Acknowledgement The authors are much indebted to Prof. T. Reichstein (Basel) for sending them the authentic samples of mallogenin and sarmentogenin and for helpful advice. They express their gratitude to Dr. L. Masler (Bratislava) and Prof. R. Tschesche (Bonn)for their kinds supply of the authentic samples of syriogenin and xysmalogenin. Thanks are also due to Mr. Yoshida for the collection of the plant material and to Miss Imai for mass spectra measurement.

[Chem. Pharm. Bull. 18(4) 831—834 (1970)]

UDC 547.466.2.03 : 541.653

Optical Rotatory Dispersion of Nitrobenzene Derivatives. II.[1] N-*o*-Nitrobenzoyl L-α-Amino Acids

UKON NAGAI[2] and MASATERU KURUMI[2a]

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received November 4, 1969)

In the previous work,[1] the optical rotatory dispersion (ORD) of *o*-nitrobenzoates of optically active secondary alcohols was studied. The relation between the sign of their Cotton effect and their absolute configuration was found and interpreted by assuming unsymmetrical twisting of the nitrobenzene chromophore induced by the neighbouring asymmetric center. In this paper, the ORD behaviour of similar derivatives of α-amino acids was examined.

Nine N-*o*-nitrobenzoyl L-α-amino acids were prepared and their ORD curves were measured in methanol. They showed Cotton effect centered at 330 nm, whose first extrema appeared near 370 nm and the second near 310 nm. Their molecular amplitudes with sign are presented in Table I together with those of the corresponding carboxylate ions and some methyl esters.

TABLE I. Molecular Amplitudes of N-*o*-Nitrobenzoyl L-α-Amino Acids and Their Methyl Esters

Compound No.	Parent amino acid	Free acid[a]	Carboxylate ion[b]	Methyl ester[c]
I	lysine[d]	−48.2	−34.5 (+14.3)	−33.9 (+13.7)
II	leucine	−46.2	+ 7.8 (+54.0)	
III	valine	−42.9	− 2.6 (+40.3)	−24.9 (+18.0)
IV	isoleucine	−41.6 −33.4[e]	− 2.4 (+39.2)	−28.2 (+13.4)
V	alanine	−36.1	− 1.6>[f]	
VI	serine	−35.6		
VII	tyrosine[d]	−12.5	+ 7.5<[f]	
VIII	phenylalanine	−11.3	+15.8 (+27.1)	− 0.9 (+10.4)
IX	tryptophan	− 0.5	+27.5 (+28.0)	

The values in parentheses mean the difference from those of the corresponding free acids.
a) measured in methanol *b*) measured in 4% $NaHCO_3$
c) measured in methanol *d*) bis (*o*-nitrobenzoyl) derivative
e) measured in chloroform
f) The molecular rotation values at the first extrema divided by 100 are presented since the second extrema were not determined.

1) Part I: U. Nagai and H. Iga, *Tetrahedron*, **26**, 725 (1970).
2) Location: *Kita-12, Nishi-6, Sapporo*; *a*) Present address: *Research Laboratory, Torii Seiyaku Co., Ltd., 3-14-3 Minamiyahata, Ichikawa.*

Following facts can be pointed out from Table I. First, all the compounds so far examined show negative Cotton effect. This fact is useful in determining the absolute configuration of an α-amino acid. But, it seems peculiar since these compounds have S-configuration and are expected to exhibit positive Cotton effect by analogy with *o*-nitrobenzoyl esters. However, this is not surprising because the effective bulkiness of a carbonyl group (sp^2-carbon) may be smaller than that of an alkyl residue (sp^3-carbon).

Second, large positive shift of the molecular amplitude is observed when measured in an alkaline solution. Third, the methyl esters exhibit slightly less negative Cotton effect than those of the corresponding free acids. These facts can be rationalized as follows on the assumption that an N-*o*-nitrobenzoyl amino acid is a mixture of conformers A and B (Fig. 1) by analogy with *o*-nitrobenzoyl esters.[1] The conformer A in free acid form can be stabilized by the hydrogen bond between the carboxyl group and the amide carbonyl group, and the conformer A in carboxylate ion form is destabilized by the Coulomb repulsion between the carboxylate ion and the polarized amide carbonyl group. According to the explanation adopted in the previous paper,[1] the conformer A (B) contribute negatively (positively) to the Cotton effect through the induced unsymmetrical twisting of the nitrobenzene chromophore.

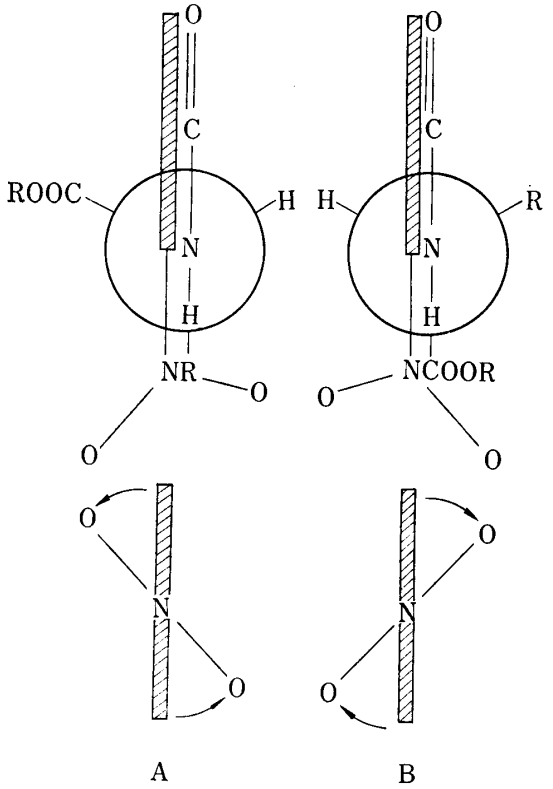

Fig. 1. Two Possible Conformers of an N-*o*-Nitrobenzoyl L-α-Amino Acid and Their Projections which indicate the Favoured Twisting Direction of Nitrobenzene Chromophore viewed from the Nitro Group Side

Shaded Area means the Benzene Ring.

Fourth, derivatives of aliphatic amino acids exhibit more negative Cotton effect than those of aromatic amino acids and the trend is retained in the series of methyl esters and of carboxylate ions, too. This fact suggests that the conformer B in the latter compounds is more stabilized than in the former ones. It seems peculiar from the viewpoint that the bulkiness of their side chains would not differ significantly in both groups. Therefore, the presence of some unknown factor which stabilizes the conformer B in the aromatic derivatives is suggested. But, no plausible reason to explain the fact could not be found. Further investigation is necessary.

In connection with our findings, it is interesting to note that the reversal in sign of the Cotton effect is observed between the xanthates of secondary alcohols of S-configuration[3] and N-methylmercaptothiocarbonyl derivatives of L-α-amino acids,[4] and that solvent effect changes the sign of the Cotton effect of N-methylmercaptocarbonyl derivatives of α-amino acids and α-phenethylamine but not that of the *sec*-butylamine derivative.[5]

Compound (IV) showed negative Cotton effect ($a=-33.4$) when measured in chloroform, so there seems to be no appreciable solvent effect in our case.

3) B. Sjöberg, D.J. Cram, L. Wolf, and C. Djerassi, *Acta Chem. Scand.*, **16**, 1079 (1962).
4) C. Djerassi, H. Wolf, and E. Bunnenberg, *J. Am. Chem. Soc.*, **84**, 4552 (1962).
5) Sh. Yamada, K. Ishikawa, and K. Achiwa, *Chem. Pharm. Bull.* (Tokyo), **13**, 892 (1965); *idem*, *ibid.*, **14**, 921 (1966).

Experimental[6]

General Procedure to Prepare *o*-Nitrobenzoyl L-α-Amino Acids and Their Methyl Esters——An L-α-amino acid was dissolved in a slight excess of 1N NaOH. To the solution an equivalent amount of *o*-nitrobenzoyl chloride was added dropwise in an ice-water bath under vigorous stirring. During the addition the solution was kept slightly alkaline by adding 1N NaOH at intervals. Stirring was continued until the oily particles of the chloride disappeared. The reaction mixture was then acidified with 4N HCl to give oily or crystalline precipitates. The oily precipitates crystallized by cooling and scratching the wall of the vessel. They were collected by filtration and recrystallized from a suitable solvent described in the respective item. Their methyl esters were prepared by treating the solution of the acids in ether or CH_2Cl_2 with CH_2N_2 in ether.

N,N'-Bis(*o*-nitrobenzoyl)-L-lysine (I)——mp 141—144°, from MeOH–ether. ORD (c=0.041, MeOH) [α] (mμ): −64.1° (500), −84.0° (450), −271° (400), −399° (372) (trough), +690° (316) (peak); (c=0.064, 4% $NaHCO_3$): −37.5° (550), −59.3° (500), −84.5° (450), −200° (400), −281° (372) (trough), +493° (316) (peak). *Anal.* Calcd. for $C_{20}H_{20}O_8N_4$: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.06; H, 4.55; N, 12.59.

Methyl Ester of I——mp 143—145°, from MeOH–EtOAc. ORD (c=0.051, MeOH) [α] (mμ): −78.0° (590), −88.0° (550), −98.0° (500), −118° (450), −236° (400), −295° (374) (trough), +315° (316) (peak), 0° (300). *Anal.* Calcd. for $C_{21}H_{22}O_8N_4$: C, 55.02; H, 4.84; N, 12.22. Found: C, 55.17; H, 5.07; N, 12.10.

N-*o*-Nitrobenzoyl-L-leucine (II)——mp 111—112°, from $CHCl_3$–benzene. ORD (c=0.111, MeOH) [α] (mμ): −90.0° (550), −144° (500), −224° (450), −665° (400), −745° (365) (trough), +910° (310) (peak); (c=0.079, 4% $NaHCO_3$): −17.7° (590), −17.7° (550), −18.9° (500), −15.2° (450), +29.0° (400), +101° (366) (peak), −177° (318) (trough), −101° (310). *Anal.* Calcd. for $C_{13}H_{16}O_5N_2$: C, 55.71; H, 5.75; N, 10.00. Found: C, 55.26; H, 5.78; N, 9.75.

N-*o*-Nitrobenzoyl-L-valine (III)——mp 153—155°, from acetone–benzene. ORD (c=0.036, MeOH) [α] (mμ): −55.6° (500), −111° (450), −389° (400), −556° (372) (trough), +1055° (310) (peak), +389° (300); (c=0.044, 4% $NaHCO_3$): −36.8° (450), −73.6° (400), −96.5° (374) (trough), 0° (324) (peak), −115° (315). *Anal.* Calcd. for $C_{12}H_{14}O_5N_2$: C, 54.13; H, 5.30; N, 10.52. Found: C, 54.16; H, 5.38; N, 10.31.

Methyl Ester of III——mp 100—102°, from ether–hexane. ORD (c=0.113, MeOH) [α] (mμ): −54.8° (590), −70.7° (550), −91.9° (500), −159° (450), −390° (400), −538° (372) (trough), +631° (318) (peak), +355° (300). *Anal.* Calcd. for $C_{13}H_{16}O_5N_2$: C, 55.71; H, 6.17; N, 9.52. Found: C, 55.79; H, 5.78; N, 9.74.

N-*o*-Nitrobenzoyl-L-isoleucine (IV)——mp 169—170° from acetone–benzene. ORD (c=0.039, MeOH) [α] (mμ): −103° (590), −103° (550), −154° (500), −205° (450), −461° (400), −564° (370) (trough), +923° (313) (peak), +308° (300); (c=0.180, $CHCl_3$): −41.0° (500), −68.9° (450), −232° (400), −349° (372) (trough), +840° (315) (peak), +517° (305); (c=0.081, 4% $NaHCO_3$): −37.0° (375) (trough), +49.4° (330) (peak). *Anal.* Calcd. for $C_{13}H_{16}O_5N_2$: C, 55.71; H, 5.75; N, 10.00. Found: C, 55.86; H, 5.75; N, 10.02.

Methyl Ester of (IV)——mp 98—100°, from ether–hexane. ORD (c=0.113, MeOH) [α] (mμ): −63.4° (590), −77.9° (550), −92.0° (500), −150° (450), −359° (400), −512° (372) (trough), +631° (318) (peak), +519° (310). *Anal.* Calcd. for $C_{14}H_{18}N_2O_5$: C, 57.13; H, 6.17; N, 9.52. Found: C, 57.31; H, 6.27; N, 9.74.

N-*o*-Nitrobenzoyl-L-alanine (V)——mp 159—162°, from ether. ORD (c=0.033, MeOH) [α] (mμ): −60.6° (450), −364° (400), −546° (370) (trough), +970° (315) (peak), 0° (300); (c=0.144, 4% $NaHCO_3$): −21.5° (590), −27.8° (500), −34.7° (450), −41.7° (400), −69.4° (370) (trough). *Anal.* Calcd. for $C_{10}H_{10}O_5N_2$: C, 50.42; H, 4.23; N, 11.76. Found: C, 50.40; H, 4.58; N, 11.72.

N-*o*-Nitrobenzoyl-L-serine (VI)——mp 164—166°, from acetone–benzene. ORD (c=0.050, MeOH) [α] (mμ): −40° (590), −50° (550), −60° (550), −100° (450), −280° (400), −440° (377) (trough), +960° (315) (peak), +240° (300). *Anal.* Calcd. for $C_{10}H_{10}O_6N_2$: C, 47.25; H, 3.97; N, 11.02. Found: C, 47.45; H, 3.83; N, 11.15.

N,O-Bis(*o*-nitrobenzoyl)-L-tyrosine (VII)——mp 179—182°, from acetone–ether. ORD (c=0.108, MeOH) [α] (mμ): −27.9° (590), −37.2° (500), −93.0° (400), −130° (375) (trough), +130° (315) (peak); (c=0.090, 4% $NaHCO_3$): +22.2° (590), +27.8° (500), +111° (400), +156° (370) (peak), 0° (335). *Anal.* Calcd. for $C_{23}H_{17}N_3O_9$: C, 57.62; H, 3.57; N, 8.77. Found: C, 57.25; H, 3.46; N, 8.57.

N-*o*-Nitrobenzoyl-L-phenylalanine (VIII)——mp 100—102°, from benzene. ORD (c=0.045, MeOH) [α] (mμ): −22.5° (550), −22.5° (500), −45.0° (450), −90.0° (400), −112° (380) (trough), +202° (315) (peak), +67.5° (300); (c=0.56, 4% $NaHCO_3$): +32.4° (590), +37.8° (550), +50.5° (500), +83.0° (450), +189° (400), +343° (362) (peak), −108° (310) (trough). *Anal.* Calcd. for $C_{16}H_{14}O_5N_2$: C, 61.14; H, 4.49; N, 8.91. Found: C, 61.07; H, 4.47; N, 8.78.

Methyl Ester of VIII——mp 94—96°, from ether–hexane. ORD (c=0.096, MeOH) [α] (mμ): −26.1° (590), −28.2° (550), −31.4° (500), −41.8° (450), −52.2° (400), −62.9° (370) (trough), −34.5° (314) (peak). *Anal.* Calcd. for $C_{17}H_{16}O_5N_2$: C, 62.19; H, 4.91; N, 8.53. Found: C, 62.23; H, 4.98; N, 8.64.

N-*o*-Nitrobenzoyl-L-tryptophan (IX)——mp 201—203°, from acetone–benzene. ORD (c=1.020, MeOH) [α] (mμ): +13.8° (590), +14.8° (550), +19.7° (500), +27.6° (450), +35.5° (420), +25.6° (384) (trough),

6) All melting points are not corrected. ORD spectra were measured with JASCO ORD/UV-5 Optical Rotatory Dispersion Recorder at room temperature ranging 10—30°.

+39.2° (340). CD (c=0.215, MeOH) [θ] (mμ): 0 (400), −108 (345) (negative maximum), −32.4 (330). ORD (c=0.063, 4% $NaHCO_3$) [α] (mμ): +25.4° (590), +38.2° (550), +57.2° (500), +102° (450), +286° (400), +429° (370) (peak), −349° (318) (trough), −254° (310). *Anal.* Calcd. for $C_{18}H_{15}O_5N_3$: C, 61.19; H, 4.28; N, 11.89. Found: C, 61.13; H, 4.21; N, 12.03.

Acknowledgement We thank Prof. H. Mitsuhashi for his kind encouragement. Thanks are also due to Mrs. T. Tohma, Miss A. Maeda, and Miss H. Kakizaki for elemental analyses.

[Chem. Pharm. Bull. 18(4) 834—838 (1970)]

UDC 615.31.015 : 615.281.07

General Base Catalyzed Hydrolysis of Furylmethylketone Isonicotinoylhydrazone[1]

Kiichiro Kakemi, Hitoshi Sezaki, Kikuo Iwamoto, and Hiroshi Kobayashi

Faculty of Pharmaceutical Sciences, Kyoto University[2]

(Received November 4, 1969)

Aminolysis of esters[3,4] and a number of acyl transfer reactions of acetylimidazole, including hydrolysis[5] have been reported to be subject to classical general base catalysis. Schowen and Zuorick[6] have reported the superimposed general-base catalysis by glycine-sodium glycinate buffer solution in the hydrolysis of anilides. Schwartz has recently reported the participation by general base catalysis in the aminolysis of benzylpenicillin by aliphatic diamines.[7]

However, very little work has been reported concerning the general catalysis of hydrolysis of hydrazones.

In our previous investigation on the hydrolysis of antitubercular agents like furylmethylketone isonicotinoylhydrazone (FKI)[8] and other isoniazid hydrazones in biological media,[9] amino acids such as glycine, asparagine, and glutamic acid exhibited a dominant effect to accelerate the degradative reaction and the data seemed to support the view that nonprotonated amino group acts as catalytic species.

To test this hypothesis, studies were conducted of the reaction of FKI with a series of amino acids and a few aliphatic amines, and kinetic deuterium solvent isotope effect was tested for the certification of general catalysis. An investigation of the mechanism of the catalytic reaction should aid in a better understanding of biopharmaceutical mechanisms involving such compounds.

Experimental

Materials——FKI (Daiichi Seiyaku Co., Ltd.) was used as received. Amino acids, aliphatic amines, and all other chemicals were of reagent grade. Deuterium oxide (Merck Sharp & Dohme of Canada Ltd.) was more than 99.7% in its isotopic purity.

1) This paper forms Part III of "Studies on the Stability of Drugs in Biological Media," Preceding paper, Part II: K. Kakemi, H. Sezaki, K. Iwamoto, and Y. Sano, *Chem. Pharm. Bull.* (Tokyo), **17**, 1623 (1969).
2) Location: *Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto.*
3) J.F. Bunnett and D.T. Davis, *J. Am. Chem. Soc.*, **82**, 665 (1960).
4) W.P. Jenks and J. Carriuolo, *J. Am. Chem. Soc.*, **82**, 675 (1960).
5) W.P. Jenks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272, 1280 (1959).
6) R.L. Schowen and G.W. Zuorick, *J. Am. Chem. Soc.*, **88**, 1223 (1966).
7) M.A. Schwartz, *J. Pharm. Sci.*, **57**, 1209(1968).
8) K. Kakemi, H. Sezaki, N. Takasugi, and K. Iwamoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1481 (1968).
9) K. Kakemi, H. Sezaki, K. Iwamoto, and Y. Sano, *Chem. Pharm. Bull.* (Tokyo), **17**, 1623 (1969).