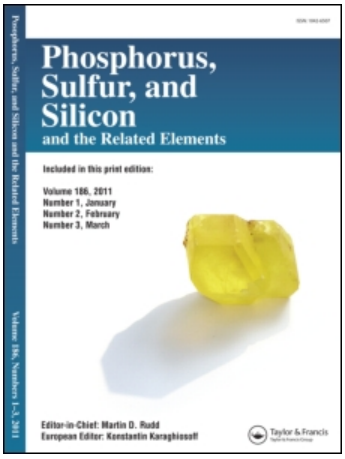

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

SYNTHESIS OF N-(HYDROGEN METHYLPHOSPHONOTHIONYL), N-(O-METHYL METHYLPHOSPHONOTHIONYL) AMINO ACID METHYL ESTERS AND THEIR ANTIMICROBIAL ACTIVITIES

F. A. Kora[a]; S. A. Abdel-ghaffar[a]; A. M. El-naggar[a]; N. S. Fodah[a]
[a] Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt

To cite this Article Kora, F. A. , Abdel-ghaffar, S. A. , El-naggar, A. M. and Fodah, N. S.(1994) 'SYNTHESIS OF N-(HYDROGEN METHYLPHOSPHONOTHIONYL), N-(O-METHYL METHYLPHOSPHONOTHIONYL) AMINO ACID METHYL ESTERS AND THEIR ANTIMICROBIAL ACTIVITIES', Phosphorus, Sulfur, and Silicon and the Related Elements, 88: 1, 245 — 250
To link to this Article: DOI: 10.1080/10426509408036928
URL: http://dx.doi.org/10.1080/10426509408036928

Phosphorus, Sulfur, and Silicon, 1994, Vol. 88, pp. 245–250
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SYNTHESIS OF N-(HYDROGEN METHYLPHOSPHONOTHIONYL), N-(O-METHYL METHYLPHOSPHONOTHIONYL) AMINO ACID METHYL ESTERS AND THEIR ANTIMICROBIAL ACTIVITIES

F. A. KORA, S. A. ABDEL-GHAFFAR, A. M. EL-NAGGAR and N. S. FODAH

Chemistry Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt

(Received September 30, 1993; in final form January 13, 1994)

The synthesis of some N-(hydrogen methylphosphonothionyl) amino acid methyl esters (II–VII) and N-(O-methyl methylphosphonothionyl) amino acid methyl esters (VIII–XIII) is described. Reaction of methyl iodide with compounds (II–XIII) afforded the corresponding methyl iodide quaternary salt derivatives (XIV–XXV). All the synthesized compounds were found to possess high and moderate antimicrobial activities towards a number of microorganisms.

Key words: N-(hydrogen methylphosphonothionyl); N-(O-methyl methylphosphonothionyl); amino acid esters and antimicrobial activities.

Phosphinothricin is produced by various streptomycete species as a component of execrated antibacterial peptides.[1–4] Glyphosate has achieved tremendous success as a broad spectrum, non-selective herbicide, while exhibiting very low mammalian toxicity.[5–8] Moreover, some phosphorylamino acid derivatives were used pharmaceutically as antihypertensive.[9] In consideration of these facts and because of the increasing realization of the importance of phosphate compounds in biological system, we have undertaken the preparation of some N-(hydrogen methylphosphonothionyl)amino acid methyl esters (II–VII), N-(O-methyl methylphosphonothionyl) amino acid methyl esters (VIII–XIII) and their methyl iodide quaternary salt (XIV–XXV) for their biological evaluation (cf. Table I).

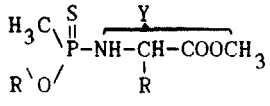
The method described in the present investigation is based on the reaction of methylphosphonothioic dichloride (I) with an amino acid ester in organic solvent then treating the resulting intermediate with water (or methanol) to obtain the desired compounds (II–XIII). Thus an amino acid methyl ester was first liberated from its hydrochloride salt. The free ester was then reacted with methylphosphonothioic dichloride (1:1) in benzenetriethylamine medium for 4 hours, then water (or methanol) was added dropwise to the reaction mixture. The time required for completion of the reaction was monitored by TLC. N-(Hydrogen methylphosphonothionyl) and N-(O-methyl methylphosphonothionyl) amino acid methyl es-

TABLE I

Physical data of various N-(hydrogen methylphosphonothionyl) and N-(O-methyl methylphosphonothionyl) amino acid methyl ester derivatives and their corresponding methyl iodide quaternary salts (II–XXV)

Comp.	Y	R	Yield	M.P	R_f	$[\alpha]_D^{20}$	Molecular Formula	Elemental Analysis% Calculated N	S	Found N	S
				Compounds of Type A							
II	Gly.OMe	H	62	-	0.80	-	$C_4H_{10}NO_3SP$	7.65	17.49	7.59	17.36
III	L-Ala.OMe	H	56	-	0.70	+35	$C_5H_{12}NO_3SP$	7.11	16.24	7.20	16.11
IV	DL-Ala.OMe	H	65	-	0.75	-	$C_5H_{12}NO_3SP$	7.11	16.24	7.16	16.09
V	L-Val.OMe	H	46	-	0.83	+30	$C_7H_{12}NO_3SP$	6.22	14.22	6.31	14.28
VI	L-Phe.OMe	H	65	-	0.75	+48	$C_{11}H_{16}NO_3SP$	5.13	11.72	5.33	11.63
VII	DL-Ph.-OMe	H	68	-	0.60	-	$C_{17}H_{16}NO_3SP$	5.13	11.72	5.30	11.60
VIII	Gly.OMe	CH_3	56	41-43	0.91	-	$C_5H_{12}NO_3SP$	7.11	16.24	7.02	16.12
IX	L-Ala.OMe	CH_3	42	-	0.85	+20	$C_6H_{14}NO_3SP$	6.64	15.17	6.73	15.09
X	DL-Ala.OMe	CH_3	58	-	0.80	-	$C_6H_{14}NO_3SP$	6.64	15.17	6.51	15.30
XI	L-Val.OMe	CH_3	61	-	0.87	+15	$C_8H_{18}NO_3SP$	5.86	13.39	5.68	13.47
XII	L-Phe.OMe	CH_3	55	35-37	0.90	+22.8	$C_{12}H_{18}NO_3SP$	4.88	11.15	4.78	11.23
XIII	DL-Phe.OMe	CH_3	51	40-42	0.82	-	$C_{12}H_{18}NO_3SP$	4.88	11.15	4.77	11.28
				Compounds of Type B							
XIV	Gly.OMe	H	72	41-43	0.62	-	$C_5H_{13}NO_3SPI$	4.31	9.85	4.27	9.92
XV	L-Ala.OMe	H	76	55-75	0.85	+42	$C_6H_{15}NO_3SPI$	4.13	9.44	4.03	9.30
XVI	DL-Ala.OMe	H	72	34-36	0.76	-	$C_6H_{15}NO_3SPI$	4.13	9.44	4.02	9.33
XVII	L-Val.OMe	H	57	36-38	0.61	+36	$C_8H_{19}NO_3SPI$	3.81	8.72	3.92	8.68
XVIII	L-Phe.OMe	H	76	45-47	0.77	+34	$C_{12}H_{19}NO_3SPI$	3.37	7.71	3.30	7.62
XIX	DL-Phe.OMe	H	75	58-60	0.68	-	$C_{12}H_{19}NO_3SPI$	3.37	7.71	3.28	7.60
XX	Gly.OMe	CH_3	72	51-53	0.63	-	$C_6H_{15}NO_3SPI$	4.13	9.44	4.03	9.38
XXI	L-Ala.OMe	CH_3	68	45-47	0.82	+28	$C_7H_{17}NO_3SPI$	3.97	9.07	4.12	8.97
XXII	DL-Ala.OMe	CH_3	76	48-50	0.75	-	$C_7H_{17}NO_3SPI$	3.97	9.07	4.16	8.91
XXIII	L-Val.OMe	CH_3	65	-	0.68	+26	$C_9H_{21}NO_3SPI$	3.68	8.39	3.78	8.47
XXIV	L-Phe.OMe	CH_3	72	55-57	0.70	+22	$C_{13}H_{21}NO_3SPI$	3.26	7.46	3.22	7.55
XXV	DL-Phe.OMe	CH_3	76	60-62	0.75	-	$C_{13}H_{21}NO_3SPI$	3.26	7.46	3.30	7.39

ters (II–XIII) were obtained as chromatographically homogeneous materials in (42–68%) yields.



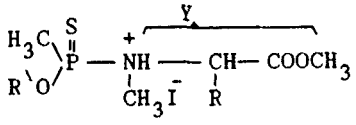
Compounds of type A
(II-XIII)

Syntheses of quaternary salts were carried out by the action of methyl iodide, N-(hydrogen methylphosphonothionyl) amino acid methyl esters (II–VII) or N-(O-methyl methylphosphonothionyl) amino acid methyl esters (VIII–XIII), in benzene, to produce their corresponding methyl iodide quaternary salts (XIV–XIX)

and (XX–XXV), respectively. The time required for completion of the reaction (2–3 hours) was monitored by TLC.

The methyl iodide quaternary salts (XIV–XXV) were easily isolated, recrystallized and obtained as chromatographically homogeneous solid materials in good yields (57–76%).

The isolated products (XIV–XXV) were found to be freely soluble in water and alcohols. The structure of the synthesized compounds (II–XXV) was confirmed by elemental analysis, IR and NMR spectra.



Compounds of type B
(XIV–XXV)

Biological Screening Results

The antimicrobial activities of the synthesized compounds (II–XXV) were tested using the hole plate and filter paper disk methods.[11–14]

The microorganisms used included gram-positive, gram-negative microorganism and selected fungi: *Escherichia coli* (NRRL-B-210), *Staphylococcus aureus* (ATCC-6538-P), *Listomo cytogenes* (b-4), *Aspergillus niger* (PP-29) and *Shigella sonnie* (2M).

N-(Hydrogen methylphosphonothionyl) glycine methyl ester (II) and the corresponding L-Ala.OMe (III), DL-Ala.OMe (IV), L-Val.OMe (V), L-Phe.OMe (VI) and DL-Phe.OMe (VII) showed high biological activities towards *Shig. sonnei, List. cytogenes* and *Asp. niger* with MIC 15–100 μg/ml (cf. Table II).

Replacement of the hydroxy hydrogen atom of N-(hydrogen methylphosphonothionyl) amino acid methyl ester derivatives (II–VII), by methyl group i.e. N-(O-methyl methylphosphonothionyl) amino acid methyl esters (VIII–XIII) reduced the biological activity two to eight times towards *Shig. sonnei, List. cytogenes* and *Asp. niger* while varying the activity towards *E. coli* and *Staph. aureus* (cf. Table II).

Conversion of N-(hydrogen methylphosphonothionyl) amino acid methyl esters (II–VII) into their corresponding methyl iodide quaternary salts (XIV–XIX) led to compounds with the highest biological activities especially towards *Shig. sonnei, List. cytogenes* and *Asp. niger*. The methyl iodide quaternary salts of Gly- (XIV) and L-Ala (XV) derivatives were found to possess the highest biological action against all the tested microorganisms, while their corresponding DL-Ala (XVI), L-Val (XVII). L-Phe (XVIII) and DL-Phe (XIX) quaternary salt derivatives showed high biological activities as compared with their corresponding N-(hydrogen methylphosphonothionyl) derivatives (II–VII) specially towards *Shig. sonnei, List. cytogenes* and *Asp. niger* (cf. Table II).

On the other hand, the methyl iodide quaternary salts of N-(O-methyl methylphosphonothionyl) amino acid methyl esters (XX–XXV) showed modified bio-

TABLE II

Antimicrobial activities (A) and minimal inhibitory concentration (MIC μg/ml) of the antimicrobial active compounds*

Comp.	Esch. coli		Staph. aureus		Shig. sonnei		Listomocytogenes		Asp. niger	
	A	MIC	A	MIC	A	MIC	A	MIC	A	MIC
II	+++	150	+++	135	++++	35	++++	30	++++	50
III	+++	110	+++	150	++++	50	++++	45	++++	75
IV	+++	150	++	175	++++	35	++++	50	++++	75
V	++++	25	++	200	++++	50	++++	60	++++	100
VI	++++	50	+++	150	++++	15	++++	30	++++	60
VII	+++	125	+++	150	++++	15	++++	35	++++	60
VIII	+++	75	++++	50	+++	115	+++	125	++	200
IX	+++	125	+++	75	+++	125	+++	150	++	225
X	+++	150	+++	110	+++	150	+++	150	++	225
XI	+++	130	+++	110	+++	125	++	175	++	250
XII	+++	120	+++	90	+++	120	++	175	++	225
XIII	+++	125	+++	100	+++	120	++	175	++	250
XIV	+++	75	+++	90	++++	5	++++	5	++++	7
XV	+++	75	+++	110	++++	7	++++	5	++++	10
XVI	+++	100	+++	115	++++	10	++++	7	++++	10
XVII	+++	125	+++	135	++++	20	++++	15	++++	15
XVIII	+++	150	++	160	++++	7	++++	5	++++	35
XIX	+++	150	++	175	++++	7	++++	7	++++	35
XX	++++	10	++++	5	++++	25	++++	30	++++	75
XXI	++++	15	++++	7	++++	30	++++	30	++++	75
XXII	++++	15	++++	10	++++	30	++++	40	+++	125
XXIII	++++	20	++++	15	++++	35	++++	50	+++	125
XXIV	++++	15	++++	15	++++	25	++++	45	+++	100
XXV	++++	25	++++	20	++++	25	++++	50	+++	100

* Standard antibiotics used are:

a) Polymixin B (300 μg/ml). b) Colimycin (10 μg/ml).

c) Neomycin (30 μg/ml). d) Kanamycin (30 μg/ml).

The standard antibiotics were purchased from Bio Merieux.

logical activities towards all the tested microorganisms, with special reference to *E. coli* and *Staph. aureus*, compared with that of their corresponding N-(O-methyl methylphosphonothionyl) amino acid methyl esters (VIII–XIII).

The present investigation revealed that:

(i) Methyl iodide quaternary salts of N-(hydrogen methylphosphonothionyl) amino acid methyl esters (XIV–XIX) were found to possess the highest biological action among the compounds under investigation.

(ii) N-(Hydrogen methylphosphonothionyl) amino acid methyl esters (II–VII) possess pronounced and higher biological action than their corresponding N-(O-methyl methylphosphonothionyl) amino acid methyl esters (VIII–XIII) while the latter compounds were found to have less biological action as compared to their corresponding methyl iodide quaternary salts (XX–XXV).

EXPERIMENTAL

Melting points are uncorrected. Thin layer chromatography (R_f value) was performed on Silica Gel-G1 plastic sheets, using butanol:acetic acid:water (4:1:1) as the solvent system and iodine-potassium iodide (20 gm in 100 ml) mixture, ninhydrin and benzidine as a detection reagent. IR spectra in KBr on a Perkin-Elmer 983 (ν_{max} in cm^{-1}), NMR spectra in DMSO-d_6 on Jeol FX- [90 MHz] spectrophotometer using TMS as internal standard (chemical shift δ in ppm). Optical rotations $[\alpha]_D^{20}$ (c = 0.5 in ethanol) were measured on a Bellingham-Stanely polarimeter at 20°C using 1 dm tube, λ_{max} 589 nm.

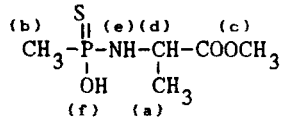
Methylphosphonothioic dichloride (I) was prepared as the procedure described earlier.[10]

General Procedure for the Synthesis of N-(hydrogen methylphosphonothionyl) amino acid methyl esters and N-(O-methyl methylphosphonothionyl) amino acid methyl esters (II–XIII Compounds of Type A): Amino acid methyl ester hydrochloride (0.11 mole) was suspended in benzene (50 ml) containing (0.11 mole) triethylamine. The mixture was stirred for 30 minutes at room temperature and the formed triethylammonium chloride was filtered off. The filterated was added dropwise during 1 hour at 10°C to benzene containing (0.1 mole) of methylphosphonothioic dichloride and triethylamine (0.2 mole). The reaction mixture was stirred for 4 hours at 50°C, cooled to 10°C and water (or methanol) was added dropwise with stirring in a period of 1 hour. The mixture was stirred for 1 hour at 50°C and cooled. The by product triethylammonium chloride was filtered off and benzene evaporated under reduced pressure. The residual oily products were purified by dissolving in benzene and precipitated with n-hexane.

The products (II-XIII) were chromatographically homogeneous when developed with benzidine or iodine solution, while gave negative ninhydrin reaction.

IR spectra of N-(hydrogen methylphosphonothionyl), N-(O-methyl methylphosphonothionyl) amino acid methyl esters (II–XIII) showed bands at: 1296 (P—CH_3); 765 (P=S); 3300, 1020 (P—OH); 1040 (P—OCH_3); 1006 (P—N) and 1730 cm^{-1} ($COOCH_3$) and other characteristic bands for the remaining residues thereby confirming their structures.

^{1}H NMR spectrum of N-(hydrogen methylphosphonothionyl)-L-alanine methyl ester (III) showed the following signals in the intensity ratio 3:3:3:1:1 as appeared from upfield side to downwards, these signals correspond to the environments designated by the letters (a), (b), (c), (d), (e) and (f) respectively.



(III)

The signal of the methyl(a) protons appeared as a doublet at δ = 0.7–0.85 ppm ($J_{Ha\text{-}Hd}$ = 14 Hz), the methyl(b) appeared as doublet at δ = 1.46–1.6 ppm ($J_{CH_3\text{-}P}$ = 11 Hz), the methoxy(c) appeared as sharp singlet signal at δ = 3.4 ppm, the methine proton(d) appeared as multiplet at δ = 4.2–4.6 ppm, the imine proton(e) appeared as broad at δ = 4.8–5.2 ppm and the acidic proton(f) of P—OH appeared as a broad at δ = 10.9 ppm. The corresponding N-(O-methyl methylphosphonothionyl)-L-alanine methyl esters (IX) showed the presence of a doublet at δ = 2.7 ppm of P—OCH_3 and no characteristic peaks for P—OH thus confirming their assigned structures. The amino acid derivatives (II–XIII) gave identified IR and NMR spectra confirming their assigned structures.

General Procedure for the Synthesis of Methyl Iodide Quaternary Salt Derivatives (XIV–XXV Compounds of Type B). Any of the synthesized phosphorus compounds (II–XIII) (0.05 mole) and methyl iodide (0.06 mole) in benzene was stirred for 2–3 hours at 40°C. The oily product formed was separated, washed with benzene and recrystallized from ethanol-benzene.

All the products were chromatographically homogeneous when developed with benzidine or iodine solutions.

IR spectra of methyl iodide quaternary salts (XIV–XXV) showed bands at: 1296 (P—CH_3); 765 ($\overset{+}{P}$=S); 3300, 1020, (P—OH); 1040 (P—OCH_3); 1006 (P—N); 1728 ($COOCH_3$) and 1630 cm^{-1} (NCH_3) and other characteristic peaks for the remaining amino acid moieties thereby confirming their structures.

^{1}H NMR spectrum of methyl iodide salt (XVI) showed signals at δ: 4.8–5.2 ppm (broad, NH); 4.2–4.6 ppm (m, CH); 3.7 ppm (s, $COOCH_3$); 10.8–10.9 ppm (broad, OH); 1.46–1.60 ppm (d, P—CH_3); 0.70–0.85 ppm (d, CH_3 alanyl) and 2.3 ppm (s, N—CH_3) while the corresponding N-(O-methyl methylphosphonothionyl) derivative (XXII) showed the presence of signal at 2.7 (s, P—OCH_3) and no characteristic peaks for (P—OH) thereby, confirming their assigned structures. The synthesized compounds (XIV–XXV) gave identified IR and NMR spectra confirming their structures.

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