to separate *cis* and *trans* isomer pair of compounds such as 1,8-bis(3-chlorophenyl)naphthalene or 1,8-bis(3-carbomethoxyphenyl)naphthalene were unsuccessful,[2] presumably owing to the low energy barrier which was not enough for their separation.[3] Recently, Clough and Roberts resolved *cis* and *trans* isomers of 1,8-di(*o*-tolyl)naphthalene, which were stable in the crystalline state, but had a half-life in solution of about only 1 day at room temperature.[4]

We have found that 1,8-di(1-naphthyl)naphthalene could be resolved to *cis* and *trans* rotamers which were stable in solid state as well as in solution, but were interconversible at temperatures above their melting points.

1,8-Di(1-naphthyl)naphthalene (**1**) was prepared using Kharash-type Grignard cross-coupling reaction[5] analogously to the preparation of 1,8-di(*o*-tolyl)naphthalene,[4,5b] but under modified reaction conditions in the presence of different catalyst. Thus 1-naphthylmagnesium iodide (8 fold excess) was directly coupled with 1,8-diiodonaphthalene in an ether-benzene mixture under refluxing in the presence of *N*,*N'*-bis(1-methyl-3-oxobutylidene)-ethylenediaminatonickel(II) as a catalyst.[6] Two isomers **1a** and **1b** were isolated from



1a **1b**

Chart 1

the reaction product by column chromatography on alumina, followed by recrystallization from a benzene–ethanol mixture in 12% and 24% yields, respectively. The more soluble needles **1a** (mp 155.4—156.8 °C) and less soluble plates **1b** (mp 173.3—174.2°C) in the same mixture were confirmed to have proposed composition by mass spectral and elemental analysis.

The proton magnetic resonance spectrum (^{1}H-NMR) of **1b**, measured at 80 MHz in $CDCl_3$ solution, reveals a quartet (2H, δ 8.13 and 7.94), a multiplet (12H, δ 7.64—7.10), and an ABX pattern (6H, ν_3 6.43, ν_2 6.27, ν_4 7.03; $J_{2,3}=7.0$ Hz, $J_{3,4}=8.5$ Hz, $J_{2,4}=1.4$ Hz), which are assignable to naphthylene protons of C_4, C_5, those of C_2, C_3, C_6, C_7 and naphthyl protons of C_5, C_6, C_7, C_8, and those of C_2, C_3, C_4, respectively. Although the chemical shift of naphthyl-C_4 protons (δ 7.03) is nearly comparable to that of phenyl protons of 1,8-diphenylnaphthalene (δ 6.89),[7] the signals of naphthyl-C_2, C_3 protons are shifted appreciably toward higher field, presumably owing to the ring current effects caused by the parallel naphthyl rings. Analogous but more prominent upfield shifts over 1 ppm have been observed in the spectrum of *trans*-[2.2](1,4)anthracenophane, in which C_2,C_3-protons were located nearly above the opposed anthracene ring.[8] The spectrum of **1a**, however, shows quite different signal pattern of naphthyl-C_2,C_3,C_4 protons (δ 7.00—6.65), but similar signals for other protons in the spectrum of **1b**. Although an assignment of the former pattern is difficult, lack of the characteristic signals of **1b** in the high field region (δ 6.50—6.14) reflects undoubtedly the absence of protons located nearly above the opposite naphthalene ring. These spectral aspects on both **1a** and **1b** are obviously compatible to the conformations as *cis* and *trans* rotamers, respectively.[9] Both isomers are stable even in a solution ($CDCl_3$) over two weeks at room temperature.

The thermoanalytical data of the two isomers were obtained by differential scanning calorimetry.[10] As shown in Fig. 1, the DSC curve of **1a** displayed two endothermic peaks at 158°C and 173°C, having approximate intensities of 2: 1. After cooling, the second run

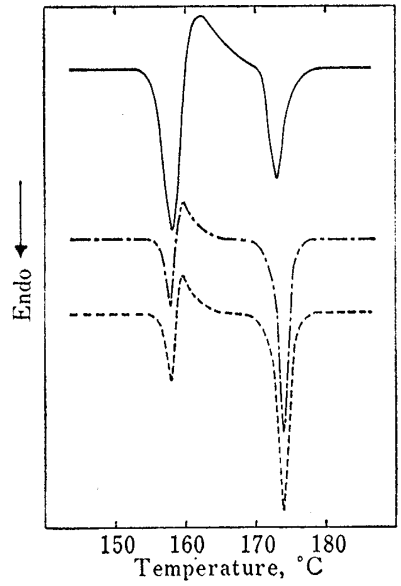


Fig. 1. DSC Curves of **1a**

———: run 1,
–·–·–: run 2,
-------: run 3.

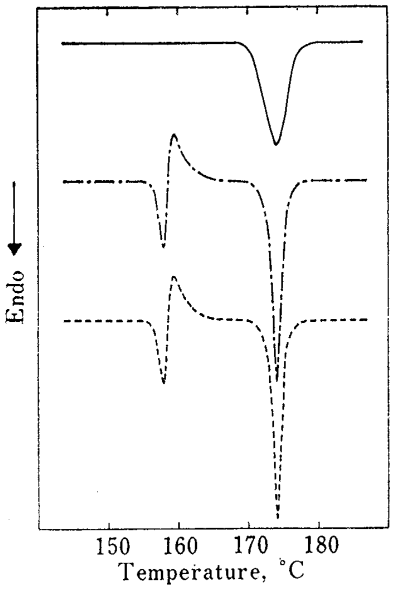


Fig. 2. DSC Curves of **1b**

———: run 1,
–·–·–: run 2,
-------: run 3.

of the melt also showed two peaks at practically the same temperatures but having reverse intensities of 1: 5. Further run of the melt did not exhibit any appreciable change compared with that of the second. The DSC curve of **1b**, however, displayed only one peak at 174°C (Fig. 2). The second run of the melt showed substantially the same DSC curve as that of the second run of **1a**, having two peaks at 157°C and 173°C with approximate intensities of 1: 5. After the second run the melt showed reproducible DSC curve. In addition, ^{1}H-NMR spectra of both melts of each second run of **1a** and **1b** are superimposable, and are compatible to that of a mixture of **1a** and **1b**. From these experiments, interpretations of DSC curves may easily be deducible. Thus, the first endothermic peak together with subsequent broad exothermic peak probably indicates the melting of **1a**, transition of **1a** to **1b**, and crystallization of **1b**. Further, the second endothermic peak probably indicates the melting of **1b** together with partial reverse transition of **1b** to **1a**.

On the basis of the foregoing results, it was clarified that **1a** (*cis*) and **1b** (*trans*) were interconversible by heating, and that either of **1a** and **1b** formed same equilibrium mixture of a ratio of approximately 1: 4.

The preceding analysis of DSC curves was verified by the following experiments (Fig. 3). The first scanning of the melt, which consisted of the equilibrium mixture of **1a** and **1b**, was stopped at 169°C (just before the second peak). After cooling, the next run of the melt displayed only one peak at 174°C, indicating that the melt used in the present scanning consisted of pure **1b**. The third run of the melt showed two endothermic peaks at practically the same temperatures (158°C and 174°C) as in the case of the second run of **1b** (Fig. 2), indicating the melt used consisted of the equilibrium mixture of **1a** and **1b**. Above thermoanalytical results were compatible with the ^{1}H-NMR data of the melts used.

These experiments provide conclusive proof that we can readily get pure *trans* rotamer **1b** from the equilibrium mixture of both rotamers by heating up to 169°C.

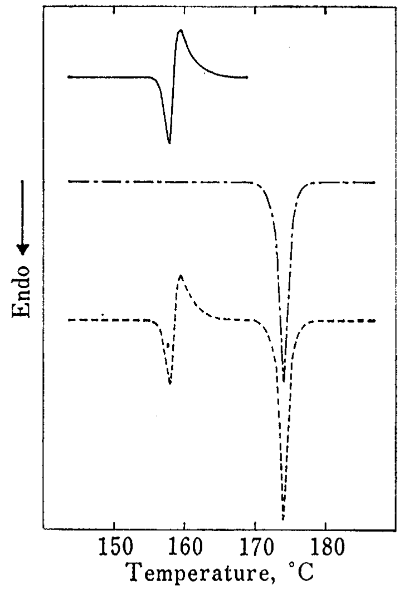


Fig. 3. DSC Curves of Equilibrium Mixture of **1a** and **1b**

———: run 1,
—·—·—: run 2,
-------: run 3.
Run 1: Heating was stopped at 169°C.

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9) Whereas the *cis* rotamer **1a** would be achiral, the *trans* rotamer **1b** would be a racemic mixture. Identification of antipode by resolution was not attempted.
10) The heating rate was 5°C/min throughout this work.
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Human Renal Kallikrein: Purification and Some Properties[1]

Human renal kallikrein (HRK, E.C. 3.4.21.8) was purified about 540-fold by using chromatographic techniques with an overall yield of 4.6% from crude preparation of HRK being prepared with acetone activation of human kidney cortex extract. The activities of this kallikrein were 3.2 KU/A_{280} of vasodilator and 0.27 μmol/min/A_{280} of TAME esterolytic activities. This purified HRK was observed to be nearly single band with faint subband on disc gel electrophoresis. The approximate molecular weight of HRK was estimated to be 4.7 to 4.9×10^4 by gel filtration on Sephadex G-100 and G-150 columns.

Keywords——purification of kallikrein; human kidney; vasodilator activity; esterolytic activity; acetone activation

The functional relationship between the renal and urinary kallikreins (E.C. 3.4.21.8) has been studied by a few investigators.[2] Recently, the renal kallikreins were purified with rat and dog, and their biochemical and physiological properties have been getting revealed.[3] On the other hand, the study on the human renal kallikrein (HRK) was somewhat difficult becaues of its low content in the human gland. In order to get a clue to elucidate the renal and urinary kallikrein system in human, the purification of HRK is at first important.

The present paper deals with the purification and some properties of HRK.

Materials and Methods

Materials——The human kidney, starting material in this study, was washed and perfused with saline, and stored at −20°C.

DEAE-cellulose was obtained from Green Cross Drug Mfg. Co., Osaka, Japan. Sephadex G-100 and G-150 were supplied from Pharmacia AB, Sweden. N-α-Tosyl-L-arginine methyl ester (TAME) and synthetic bradykinin from Protein Research Foundation, Osaka, Japan, were used.

Assay of Kallikrein Activities——Vasodilator activity was assayed by measuring the increase in femoral arterial blood flow following the injection of samples in the dog,[4] and was expressed as kallikrein unit(KU). Esterolytic activity was measured by the fluorometric method[5] using TAME as substrate and was expressed in terms of μmol TAME hydrolyzed/min at pH 8.0, 30°C.

Protein Determination——The absorbancy at 280 nm in a 1 cm width cuvette was routinely measured to estimate protein concentrations of various solutions.

Disc Electrophoresis——This was performed to the modified method of Davis,[6] using 7% (w/v) polyacrylamide gel columns and 0.04 M Tris-glycinate buffer at pH 8.6.

Results and Discussion

The activation of HRK by acetone was investigated under the same method described in the previous paper with dog renal kallikrein,[3b] using acetone concentration of 0 to 50% (v/v). The maximum relative activity, measured by vasodilator assay, was found at 30% (v/v) as approximately 1.4 times more than that of the water extract without activation treatment. Therefore, the acetone concentration for activation was fixed at 30% (v/v)