The Preparation and Some Properties of *N*-Monomethylated L-Amino Acids

By Mitsuo EBATA, Yasuo TAKAHASHI and Hideo OTSUKA

Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka

(Received January 10, 1966)

N^{α}-Monomethylated amino acids which can be grouped as the unusual amino acids have been found in natural products. Most of them are present in a series of antibiotics which have been discovered and elucidated during the last several years. They are *N*-methyl-L-isoleucine, *N*-methyl-L-valine (Enniatins);[1,2] sarcosine, *N*-methyl-L-alanine, *N*-methyl-L-valine, *N*-methyl-L-isoleucine (Actinomycins);[3] *N*-methyl-L-phenylglycine (L-α-phenylsarcosine), *N*, β-dimethyl-L-leucine (Etamycin);[4] *N*, *N'*-dimethyl-L-cystine, *N*-methyl-L-valine, *N*-methyl-L-alloisoleucine, *N*, γ-dimethyl-L-alloisoleucine (Quinoxaline antibiotics);[5-7] *N*-methyl-L-phenylalanine (Staphylomycin)[8] and *N*-methyl-L-leucine (Sporidesmolides).[9] Two of the *N*-methylamino acids, *N*-methyl-D-tyrosine (D-surinamine) and *N*-methyl-L-tryptophan (L-abrin),

1) Pl. A. Plattner and U. Nager, *Helv. Chim. Acta*, **31**, 665, 2192 (1948).
2) M. M. Shemyakin, Yu, A. Ovchinnikov, A. A. Kiryushkin and V. I. Ivanov, *Tetrahedron Letters*, **7**, 301 (1962).
3) H. Brockmann, *Angew. Chem.*, **72**, 939 (1960); *Ann. N. Y. Acad. Sci.*, **89**, 323 (1960).
4) J. C. Sheehan, H. G. Zachan and W. B. Lawson, *J. Am. Chem. Soc.*, **80**, 3349 (1959).
5) H. Otsuka and J. Shoji, *J. Antibiotics (Tokyo)*, **A16**, 52 (1963).
6) H. Otsuka and J. Shoji, ibid., **A18**, 134 (1965).
7) J. Shoji, K. Tori and H. Otsuka, *J. Org. Chem.*, **30**, 2772 (1965).
8) H. Vanderhaeghe and G. Permentier, *J. Am. Chem. Soc.*, **82**, 4414 (1960).
9) D. W. Russell, *Biochim. Biophys. Acta*, **45**, 411 (1960).

TABLE II. SOME PROPERTIES OF

N-Methylamino acid	Formula	Yield* %	M. p. °C
N-Methyl-L-alanine	$C_4H_9O_2N$	19	260—263
N^{α}-Methyl-L-arginine	$C_7H_{16}O_2N_4$	26	260
N-Methyl-L-aspartic acid	$C_5H_9O_4N \cdot {}^1/_2H_2O$	28	168
N-Methyl-L-cysteic acid	$C_4H_9O_5NS$	24	219—220
N, *N'*-Dimethyl-L-crystine	$C_8H_{16}O_4N_2S_2 \cdot 1/2H_2O$	34	206—208 dec.
N-Methyl-L-glutamic acid	$C_6H_{11}O_4N \cdot {}^3/_4H_2O$	3	131
N-Methyl-glycine(sarcosine)	$C_3H_7O_2N$	—	215 dec.
N-Methyl-L-isoleucine	$C_7H_{15}O_2N$	31	240
N-Methyl-L-leucine	$C_7H_{15}O_2N$	42	220 sub.
N^{α}-Methyl-L-lysine(hydrochloride)	$C_7H_{16}O_2N_2 \cdot HCl$	38	234—235
N-Methyl-L-phenylalanine	$C_{10}H_{13}O_2N$	14	233—237 dec.
N-Methyl-L-serine	$C_4H_9O_3N$	27	194
N-Methyl-L-threonine	$C_5H_{11}O_3N$	14	240
N-Methyl-L-tyrosine(L-surinamine)	$C_{10}H_{13}O_3N \cdot {}^1/_8H_2O$	34	149
N-Methyl-L-valine	$C_6H_{13}O_2N$	36	300

* Yield was calculated from each parent amino acid.
** As *N*-methylamino acid/parent amino acid. Solvent, BuOH : AcOH : H_2O=4 : 1 : 2; temperature,
*** Color yield ratio=Ninhydrin color yield of *N*-methylamino acid/ninhydrin color yield of original
I Estimated from the analytical chart of the automatic amino acid analyzer. The reaction was
II Measured by the method of Moore and Stein.[23] The conditions of the reaction was as described
a In 6 N HCl, acetic acid (1 : 1) b In 5 N HCl

were found in cabbage tree bark[10] and in *Abrus precatorius*[11-13] respectively. It, therefore, can not be doubted that *N*-methylamino acids play a very important role as constituents of peptide or depsipeptide antibiotics.

For the synthesis of the *N*-methylamino acids, however, there is still no simple and generally applicable method except for the method of Quitt et al.,[14] in which the temporary benzylation and the reductive methylation are involved. They have demonstrated that this method is an excellent one by preparing *N*-methylated L-alanine, L-valine, L-leucine, L-phenylalanine, L-serine, L-lysine and L-arginine. However, it has been considered that the kind of *N*-methylamino acids prepared was not sufficient to claim that this is a generally-applicable method.

In the present work the authors have shown that this method is, certainly, a generally-applicable simple method for the preparation of the most *N*-methylamino acids; they have shown this by preparing *N*-monomethyl derivatives of aspartic acid, glutamic acid, isoleucine, threonine and tyrosine, together with the amino acids described above. However, a few exceptions or limitations have been found; namely, the *N*-methylations of histidine and of tryptophan were not achieved. The final products obtained gave large values of negative optical rotations in both cases, while the values of the elemental analyses agreed very nearly with those of the respective expected methylated products. The structures of the products were still obscure, since these compounds have not yet been investigated in detail. *N*-Benzyl-*N*-methyl-L-methionine was prepared in this work in a 54% yield from L-methionine. However, the debenzylation of the compound was not carried out, even in the presence of large amounts of a palladium-barium sulfate catalyst. The treatment of the compound with sodium in liquid ammonia also failed to remove the benzyl group. *N*-Benzyl-L-glutamic acid was prepared with only a 7.4% yield. This indicates that the γ-carboxyl group of glutamic acid exerts a steric hindrance when the Schiff base of glutamic acid with benzaldehyde is formed. For the synthesis of *N*, *N'*-dimethyl-L-cystine, L-thiazolidine-4-carboxylic acid was more useful as the starting material.[15] *N*-Methyl-L-cysteic acid was obtained by the oxidation of *N*, *N'*-dimethyl-L-cystine with performic acid.

The optical rotations of *N*-methylamino acids have already been described in the literature, although the reported values do not always agree. We measured the rotations in 6 N hydrochloric acid unless we otherwise state. The results obtained are listed in Table I. Among the *N*-methylamino acids prepared, we have found that arginine,

10) E. Winterstein, *Z. Physiol. Chem.*, **105**, 20 (1919).
11) T. Hoshino, *Ann.*, **520**, 31 (1935).
12) W. M. Cahill and R. W. Jackson, *J. Biol. Chem.*, **126**, 29 (1938).
13) T. Yoshida and S. Fukuyama, *J. Biochem. (Tokyo)*, **34**, 429 (1941); **36**, 249 (1944).
14) P. Quitt, J. Hellerback and K. Vogler, *Helv. Chim. Acta*, **46**, 327 (1963).
15) S. Ranter and H. T. Clarke, *J. Am. Chem. Soc.*, **59**, 200 (1937).

N-METHYL-L-AMINO ACIDS

$[\alpha]_D^{24-25}$ $c=1-2$	$[M]_D^{N\text{-Methyl amino acid}}$ minus $[M]_D^{\text{Amino acid}}$	Taste	R_f-value ratio**	Color yield ratio with ninhydrin at 570 mμ***	
				I	II
+10.4	−1.0	Slightly sweet	1.1	0.081	0.458
+34.2	+16.1	Slightly sweet	1.8	0.189	0.599
+25.3	+13.3	Sour	1.4	0.039	0.355
+ 6.7	—	—	—	0.083	0.745
+98.8	+723[b]	Tasteless	1.5	0.125	—
+30.6	+6.5	Sour	1.2	0.066	0.344
—	—	Sweet	1.1	0.190	0.473
+46.1	+14.0	Tasteless	1.1	0.014	0.599
+30.0	+26.9	Tasteless	1.1	0.064	0.894
+28.5	+29.8	Faintly sweet	1.2	0.877	1.078
+26.6	+38.9	Tasteless	1.1	0.203	—
+ 9.8	−4.2	Tasteless	1.1	0.045	0.638
−15.9	−3.3	Sweet	1.5	0.065	0.721
+29.3[a]	+58.6[a]	Tasteless	1.2	0.186	—
+29.3	+10.3	Tasteless	1.1	0.023	0.433

22°C; paper, Toyo Roshi No. 51 filter paper; ascending-frame technique was used.
amino acid.
carried out at 100°C for 15 min. at pH 4.9. The ratio, sample solution : ninhydrin solution was 1 : 0.5.
above except ninhydrin concentrations. The ratio, sample solution: ninhydrin solution was 1 : 2.

isoleucine, serine and tyrosine gave somewhat higher values than those of the literature (*N*-methyl-L-arginine, +32.9;[14] *N*-methyl-L-isoleucine, +44.8;[1] *N*-methyl-L-serine, +8.0;[14] and *N*-methyl-L-tyrosine, +19.8[16]). The optical rotations of several *N*-methylamino acids were the same or somewhat lower than those reported by Quitt et al.; nevertheless, they were still higher than those of the derivatives prepared by the classical method (*N*-methyl derivatives of alanine, aspartic acid, lysine, phenylalanine and valine).[16,17] This is one evidence that the method of Quitt et al. is a generally-applicable, simple method for the preparation of *N*-methylamino acids.

The rotational shifts of amino acids induced by *N*-methylation were also observed; they also are listed in the table. In the fact that the values for the latter compounds are invariably more positive than for those for the former, there seems to be a striking rule, as has been pointed out by Izumiya and his associates.[18] However, three exceptions to this rule appeared to occur in the cases of L-alanine, L-serine and L-threonine. We must, therefore, recall the correlation between optically-active amino acid and glycyl-amino acid. In this case, no correlation can be drawn with the introduction of a glycyl residue at the *N*-acyl position.[19-21]

The R_f-values determined upon the one-dimensional paper chromatography of *N*-methylamino acids are listed in Table I as ratios to those of the parent amino acids. The R_f-values of *N*-methylamino acids are usually higher than those of the parent amino acids. Thin-layer chromatography on silica gle G with the same solvent gave almost the same R_f-value as each of the original parent amino acids.

The tastes of *N*-methylamino acids were tested in comparison with those of the L- and D-series of amino acids. The results obtained are shown in Table I. (Of course, the properties are not nowadays important in identifying the individual amino acids.) As far as the tastes of these *N*-methylamino acids were tested, they reproduced the characteristics of the parent L-amino acid series.[22]

The effect of *N*-methylamino acids on the growth of *Escherichia coli* K 12 and *Bacillus subtilis* PCI 219 strains was investigated using the paper-disk method. None of the 14 *N*-methylamino acids tested inhibited the growth of the both microorganisms in a nutrient medium until the concentrations became 0.05 per cent (500 μg./ml.).

It is well known that ninhydrin can react not

16) E. Fischer and W. Lopschitz, *Ber.*, **48**, 377 (1915).
17) Keller-Schierlein, M. L. Michailovic and V. Prelog, *Helv. Chim. Acta*, **42**, 305 (1959).
18) N. Izumiya, A. Nagamatsu and S. Ota, *Kyushu Mem. Med. Sci.*, **4**, 1 (1953).
19) R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers, *J. Am. Chem. Soc.*, **65**, 1013 (1943).
20) P. Karrer, *Helv. Chim. Acta*, **6**, 957 (1923).
21) P. Karrer and A. Schlosser, ibid., **6**, 411 (1923).
22) A. Meister, "Biochemistry of the Amino Acids," Academic Press, New York (1957), p. 76.

only with α-amino acids, but also with *N*-monoalkylated α-amino acids at neutral pH values.[23,24] In the present work, the color yields with ninhydrin of *N*-methylamino acids were compared with those of the original amino acids by an automatic amino-acid analyzer (Hitachi KLA type 2) and by the method of Stein and Moore.[23] The results obtained are listed in Table I. Although the *N*-methylamino acids generally react with ninhydrin quantitatively, at least 20—30 min. are necessary for their complete reaction at 100°C. The color yields were considerably lower than those of the original amino acids under the conditions used, as is shown in the table. This indicates that the heating was not enough to produce the maximum reaction between ninhydrin and *N*-methylamino acids. In the ninhydrin reaction with *N*-methylamino acids, it was considered that demethylation might occur before color developing, since the absorption spectra of the ninhydrin color reaction of amino acid and *N*-methylamino acid gave the same type of curves.

The conversion of an L-amino acid to the corresponding *N*-methylamino acid is accompanied by some shift in the infrared absorption spectrum when these spectra are measured under the same conditions. Comparisons were performed in each case of amino acid esters and *N*-methylamino acid esters in the same solvent. In this work two main shifts of the absorption band were found, from 6.13 μ to 6.36 μ and from 6.58 μ to 6.80 μ, corresponding to the change from the $-NH_2^+ \cdot Cl^-$ to the $-NH^+-CH_3 \cdot Cl^-$ group.

Grateful acknowledgement is hope by made to Dr. Kaoru Kuriyama and his staff for their optical rotation measurements, to Dr. Satoshi Mizukami and his staff for their microanalyses, and to Miss Shizuko Kitamura for her technical assistance.

23) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).
24) C. E. Dalgleish, A. W. Johnson, A. R. Todd and L. C. Vinning, *J. Chem. Soc.*, **1950**, 2946.