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FLUORINE-CONTAINING AMINO ACIDS AND THEIR DERIVATIVES. 9.[1] SYNTHESIS AND BIOLOGICAL ACTIVITIES OF DIFLUOROMETHYLHOMOCYSTEINE

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Summary: A new fluorine-containing amino acid, difluoromethylhomocystein, was prepared in a chiral form. Some of its biological activities are described.

The essential amino acid methionine has diverse biochemical and physiological roles not only as a constituent of peptides and proteins but also as a component of biosynthetic agents such as S-adenosylmethionine[2]. Thus, a number of its analogs, particularly those modified at the methylmercapt moiety, have been synthesized and their biological activities, e.g., whether or not they can inhibit S-adenosylmethionine formation or whether or not they can be incorporated into proteins, have attracted the attention of organic chemists as well as biochemists.[3] One synthetic approach of current interest, incorporation of fluorine into this molecule, has led to two new analogs, trifluoromethyl[4a] and monofluoromethylhomocystein[4b]. The biological activity of trifluoromethylhomocystein has been studied to certain extent but many ambiguities still remain[3]. Another analog of this type, difluoromethylhomocystein (1), has been suggested, based on mechanistic speculation[3g], to have somewhat different biological features from the former two analogs. Very recently, a Canadian group[5] reported its synthesis for the first time, starting from (L)-N-acetylhomocysteinthiolactone.[6] But their method seemed to be quite unpractical to yield a complete racemic mixture of N-acetyldifluoromethylhomocystein. We herein report a more convenient and straightfoward chemical synthesis of the optically active (L)-isomer (1) which eliminates tedious enzymatic separation required in their method and also some of its biological activities.

As shown in the reaction Scheme, (L)-homocystine was used as a starting material in our method. It was easily reduced to (L)-homocystein sodium salt without racemization by treatment with sodium metal in liquid ammonia.[7] The salt was quite unstable in air and handled under nitrogen. It was subjected to difluoromethylation of the mercapt group in preference to other nucleophilic functional groups like the amino and free carboxylate ones despite no successful reports known for such a complex case.[8] After our careful search for the optimal reaction conditions, the reaction yielded the desired product (1) in an isolation yield of 56% as described below.

$$\underset{\text{(L)-Homocystine}}{(\text{-SCH}_2\text{CH}_2\text{CH(NH}_2\text{)CO}_2\text{H})_2} \xrightarrow{\text{Reduction}} \text{NaSCH}_2\text{CH}_2\text{CH(NH}_2\text{)CO}_2\text{H} \xrightarrow[2)\ H_3^+O]{1)\ :CF_2} \underset{\mathbf{1}}{\text{F}_2\text{CHSCH}_2\text{CH}_2\text{CH(NH}_2\text{)CO}_2\text{H}}$$

(L)-Homocystein sodium salt (2.04 g, 10.56 mmol) was dissolved in 18 ml of nitrogen-flashed dry ethanol and chlorodifluoromethane (Freon-22) was gradually introduced into this solution at room temperature during dropwise addition of an ethanol solution of potassium t-butoxide (2.03 g, 20.1 mmol/12 ml EtOH) placed in a side funnel over a period of 1-2 hrs. The reaction proceeded smoothly in an exothermic manner and the temperature gradually rose to around 40°C with precipitation of white inorganic salts. The reaction was completed by introduction of Freon-22 for an additional 1 hr after all the base had been added. The reaction mixture was quenched with 1 N aq. HCl (pH 4.3) and condensed under reduced pressure to dryness. The methanol-insoluble inorganic salts were filtered off and the mixture was condensed again, giving 1.7 g of the solidified crude product (1). Column chromatography of this over an acidic ion-exchange resin, Dowex 50W-X8, and over HP-20 afforded the pure product (1) in an overall yield of 56% from the starting salt. It showed the following physical properties: mp >195°C, $[\alpha]^{25}_D$ +23.4±0.8° (c, 0.822,

4 N-HCl), see ref. 9 for spectral data. The optical purity of 1 was unambiguously confirmed by the single clean triplet ^{1}H-NMR signal (δ 6.81, J = 56.1 Hz) of the CHF_2 group of the amide derived from the methyl ester of 1 and (*R*)-(+)-MTPA in contrary to the two well separated corresponding signals (δ 6.81 and 6.73) of the diastereomeric mixture derived from racemic 1.

The biological activities of 1 included a rather high and selective insecticidal activity against aphids. It was effective even against drug-resistant aphids, particularly Myzus persicae which is resistant to malathion and pirimicarb. This may suggest that it acts differently from those of these well known insecticides, although nothing has been clarified yet about its mechanisms. The compound also showed moderate growth inhibitory activity, IC_{50} 42 μg/ml, on A-549 (lung carcinoma, human) in vitro. We are presently conducting a study to examine its in vivo anticancer activity.

Finally, the calculated values of partition coefficient (CLOGP) by the Hansch method[10] obviously suggests that incorpolation of fluorine into the methyl group of methionine significantly enhances the hydrophobicity of the molecule.[10] As methionine often acts as an important hydrophobic amino acid residue in biologically active peptides or proteins, we are conducting studies on the modification of methionine-containing peptides with this unnatural amino acid. Our results will be reported elsewhere in the near future.

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6. We had studied the same procedure as theirs and had applied for a Japanese patent on Oct. 25th, 1988, about two months before the date on which their paper (Ref. 5) was received for publication.
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9. 200 MHz ^{1}H-NMR: δ (D_2O) 2.23 (m, 2H, β-CH_2), 2.96 (t, 2H, J = 7.8 Hz, γ-CH_2), 3.84 (t, lH, J = 6.4 Hz, α-CH), 7.07 (t, lH, J = 55.8 Hz, CHF_2); IR (KBr disc): 3400 (m), 3200-2400 (br.s), 1585 (br.s), 1513 (s), 1408 (s), 1326 (m), 1015 (s), 765 (m).; Mass m/z 186 (MH^+), 167 (MH^+ - F), 140 (M^+ - COOH), 134 (M^+ - CHF_2), 111 ($F_2CHSCH_2CH_2$), 97 (F_2CHSCH_2), 74 (M^+ - $F_2CHSCH_2CH_2$). (Found: C, 32.31; H, 4.90; N, 7.54; F, 20.21; S, 17.60. Calcd for $C_5H_9O_2NF_2S$: C, 32.43; H, 4.90; N, 7.56; F, 20.52; S, 17.31.)
10. MedChem Software Release 3.54 (Daylight Chemical Information Systems, Inc. USA) was used in the calculation of CLOG P values for the following analogs: For references see:(a) Hansch, C.; Leo A. "Substituent Constants for Correlation Analysis in Chemistry and Biology" John-Wiley & Sons, Inc., New York, **1979**, 18-43; (b) Chou, J. T.; Jurs, P. C. *J. Chem. Inf. Comput. Sci.*, **1979**, *19*, 172.

$XYCH_2CH_2CH(NH_2)COOH$

CLOGP : XY = CF_3S (-1.742); CHF_2S (-2.405); CH_2FS (-2.678); MeS (-2.851); EtS (-2.322); CF_3CH_2 (-2.003); Et (-1.412); CF_3O (-2.772); MeO (-3.881).

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