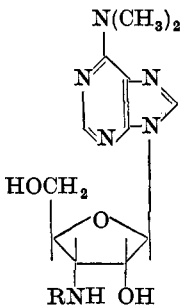
Journal of Medicinal and Pharmaceutical Chemistry

VOL. 2, No. 4 (1960)

The Preparation of Various Amides of *p*-Methoxy-L-phenylalanine

HENRY M. KISSMAN* and B. R. BAKER†, *Organic Chemical Research Section, Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York*

As discussed in the preceding papers,[1,2] chemical removal of the *p*-methoxy-L-phenylalanyl moiety from the antibiotic puromycin (I) affords a degradation product, the aminonucleoside (II),[3] which does not show the antibacterial[4] activity of the parent



(I); R = *p*-$CH_3OC_6H_4CH_2CHCO$—
NH$_2$

(II); R = H

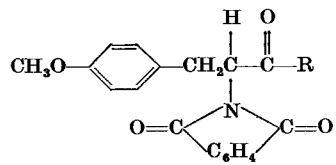
antibiotic. This fact suggested that this activity involved the presence of the *p*-methoxy-L-phenylalanyl moiety and, in order to elaborate this hypothesis further, it was desirable to prepare certain simple derivatives of this amino acid.‡ In puromycin (I), the amino acid is joined to the remainder of the molecule (i.e.

* To whom inquiries concerning this paper should be addressed.

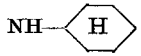
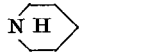
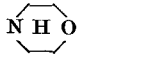
† Stanford Research Institute, Menlo Park, California.

‡ In one of the accompanying papers[1] we have described the preparation of a number of biologically active esters of substituted phenylalanine derivatives.

Table I. *p*-Methoxy-*N*-phthaloyl-L-phenylalanine amides

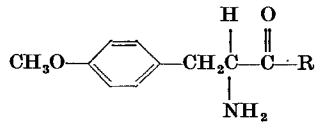


(III)

No.	R	Empirical formula	Yield, %	m.p., °C[a]	Optical rotation[c] $[\alpha]_D^{24-25}$, °	*c*[b]	Analysis, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
III a	NH_2	$C_{18}H_{16}N_2O_4$	73	182–183[b]	−128	1·4	66·66	4·97	8·64	66·86	5·27	8·99
III b	$NHCH_2CH_2OH$	$C_{20}H_{20}N_2O_5 \cdot \frac{1}{2}H_2O$	84	90–93	−116	1·2	63·63	5·61	7·42	63·90	5·50	7·40
III c	$NH(CH_2)_8CH_3$	$C_{27}H_{34}N_2O_4$	46	127–130[c]	−81	0·3	71·97	7·61	6·22	72·22	7·76	6·21
III d	$NHCH_2C_6H_5$	$C_{25}H_{22}N_2O_4$	73	173–174[d]	−120	2·0	72·45	5·35	6·76	72·62	5·46	6·91
III e		$C_{24}H_{26}N_2O_4$	90	150–152	−76	1·1	70·91	6·45	6·89	71·21	6·74	6·82
III f	NHC_6H_5	$C_{24}H_{20}N_2O_4$	89	184–185	−107	1·4	71·98	5·03	7·00	71·77	4·78	6·83
III g		$C_{22}H_{24}N_2O_4$	91	–[e]	−135	1·5	69·45	6·36	7·36	69·07	6·43	7·11
III h		$C_{23}H_{24}N_2O_4$	86	122–123	−129	1·0	70·39	6·16	7·14	70·44	6·47	7·32
III i		$C_{22}H_{22}N_2O_5$	91	167–168[f]	−152	1·3	66·99	5·62	7·10	67·17	6·03	7·34

[a] Recrystallised from methylene chloride–ether unless otherwise stated. [b] In chloroform. [c] Recrystallised from ether. [d] Recrystallised from benzene. [e] Could not be obtained in crystalline form. [f] Recrystallised from methylene chloride–hexane.

Table II. *p*-Methoxy-L-phenylalanine amides



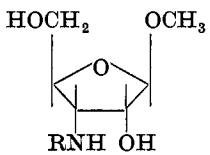
(IV)

No.	R	Empirical formula	Yield, %	m.p., °C[a]	Method of isolation[b]	Optical Rotation $[\alpha]_D^{24-25,°}$	c[c]	Analysis, % Calcd. C	H	N	Found C	H	N
IV a	NH_2	$C_{10}H_{14}N_2O_2$	66	102–103	A	−60	1·2	61·83	7·27	14·42	61·92	7·33	14·65
IV b	$NH_2CH_2CH_2OH$	$C_{12}H_{18}N_2O_3$	51	73–74	A	−65	2·1	60·48	7·61	11·76	60·85	7·73	11·46
IV c	$NH(CH_2)_8CH_3$	$C_{19}H_{32}N_2O_2$	58	67–68[d]	B	−48	1·1	71·21	10·07	8·74	71·28	10·01	8·76
IV d	$NHCH_2C_6H_5$	$C_{17}H_{20}N_2O_2$	66	89–90	B	−54	2·4	71·80	7·09	9·85	71·69	7·14	9·56
IV e	NH— 	$C_{16}H_{24}N_2O_2$	64	75–76[e]	B	−52	0·6	69·73	8·75	10·14	69·51	8·83	9·99
IV f	NHC_6H_5	$C_{16}H_{18}N_2O_2$	78	119–120[e]	B	−136[f]	0·8	71·09	6·71	10·36	70·85	7·02	10·33
IV g		$C_{14}H_{22}O_2N_2 \cdot \frac{1}{2}H_2O$	53	~[g]	A	+49	1·3	64·83	8·94	10·80	64·72	9·34	11·01
IV h		$C_{15}H_{22}N_2O_2 \cdot CH_3COOH$	56	92–95[h]	–[i]	+50	0·9	63·33	8·13	8·69	62·74	8·25	8·71
IV i		$C_{14}H_{20}N_2O_3 \cdot \frac{1}{2}H_2O$	81	~[k]	B	–	–	61·52	7·74	10·25	61·85	7·74	9·87
	 picrate	$C_{14}H_{20}N_2O_3 \cdot C_6H_3N_3O_7$	63	187–188[l] (d.)	~	+43[m]	0·2	48·68	4·70	14·19	48·60	5·00	13·86

[a] Recrystallised from methylene chloride–ether unless stated otherwise.
[b] For details see experimental section.
[c] In chloroform unless stated otherwise.
[d] Recrystallised from hexane.
[e] Recrystallised from hexane–ether.
[f] $[\alpha]_D^{26}$ +16° (ethanol). M. Bovarnick and H. T. Clarke[8] report m.p. 121–123°, $[\alpha]_D^{25}$ +34°, (95% ethanol) for this compound.
[g] Obtained by molecular distillation in 110–120° bath/0·5 mm as viscous hygroscopic liquid.
[h] Recrystallised from ether.
[i] Isolated directly as acetate salt.
[j] The substance was hygroscopic and could not be purified to give better combustion values.
[k] Obtained by molecular distillation in 160–170° bath/0·5 mm as a hygroscopic oil. Calcd. for $\frac{1}{2}H_2O$ 3·33%; found 3·93% (Karl Fischer).
[l] Recrystallised from chloroform–methanol

II) through an amide linkage and therefore it was most relevant to prepare and test several amides of *p*-methoxy-L-phenylalanine.

For the preparation of most of the amides described in this paper, the *N*-phthaloyl derivative of *p*-methoxy-L-phenylalanine[5] was converted to the acid chloride by a variation of the literature method.[6] The acyl halide was not purified *per se* but was allowed to react with an excess of the requisite amine to give the *p*-methoxy-*N*-phthaloyl-L-phenylalanine amides (III) listed in Table I. Removal of the phthaloyl group with hydrazine hydrate in ethylene glycol monomethyl ether, followed by treatment with glacial acetic acid, yielded the desired *p*-methoxy-L-phenylalanine amides (IV) which were either crystalline solids or could be char-



(V); R = *p*-$CH_3OC_6H_4CH_2CHCO$—
NH_2

(VI); R = H

acterized as acetate or picrate salts. These compounds are described in Table II. *p*-Methoxy-L-phenylalaninamide (IVa) could be obtained by the sequence just described but was prepared more conveniently *via* the reaction of methyl *p*-methoxy-L-phenylalaninate hydrochloride[3] with methanolic ammonia.

It was of particular interest to prepare the *p*-methoxy-L-phenylalanine amide (V) in which the amine component was methyl 3-amino-3-deoxy-β-D-ribofuranoside (VI).[7] Compound (V) represents an analogue of puromycin (I) in which the purine portion has been replaced by a methoxy group. The amide (V) was prepared *via* the condensation in dimethylformamide solution of (VI) with the mixed anhydride obtained from *N*-carbobenzoxy-*p*-methoxy-L-phenylalanine[3] and ethyl chlorocarbonate. The intermediate carbobenzoxy derivative was not characterized as such but was hydrogenolized over palladium-on-carbon catalyst to afford methyl 3-deoxy-3-(*p*-methoxy-L-phenylalanylamino)-β-D-ribofuranoside (V) in 38 per cent yield (over-all from VI).

Biological Activities

The *p*-methoxy-L-phenylalanine amides described in this paper were tested for *in vitro* antibacterial and antifungal activity by the agar dilution technique against the organisms described in the accompanying paper.[1] In contrast to the *p*-alkoxy-L-phenylalanine ester series, members of which showed substantial activity in these tests,[1] none of the amides had activity at a concentration of less than 500 μg/ml. It is of interest that methyl 3-deoxy-3-(*p*-methoxy-L-phenylalanylamino)-β-D-ribofuranoside (V) was inactive in the antifungal and antibacterial tests and also when tested against *Trypanosoma equiperdum* in mice and against the transplanted mammary adenocarcinoma in C_3H mice. Both puromycin (I) and the aminonucleoside (II) showed activities in the last two tests.*

Experimental†

p-*Methoxy*-N-*phthaloyl*-L-*phenylalanyl chloride*.[6] Transformation of *p*-methoxy-*N*-phthaloyl-L-phenylalanine[5] to the corresponding acid chloride was brought about with phosphorous pentachloride in ether. The acid chloride was not isolated as such but was converted to amide as shown in the following example.

N - (p - *Methoxy* - N - *phthaloyl* - L - *phenylalanyl*) - *cyclohexylamine* (*IIIe*). Dried *p*-methoxy-*N*-phthaloyl-L-phenylalanine (6·5 g, 20 mmole) was suspended in absolute ether (80 ml) and the mixture was stirred in an ice bath for 10 min when phosphorous pentachloride (4·3 g, 21 mmole) was added. Stirring was continued for 20 min in the ice bath and for 30 min at room temperature. The clear solution was evaporated and the residue was evaporated twice with small portions of toluene. The residual gum was dissolved in chloroform (100 ml) and to the cooled, stirred solution cyclohexylamine (5·05 ml, 44 mmole) was

* We would like to thank the following for these testing results: Mr. A. C. Dornbush (antibacterial and antifungal testing), Dr. R. I. Hewitt (antitrypanosomiasis testing), Miss S. L. Halliday and Dr. J. J. Oleson (antitumour testing) of these Laboratories.

† Melting points were taken on a Kofler micro hot-stage and are corrected. Solutions were evaporated under reduced pressure unless stated otherwise. Magnesium sulphate was used as drying agent.

added. The mixture was stirred at room temperature for 1 h and was then extracted with successive portions of water, 1 N hydrochloric acid, saturated aqueous sodium bicarbonate solution and water. The dried solution was evaporated and the residue was triturated with ether and filtered to yield, after drying *in vacuo*, 7·34 g of product (90 per cent), m.p. 148–150°. For analysis the material was crystallized from methylene chloride–ether.*

Amides of p-*Methoxy*-L-*phenylalanine.* Removal of the phthaloyl blocking group was carried out with hydrazine hydrate and acetic acid. The reaction mixture was worked up by two different methods depending on whether the product was water soluble (Method A) or water insoluble (Method B), as shown in the following examples.

Method A

N-(p-*Methoxy*-L-*phenylalanyl*)-*2-hydroxyethylamine* (*IV b*). Hydrazine hydrate (0·206 ml, 4·29 mmole) was added to a hot solution of *N*-phthaloyl derivative (III b) (1·58 g, 4·29 mmole) in ethylene glycol monomethyl ether (3·4 ml), and the mixture was heated with occasional shaking on the steam bath for 12 min. To the thick, white suspension was added glacial acetic acid (0·86 ml, 15 mmole) in ethylene glycol monomethyl ether (2·5 ml) and the mixture was heated on the steam bath for another 15 min and was then evaporated. The residue was triturated with methanol, filtered, and the precipitate was washed further with a little methanol. Filtrate and washings were combined and evaporated. The residue was taken up in water (25 ml), filtered from a small amount of insoluble material, and the filtrate was stirred with Amberlite IRA-400 anion exchange resin† (freshly washed with 2 N sodium hydroxide solution and then with distilled water until the supernatant was neutral) until the solution reached pH 9. The resin was removed by filtration and was washed thoroughly with distilled water. Filtrate and washings were combined and

* The compounds described in Table I were prepared in a similar manner. Yields were calculated from *p*-methoxy-*N*-phthaloyl-L-phenylalanine.

† Amberlite IRA-400 is the registered trademark of the Rohm and Haas Company for a strongly basic anion exchange resin.

evaporated at 35–40°. The residue was further dried over phosphorus pentoxide *in vacuo* to afford 1·06 g of glass. This was dissolved in methylene chloride, and ether was added at the boiling point until crystallization started. The mixture was then cooled in an ice bath, the crystalline solid was collected, washed with ether and dried to afford 0·52 g (51 per cent), m.p. 72–73°.

Method B

N-(p-*Methoxy*-L-*phenylalanyl*)-*benzylamine* (*IV d*). The *N*-phthaloyl derivative (III d) (0·414 g, 1 mmole) was treated with hydrazine hydrate and glacial acetic acid as described above. After evaporation of the methanol solution, the residue was mixed with 0·5 N hydrochloric acid (15 ml) and filtered. The precipitate was washed with a little more acid and the combined filtrate and washings were saturated with sodium bicarbonate. The mixture was extracted several times with 5 ml portions of chloroform and the combined extracts were washed with a little water, dried and evaporated. The crystalline residue was recrystallized from methylene chloride–ether to afford 0·187 g of product (66 per cent), m.p. 89–90°.

p-*Methoxy*-L-*phenylalaninamide* (*IV a*) *from methyl* p-*methoxy*-L-*phenylalaninate hydrochloride*. Methyl *p*-methoxy-L-phenylalaninate hydrochloride[3] (53·4 g, 0·218 mole) was added to dry methanol (1000 ml) which had been saturated with ammonia at 0° and the solution (protected from atmospheric moisture) was allowed to stand at room temperature for 5 days and was then evaporated. The residue was mixed with water (300 ml) and was filtered from a small amount of undissolved precipitate. The filtrate was saturated with sodium bicarbonate. The product crystallized suddenly to give a very thick suspension which could be filtered only after the addition of chloroform (100 ml). The precipitate was washed with a little water and was dried *in vacuo* (25 g, m.p. 102–103°). The layers in the filtrate were separated and the aqueous phase was extracted several times with chloroform. The combined chloroform extracts were dried and evaporated. The crystalline residue was triturated with a small amount of ether and filtered to afford another 9·5 g of product, m.p. 102–104°. Identity with (IVa), prepared from the *N*-phthaloyl

derivative (IIIa) by method A, was established by mixture melting point. The total yield (34·5 g) was 81 per cent.

Anal. Calcd. for $C_{10}H_{14}N_2O_2$: C, 61·83; H, 7·27; N, 14·42. Found: C, 61·64; H, 7·18; N, 14·29.

Methyl 3-deoxy-3-(p-*methoxy*-L-*phenylalanylamino*)-*β*-D-*ribofuranoside* (*V*). Ethyl chlorocarbonate (0·88 ml) was added to a stirred, cooled solution of *N*-carbobenzoxy-*p*-methoxy-L-phenylalanine (3·29 g, 8·4 mmole)[3] in dimethylformamide (15 ml) and triethylamine (1·24 ml). Stirring and cooling were continued for 10 min and the suspension was then added in portions to a stirred, cooled solution of methyl 3-amino-3-deoxy-*β*-D-ribofuranoside[7] (1·14 g, 7 mmole) in dimethylformamide (15 ml) and triethylamine (2 ml). The mixture was kept at 0° over-night and was then evaporated at 60–70°. The residue was mixed with chloroform and water and the phases were separated. The organic phase was washed with saturated sodium bicarbonate solution and with water, and was then dried and evaporated. The residue, which crystallized when triturated with ether, was collected by filtration, washed with ether and dried *in vacuo* to give 2·15 g of product (64 per cent), m.p. 157–158°. This material could not be recrystallized to give acceptable combustion values and 1·85 g (3·9 mmole) of it was therefore converted to the final product (V) by suspension in absolute alcohol (50 ml) to which had been added 1 g of palladium-on-carbon catalyst (10 per cent) suspended in ethylene glycol monomethyl ether (10 ml). Hydrogen was bubbled through the solution until the exit gases no longer contained carbon dioxide (CO_2 was detected with aqueous barium hydroxide solution). The suspension was filtered through diatomaceous earth and the filtrate was evaporated. The residue crystallized when triturated with ether containing a few drops of methylene chloride; yield, 0·9 g, m.p. 123–125°. Recrystallization from methylene chloride–ether gave 0·78 g (38 per cent over-all from methyl 3-amino-3-deoxy-*β*-D-ribofuranoside), m.p. 122–123°; $[\alpha]_D^{25} + 26·7°$ (*c*, 1·1 in chloroform).

Anal. Calcd. for $C_{16}H_{24}N_2O_6$: C, 56·46; H, 7·11; N, 8·23. Found: C, 56·23; H, 7·28; N, 8·55.

Summary. Various amides of *p*-methoxy-L-phenylalanine were prepared as simple analogues of the antibiotic puromycin. These amides did not show significant *in vitro* antibacterial and antifungal activities.

Acknowledgement. We would like to thank Mr. L. Brancone and staff for microanalyses, Mr. W. Fulmor and staff for polarimetric work and Mr. W. McEwen and staff for the large-scale preparations of certain intermediates.

(*Received 15 February, 1960*)

References

[1] Allen, G. R. Jr., Baker, B. R., Dornbush, A. C., Joseph, J. P., Kissman, H. M. and Weiss, M. J. This Journal, **2,** 391 (1960)

[2] Small, A. M., Kissman, H. M., Joseph, J. P. and Weiss, M. J. This Journal, **2,** 375 (1960)

[3] Baker, B. R., Joseph, J. P. and Williams, J. H. *J. Amer. chem. Soc.*, **77,** 1 (1955)

[4] Porter, J. N., Hewitt, R. I., Hesseltine, C. W., Krupka, G., Lowery, J. A., Wallace, W. S., Bohonos, N. and Williams, J. H. *Antibiotics & Chemotherapy*, **2,** 409 (1952)

[5] Thaller, V., Filipović, L. and Balenović, K. *Arh. Hem. Farm.*, **20,** 68 (1948); *Chem. Abstr.*, **44,** 6830c (1950)

[6] Balenović, K., Thaller, V. and Filipović, L. *Helv. chim. acta*, **34,** 744 (1951)

[7] Weiss, M. J., Joseph, J. P., Kissman, H. M., Small, A. M., Schaub, R. E. and McEvoy, F. J. *J. Amer. chem. Soc.*, **81,** 4050 (1959)

[8] Bovarnick, M. and Clarke, H. T. *J. Amer. chem. Soc.*, **60,** 2426 (1938)