Agric. Biol. Chem., **42** (5), 1059～1061, 1978

Racemization of L-Proline during Trifluoroacetylation

Detection of Unexpected Stereoisomers in Trifluoroacetylprolyl-valine *t*-Butyl Ester

Ichiro TOMIDA and Takahisa KUWAHARA

Department of Agriculture, Institute of Agricultural Chemistry, Shinshu University, Ina-shi, Nagano-ken

Received October 24, 1977

F. Weygand *et al.*[1] have reported an excellent method of racemization test with GLC (gas-liquid-chromatography) by using the coupling reaction between Tfa-Pro-Val-OH and H-Pro-OMe. In an attempt to pursue this method further, we wished to synthesize Tfa-L-Pro-L-Val-O_tBu (I) and Tfa-L-Pro-D-Val-O_tBu (II) which then should be converted to the corresponding Tfa-Pro-Val-OH.

However, in the condensation product from Tfa-L-Pro-OH and H-L-Val-O_tBu by the method of DCCD/HOBt,[2] we have always detected a small amount of the by-product by GLC-analysis at a t_R corresponding to the enantiomer of the L-D-compound. In this case, the Tfa-L-Pro-OH used was an ether-soluble fraction obtained from a reaction mixture of L-Pro and 2～2.5 eq mole Tfa-anhydride. Also in the condensation product between Tfa-L-Pro-OH and H-D-Val-O_tBu, a small amount of by-product was always detected at the position corresponding to the enantiomer of the L-L-compound (Fig. 1). (GLC: Hitachi Perkin Elmer K 53; column (1 m×3 m/m) packed with OV-17 (5%) impregnated on chromosorb M AW HMDS; FID-detector; carrier gas N_2 1.5 atm; column temperature, 170°C; t_R of Tfa-L-Pro-L-Val-O_tBu=6.40 min and t_R of its epimer, L-D-compound=5.40 min). The fact that those by-products were the unexpected stereoisomers *e.g.* D-L-enantiomer in the former case and D-D-enantiomer in the latter, respectively, was confirmed from the following investigation: 1) The elementary analyses of both condensation products agreed with the composition of Tfa-Pro-Val-O_tBu. 2) The pattern of GLC/MS-analyses of the both main- and sub-peaks were identical and all the fragments satisfied Tfa-Pro-Val-O_tBu structure (*m/e*: 367(M^+), 310 ($M^+-C(CH_3)_3$), 293, 266 ($M^+-COOC(CH_3)_3$)).

In order to distinguish to which enantiomer, D-D or L-L, the sub-peak in the latter condensation product above should be assigned, in other words, to decide in which amino acid, Pro or Val, the racemization had occurred, the product was treated with trifluoroacetic acid. It was followed by condensation with H-L-Pro-OMe according to the method of DCCD/HOSu[2] to give Tfa-Pro-Val-Pro-OMe, which was then subjected to GLC-analysis. Two peaks were obtained: the main- corresponded with Tfa-L-Pro-D-Val-L-Pro-OMe (t_R=4.00 min at 235°C), while the sub-peak, which should be resulted from the by-product of concern, with Tfa-D-Pro-D-Val-L-Pro-OMe (t_R=5.10 min).* The result proves that the unexpected diastereomer was Tfa-D-Pro-D-Val-O_tBu, but not L-L-isomer (I).

In order to clarify problems related to this racemization of L-Pro, we investigated the trifluoroacetylation product precisely, and found that the ether-insoluble, solid fraction consisted not of the unreacted starting material, but of some other compound. This solid substance showed after recrystallization from acetone/*n*-hexane the same mp (mp 139～141°C) as the compound described as Tfa-L-Pro-anhydride in Lit[3] (mp 139.5～140°C). Its optical rotation was also the same and nearly as small as zero ($[\alpha]_D$–2; Lit.[3] $[\alpha]_D$–4.1 (c=0.4, benzene)). The spectral data were coincident with those of Tfa-Pro-anhydride(NMR($CDCl_3$): (TMS=0.00) δ 2.1 (d, 8H) 3.7 (t, 4H) 4.5 (t, 2H, CO-CH-N); IR (KBr): 1820, 1755, 1690, 1680 cm^{-1}). This substance was, however, unexpectedly proved to be a complete racemate. Namely, the reaction mixture obtained by treating it with H-D-Val-O_tBu showed two GLC-peaks of nearly identical areas corresponding to the L-L(=D-D) and D-L (=L-D)-isomers of Tfa-Pro-Val-O_tBu. Contamination of the ether-soluble fraction, which was used as Tfa-L-Pro-OH in the above reactions, with this compound, Tfa-DL-Pro-anhydride, could have occurred inevitably, giving some difficulties in synthesizing pure compounds, I or II, as described above.

The optically pure Tfa-L-Pro-anhydride was synthesized by the condensation reaction between Tfa-L-Pro-OH and Tfa-L-Pro-chloride under mild conditions. The Tfa-L-Pro-OH used here was obtained as an oily, ether-soluble fraction (yield, 57%) by treating L-Pro with 0.9 eq mole Tfa-anhydride under ice-cooling and allowing the mixture to stand for 2 days at room temperature, followed by evaporation to dryness *in vacuo* under 45°C. The product was confirmed not to be contaminated with Tfa-DL-Pro-anhydride, for it gave a single peak in GLC after condensation with H-L-Val-O_tBu.** A portion of the Tfa-L-Pro-OH was treated

Abbreviation: DCCD, dicyclohexyl carbodiimide; DMF: dimethylformamide; HOSu, N-hydroxysuccinimide; HOBt, 1-hydroxybenzotriazole; O_tBu, *tert*-butoxy; THF: tetrahydrofuran.

* t_R of each standard diastereomer of Tfa-Pro-Val-Pro-OMe at column temperature 235°C. L-L-L, 5.60 min (q=1.00); L-L-D (=D-D-L), 5.10 (q=0.90): L-D-D (=D-L-L), 4.60 (q=0.82) and L-D-L (=D-L-D), 4.00 (q=0.71).

** Later Tfa-L-Pro-OH was again proved not to be contaminated with Tfa-DL-anhydride by a different preparation, namely, trifluoroacetylation of L-Pro in ether as described in Lit.[4]

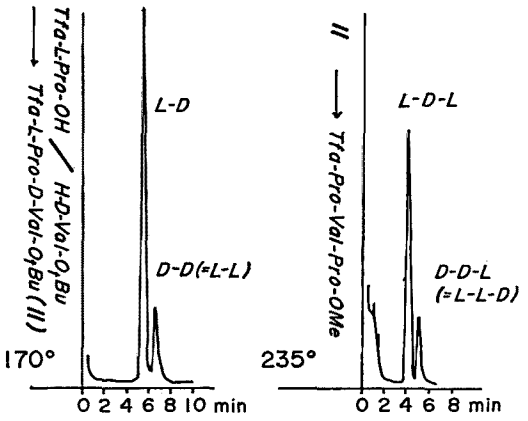

FIG. 1. GLC-analysis of the Synthesized Tfa-L-Pro-D-Val-O_tBu and the Tfa-Pro-Val-Pro-OMe Derived from It.

with thionyl chloride (1.5 eq mole) at moderate temperature (40~45°C) and Tfa-L-Pro-chloride was obtained by fractional distillation, which was also checked by GLC-analysis for its optical purity in the form of Tfa-Pro-Val-O_tBu. Tfa-L-Pro-chloride was an oil (bp 101°C/3 mmHg (yield, 72%), $[\alpha]_D^{18}$ −52.4 (c=0.8, benzene). Found. C, 36.63; H, 3.34; N, 6.23. Calcd. for $C_7H_7NO_2ClF_3$: C, 36.62; H, 3.07; N, 6.10%). The condensation reaction between the compounds gave Tfa-L-Pro-anhydride (mp 113~115°C, $[\alpha]_D^{18}$ −105.8 (c=0.5, benzene), other data s. EXPERIMENTAL). Its optical purity was established by the single peak corresponding to Tfa-L-Pro-L-Val-O_tBu in the same GLC-analysis as described above.

Some derivatives were prepared by using this compound and Tfa-DL-Pro-anhydride (s. EXPERIMENTAL).***

Tfa-L-Pro-anhydride racemized partially when heated in refluxing ethyl acetate containing a small amount of triethylamine (s. EXPERIMENTAL) and also when heated in trifluoroacetic acid. These observation makes it possible to speculate that in the trifluoroacetylation of L-Pro, a small amount of asymmetric anhydride, Tfa-L-Pro-O-CO-CF_3, should have formed at first (*cf.* Lit.[3]), which then racemized directly or *via* the Tfa-L-Pro-anhydride under the reaction conditions.

*** Mps and optical rotation of some derivatives of Tfa-L-Pro-anhydride were the same with those in Lit.[3] On the other hand, the corresponding derivatives of Tfa-DL-Pro-anhydride showed the other mps (s. EXPERIMENTAL). It appears therefore that in the Lit.[3] Tfa-L-Pro-anhydride had been isolated and was used for the preparation of those derivatives, while for the identification of Tfa-Pro-anhydride, the complete or incomplete racemate had been taken.

EXPERIMENTAL

1. Tfa-L-Pro-anhydride. The Tfa-L-Pro-OH and the Tfa-L-Pro-chloride (1: 1 molar ratio) were dissolved in ethyl acetate (35 ml for 10 mM) and triethylamine was added under ice-cooling until the solution became slightly alkaline. The reaction mixture was allowed to stand for 12 hr at room temperature. It was washed with water and then dried over anhydrous sodium sulfate. Evaporation of the solvent *in vacuo* gave a solid residue, which was washed with ethyl acetate/*n*-hexane and with ether. A crystalline substance, mp 113~115°C (yield, 48%). $[\alpha]_D^{19}$ −102.5 (c=0.5, benzene). IR (KBr): 1830, 1760, 1710, 1695 cm^{-1}. NMR ($CDCl_3$): δ 2.0~2.5 (ring methylene, 8H) 3.8 (t, N-CH_2-, 4H) 4.6 (t, α-CH, 2H). Found: C, 41.56; H, 3.41; N, 6.78. Calcd. for $C_{14}H_{14}O_5N_2F_6$ (404.3): C, 41.59; H, 3.49; N, 6.93%.

2. Tfa-L-Pro-anilide. Tfa-L-Pro-anhydride was condensed with aniline in ethanol for 0.5 hr according to the Lit.[3] A crystalline substance (from water). (yield, 69%). mp 158~159°C. $[\alpha]_D^{18}$ −42.5 (c= 0.33, THF). (Lit.[3] mp 161~162°C, $[\alpha]_D^{16}$ −50 (c= 0.33, anhyd. THF)). Found: C, 54.54; H, 4.53; N, 9.87. Calcd. for $C_{13}H_{13}O_2N_2F_3$: C, 54.55; H, 4.58; N, 9.78%.

3. Tfa-L-Pro-Gly-OEt. Tfa-L-Pro-anhydride was condensed with H-Gly-OEt (HCl) in DMF in the presence of triethylamine and stirring overnight at room temperature, followed by separation of the neutral fraction from the reaction mixture. A crystalline substance, mp 112~113°C (yield, 41%). $[\alpha]_D^{18}$ −65.0 (c= 0.9, THF). (Lit.[3] mp 112~114°C, $[\alpha]_D^{17}$ −63.2 (c= 0.87, anhyd. THF) and mp 111~113°C, $[\alpha]_D^{18}$ −68.2 (c=0.46, anhyd. THF)). Found: C, 44.65; H, 5.03; N, 9.24. Calcd. for $C_{11}H_{15}O_4N_2F_3$: C, 44.60; H, 5.10; N, 9.46%.

4. Tfa-DL-Pro-anilide. Tfa-DL-Pro-anhydride was condensed with aniline in the same manner as for the compound in 2. A crystalline substance, mp 159~161.5°C (Lit.[3] mp 157°C). Found: C, 54.42; H, 4.42; N, 9.62. Calcd. for $C_{13}H_{13}O_2N_2F_3$: C, 54.55; H, 4.58; N, 9.78%.

5. Tfa-DL-Pro-Gly-OEt. Tfa-DL-Pro-anhydride was condensed with H-Gly-OEt (HCl) in a manner similar to the compound in 3. A crystalline substance, mp 119~120°C (yield, 65%). Found: C, 44.72; H, 5.05; N, 9.46. Calcd. for $C_{11}H_{15}O_4N_2F_3$: C, 44.60; H, 5.10; N, 9.46%.

6. Racemization of Tfa-L-Pro-anhydride to Tfa-DL-Pro-anhydride. Tfa-L-Pro-anhydride was heated in refluxing ethyl acetate containing 0.5 ml triethylamine

for 0.5 hr. The resulted crystalline substance showed mp 139~140.5°C and $[\alpha]_D^{19}$ −3.3 (c=0.4, benzene). IR: identical to that of the starting material. Test in epimerization in the form of Tfa-Pro-L-Val-O_tBu by GLC, L-L/D-L=58: 42. After reflux of Tfa-L-Pro-anhydride (0.33 g) in trifluoroacetic acid (1.2 ml) at 120°C (bath temperature), racemization was detected to the extent of 12.5% after 5 min, 32% after 10 min and 49% after 0.5 hr (The extent of racemization= % of D-L-compound×2).

REFERENCES

1) F. Weygand, D. Hoffmann and A. Prox, *Z. Naturforschg.*, **23b**, 276 (1968).
2) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
3) F. Weygand, P. Klinke and I. Eigen, *ibid.*, **90**, 1896 (1957).
4) M. Horiba, H. Kitahara and A. Murano, *Agric. Biol. Chem.*, **41**, 2003 (1977).