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The Preparation of Various Esters of Certain L-Phenylalanine Derivatives and their Antifungal and Antibacterial Activity

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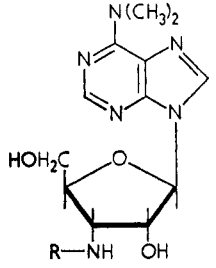
Cleavage of the 4-methoxy-L-phenylalanyl moiety from the antibiotic puromycin (I) to give the aminonucleoside (II) results in the loss of antibacterial activity.[1] This fact suggested a



(I); R = 4—$CH_3OC_6H_4CH_2CHCO$ (NH$_2$)

(II); R = H

correlation between antibacterial activity and the presence of the 4-methoxy-L-phenylalanyl moiety. Although the antibacterial activity[2] of puromycin is not particularly distinguished,§ it seemed worthwhile to pursue this observation by the preparation of certain simple derivatives of 4-methoxy-L-phenylalanine. The most pertinent derivatives are the secondary amides of 4-methoxy-L-phenylalanine, since in puromycin (I) this acid is linked to (II)

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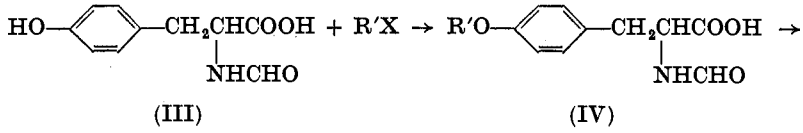
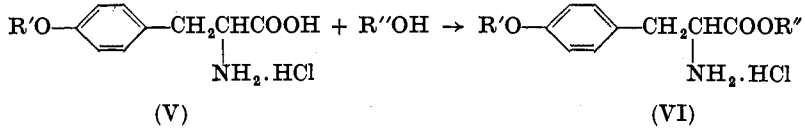
§ Puromycin is of special interest because of its antitumour,[3] trypanocidal[4] and amoebacidal[5] activities.

through an amide bond, and these amides are the subject of an accompanying paper.[6]* The present paper describes the preparation and biological activity of certain esters of L-phenylalanine and of various substituted and unsubstituted 4-alkoxy-L-phenylalanine derivatives. Contrary to our expectations, the esters proved to be considerably more active than the amides, and some were approximately as active as puromycin (I) in the *in vitro* antibacterial test.

The esters for this investigation were prepared conveniently by one of two procedures. Either the amino acid hydrochloride was heated with the esterifying alcohol and acetyl chloride (Procedure



(III) (IV)



(V) (VI)

A),[8] or the amino acid was heated with a hydrogen chloride-saturated solution of the alcohol (Procedure B).† These methods usually proceeded smoothly and in good yields, although with the longer-chain alcohols, esterification was much more sluggish, and in some instances a considerable quantity of the amino acid hydrochloride was recovered.

In the course of this study it was necessary to prepare esters of other 4-alkoxy-L-phenylalanines. It would have been most convenient to prepare these esters by a one-step procedure involving *O*-alkylation of the appropriate tyrosine ester. In fact, Abderhalden and Guggenheim[10] have reported the preferential

* Certain *N*-aminoacyl derivatives of (II) have also been prepared.[1, 7]

† Procedures A and B had been used previously for the preparation of methyl 4-methoxy-L-phenylalaninate hydrochloride[1,8] and ethyl 4-methoxy-L-phenylalaninate hydrochloride,[9] respectively.

O-alkylation of tyrosine disodium salt in unspecified yield. However, a number of attempts to alkylate the sodium salt of tyrosine butyl ester with several alkyl halides failed. Therefore, the various 4-alkoxy-L-phenylalanine esters (VI) were prepared by esterification of the corresponding acid (V). The required 4-alkoxy-L-phenylalanines (V) were synthesized by alkylation of *N*-formyl-L-tyrosine (III) followed by acid hydrolysis of the *N*-formyl blocking group.

The esters of 4-methoxy-L-phenylalanine are summarized in Table I and the esters of the other 4-alkoxy-L-phenylalanines are listed in Table II. The intermediate 4-alkoxy-*N*-formyl-L-phenylalanines (IV) and the 4-alkoxy-L-phenylalanines (V) are summarized in Tables V and VI, respectively.

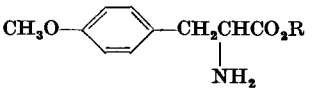
It was also of interest to prepare the esters of certain ring-substituted 4-hydroxy- and 4-alkoxy-L-phenylalanines. These esters are listed in Table III. The required 4-methoxy-3-nitro-L-phenylalanine and 3,5-diiodo-4-methoxy-L-phenylalanine were prepared from 3-nitro-L-tyrosine[12] and the commercially available 3,5-diiodo-L-tyrosine, respectively, using the procedure outlined above for the 4-alkoxy-L-phenylalanines. 3-Chloro-4-methoxy-L-phenylalanine was prepared by chlorination of *N*-acetyl-4-methoxy-L-phenylalanine[1] with sulphuryl chloride followed by hydrolysis of the acetyl blocking group. Butyl 3-amino-4-methoxy-L-phenylalaninate was obtained by catalytic hydrogenation of the corresponding nitro ester.

Finally, various esters of unsubstituted L-phenylalanine were prepared using Procedure B. These esters are summarized in Table IV.

Biological Activity

In vitro antibacterial and antifungal testing was carried out by the agar dilution technique. The results of these tests revealed a moderate level of activity against various fungi and Gram-positive bacteria, and permitted certain interesting structure–activity correlations. The compounds are classified in Groups A–G, and testing results are given in the appropriate table. The absence of figures in these tables indicates no activity at the maximum dose level (1000 μg/ml).

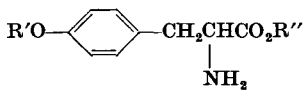
Table I. Esters of 4-methoxy-L-phenylalanine[a]



No.	R	Yield, %	m.p., °C	Recrystallization solvent	$[\alpha]_D^{24-27}$[b], °	Empirical formula	Analysis					
							Calcd.			Found		
							C	H	N	C	H	N
1	Ethyl[c]	96	195·5–196·5[d]	ethanol–ether	+24·2 E	$C_{12}H_{17}NO_3$.HCl	55·48	6·99	5·40	55·21	7·24	5·35
2	*n*-Propyl	95	170–171	*n*-propanol–ether	+27·5 E	$C_{13}H_{19}NO_3$.HCl	57·03	7·37	5·12	56·70	7·68	5·26
3	Isopropyl	63	222–224	*t*-butyl alcohol	+29·6 E	$C_{13}H_{19}NO_3$.HCl	57·03	7·37	5·12	57·02	7·60	5·17
4	*n*-Butyl	66	164–166	methylene chloride–ether	+19·1 C	$C_{14}H_{21}NO_3$.HCl	58·43	7·71	4·87	58·35	7·44	4·79
5	Isobutyl	96	175–177	acetone	+18·4 A	$C_{14}H_{21}NO_3$.HCl	58·43	7·71	4·87	58·20	7·75	5·08
6	*n*-Pentyl	94	149–150	acetone–ether	+ 5·8 A	$C_{15}H_{23}NO_3$.HCl	59·7	8·01	4·65	59·5	8·12	4·78
7	*n*-Hexyl	94	141–142	ethyl acetate	+34·3 C	$C_{16}H_{25}NO_3$.HCl	60·84	8·30	4·44	60·77	8·57	4·64
8	Cyclohexyl	90	202·0–202·5	chloroform–ether	+22·6 E	$C_{16}H_{23}NO_3$.HCl	60·70	7·68	4·45	60·93	7·62	4·43
9	5-Methylhexyl	63	124–126	acetone	+14·5 E	$C_{17}H_{27}NO_3$.HCl	61·90	8·56	4·23	61·5	8·73	4·27
10	*n*-Heptyl	58	135·0–136·5	ethyl acetate–ether	+23·3 E	$C_{17}H_{27}NO_3$.HCl	61·90	8·56	4·23	61·60	8·48	4·35
11	*n*-Octyl	77	120–122	chloroform–ether	+20·0 E	$C_{18}H_{29}NO_3$.HCl	62·87	8·79	4·07	62·62	8·60	4·19
12	*n*-Nonyl	16	130–133	benzene–heptane		$C_{19}H_{31}NO_3$.HCl[e]	63·75	9·01	3·91	63·09	9·10	4·01
13	*n*-Decyl	7	116–120	benzene–heptane		$C_{20}H_{33}NO_3$.HCl	64·6	9·22	3·76	63·3	9·12	3·96
14	3,7-Dimethyloctyl	75	121–123	acetone–ether		$C_{20}H_{33}NO_3$.HCl	64·6	9·22	3·76	64·1	8·96	3·82
15	*n*-Undecyl	58	131–132	chloroform–ether	+12·6 C	$C_{21}H_{35}NO_3$.HCl	65·4	9·44	3·63	65·3	9·23	3·64
16	*n*-Octadecyl	78	127–128	ethanol	+14·2 E	$C_{28}H_{49}NO_3$.HCl	69·4	10·4	2·89	69·8	10·5	2·93
17	Benzyl	57	204–205	*t*-butyl alcohol	+20·3 C	$C_{17}H_{19}NO_3$.HCl	63·44	6·27	4·36	63·47	6·59	4·43
18	Phenethyl	75	172·0–173·5	*t*-butyl alcohol	+ 5·3 E	$C_{18}H_{21}NO_3$.HCl	64·37	6·60	4·17	63·81	6·49	4·08
19	2-Chloroethyl	94	207–210	ethanol–ether	+ 7·7 E	$C_{12}H_{16}ClNO_3$.HCl	49·0	5·49	4·76	49·2	5·85	4·98
20	4-Chlorobutyl	94	164·5–165·5	methylene chloride–ether	+24·8 E	$C_{14}H_{20}ClNO_3$.HCl	52·18	6·57	4·35	51·86	6·94	4·54
21	Tetrahydrofurfuryl	64	171·0–172·5	chloroform–ether	+ 9·7 E	$C_{15}H_{21}NO_4$.HCl	57·06	7·02	4·44	56·78	7·09	4·40
22	2-Methoxyethyl	63	156–157	chloroform–ether	+11·8 E	$C_{13}H_{19}NO_4$.HCl	53·89	6·96	4·84	53·38	7·12	4·82
23	2-(2-Butyloxy)-ethoxyethyl	80	148–153	acetone	+ 2·0 E	$C_{18}H_{29}NO_5$.HCl	57·5	7·80	3·73	57·1	7·88	3·75

[a] Unless otherwise specified the esters in this table were prepared by Procedure A. The requisite 4-methoxy-L-phenylalanine was prepared by the method of Izumiya and Nagamatsu.[11] [b] *c*,1–2; E=ethanol, C=chloroform, A=acetone. [c] Prepared by Procedure B. [d] Karrer and co-workers[9] do not report a melting point for their preparation. [e] Chlorine analysis: Calcd., 9·91; Found, 9·94.

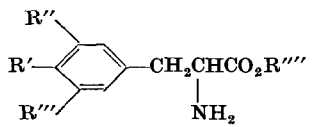
Table II. Esters of other 4-alkoxy-L-phenylalanines[a]



No.	R′	R″	Yield, %	m.p., °C	Recrystallization solvent[b]	$[\alpha]_D^{24-25^c}$, °	Empirical formula	Analysis					
								Calcd.			Found		
								C	H	N	C	H	N
24	Ethyl	*n*-butyl	78	130·0–131·5	ethyl acetate–ether	+26·2[d]	$C_{15}H_{23}NO_3.HCl$	59·69	8·02	4·64	59·79	8·06	4·54
25	*n*-Propyl	*n*-butyl	75	132–133	ethyl acetate	+25·7	$C_{16}H_{25}NO_3.HCl$	60·84	8·30	4·44	60·69	8·22	4·68
26	*n*-Propyl	*n*-pentyl	75	131–133	ethyl acetate	+21·8	$C_{17}H_{27}NO_3.HCl$	61·90	8·56	4·25	61·50	8·62	4·38
27	*n*-Propyl	*n*-hexyl	75	134–136	ethyl acetate	+18·5	$C_{18}H_{29}NO_3.HCl$	62·87	8·79	4·08	62·62	9·02	4·07
28	*n*-Butyl	*n*-butyl	72	129·5–130·0	ethyl acetate	+23·3	$C_{17}H_{27}NO_3.HCl$	61·90	8·56	4·25	61·82	8·71	4·43
29	*n*-Butyl	*n*-pentyl	73	129–131	ethyl acetate	+21·5	$C_{18}H_{29}NO_3.HCl$	62·87	8·79	4·08	62·46	8·85	4·06
30	*n*-Hexyl	*n*-propyl	82	139·5–140·0	ethyl acetate	+21·1	$C_{18}H_{29}NO_3.HCl$	62·87	8·79	4·08	63·02	8·65	4·02
31	*n*-Hexyl	*n*-butyl	29	119–120	ethyl acetate–ether	+19·7	$C_{19}H_{31}NO_3.HCl$	63·76	9·01	3·91	63·86	9·25	3·94
32	*n*-Hexyl	*n*-pentyl	74	122–124	ethyl acetate	+28·6	$C_{20}H_{33}NO_3.HCl$	64·61	9·21	3·77	64·26	8·91	4·15
33	*n*-Hexyl	*n*-hexyl	69	107–108	ethyl acetate	+ 7·4	$C_{21}H_{35}NO_3.HCl$	65·34	9·42	3·63	64·90	9·30	3·79

[a] The esters in this table were prepared by Procedure A.
[b] These esters, almost without exception, separate as gels which were dried for analysis.
[c] *c*, 1–2 in ethanol, unless otherwise specified.
[d] The solvent was chloroform.

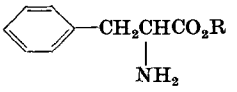
Table III. Esters of miscellaneous ring substituted derivatives of L-phenylalanine[a]



No.	R′	R″	R‴	R⁗	Yield, %	m.p., °C	Recrystallization solvent	$[\alpha]_D^{25}$[b], °	Empirical formula	Analysis					
										Calcd.			Found		
										C	H	N	C	H	N
34	MeO	Cl	H	*n*-Butyl	78	179–180	chloroform	+ 6·3 C	$C_{14}H_{20}ClNO_3.HCl$	52·18	6·57	4·35	52·45	6·69	4·66
35	MeO	Cl	H	*n*-Pentyl	80	173–175	ethyl acetate–isopropyl alcohol	+15·3 E	$C_{15}H_{22}ClNO_3.HCl$	53·58	6·90	4·17	53·68	6·98	4·24
36	HO	I	I	*n*-Butyl	71[c]	177·0–178·5	*t*-butyl alcohol–ether	+20·4 E	$C_{13}H_{17}I_2NO_3.HCl$	29·71	3·45	2·67	29·99	3·42	2·71
37	MeO	I	I	*n*-Butyl	83	141–143	acetone–ether		$C_{14}H_{19}I_2NO_3.HCl$	31·2	3·56	2·60	31·7	3·97	2·55
38	MeO	I	I	*n*-Pentyl	78	133–134	ethanol–petroleum ether[d]		$C_{15}H_{21}I_2NO_3.HCl$	32·6	3·83	2·53	33·5	3·86	2·88
39	MeO	I	I	*n*-Hexyl	80	127–129	acetone	+23·1 C	$C_{16}H_{23}I_2NO_3.HCl$	33·9	4·09	2·47	34·3	4·46	2·61
40	MeO	NO_2	H	*n*-Butyl	13	95·0–97·5	ethanol	+20·2 E	$C_{14}H_{20}N_2O_5$	56·74	6·80	9·46	56·70	7·15	9·65
41	MeO	NH_2	H	*n*-Butyl	92[e]	225			$C_{14}H_{22}N_2O_3.2HCl$	49·5	7·12	8·27	48·2	6·84	8·45
42	HO	H	H	*n*-Butyl	97	162–164	acetone	+ 4·4 A	$C_{13}H_{19}NO_3.HCl$	57·0	7·01	5·12	57·1	7·37	5·20

[a] These compounds were prepared by Procedure A unless otherwise stated. [b] *c*, 1·1–2·5; C=chloroform, E=ethanol, A=acetone. [c] Prepared by Procedure B.
[d] That fraction with b.p. 90–100°. [e] Prepared by catalytic reduction of the corresponding nitro compound; this ester could not be recrystallized.

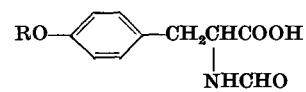
Table IV. Esters of L-phenylalanine[a]



No.	R	Yield, %	m.p., °C	Recrystallization solvent	$[\alpha]_D^{25\cdot27}$[b], °	Empirical formula	Analysis					
							Calcd.			Found		
							C	H	N	C	H	N
43	Allyl	46	155·5–157·5	ethyl acetate	+21·9	$C_{12}H_{15}NO_2.HCl$	59·62	6·67	5·80	59·37	6·70	5·74
44	*n*-Propyl	54	148·0–149·5	ethyl acetate–ether	+34·3	$C_{12}H_{17}NO_2.HCl$	59·08	7·44	5·75	59·03	7·17	5·89
45	*n*-Butyl	75	128·5–129·5	ethyl acetate–ether	+33·9	$C_{13}H_{19}NO_2.HCl$	60·57	7·82	5·44	60·56	7·79	5·47
46	*n*-Pentyl	78	132·5–133·0	ethyl acetate	+31·8	$C_{14}H_{21}NO_2.HCl$	61·88	8·16	5·16	61·78	8·17	5·45
47	*n*-Hexyl	68	120–121	ethyl acetate	+30·0	$C_{15}H_{23}NO_2.HCl$	63·03	8·46	4·90	63·37	8·52	4·82
48	Phenethyl	60	158–160	ethyl acetate–ethanol		$C_{17}H_{19}NO_2.HCl$	67·00	6·66	4·59	66·82	6·74	4·54
49	*n*-Nonyl	35	109–110	ethyl acetate	+25·8	$C_{18}H_{29}NO_2.HCl$	65·93	9·22	4·27	65·69	9·37	4·17

[a] All esters in this table were prepared by Procedure B. [b] *c*, 1–2 in ethanol.

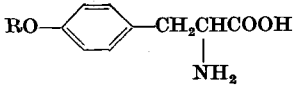
Table V. *N*-Formyl-4-alkoxy-L-phenylalanines



R	Yield, %	m.p., °C[a]	$[\alpha]_D^b$,°	Empirical formula	Analysis					
					Calcd.			Found		
					C	H	N	C	H	N
Ethyl	42	170–171	+77·4[c]	$C_{12}H_{15}NO_4$	60·75	6·37	5·91	60·70	6·35	5·95
n-Propyl	82	154–156	+65·5	$C_{13}H_{17}NO_4$	62·13	6·82	5·58	61·90	6·89	5·56
n-Butyl	70	153–154	+60·2	$C_{14}H_{19}NO_4$	63·38	7·22	5·28	63·51	7·66	4·94
n-Hexyl	73	160–161	+51·8	$C_{16}H_{23}NO_4$	65·50	7·90	4·78	65·60	7·90	4·70

[a] Recrystallized from ethanol–water. [b] *c*, 1–2 in methanol unless otherwise specified; measured at 25–26°. [c] The solvent was enthanol.

Table VI. 4-Alkoxy-L-phenylalanines

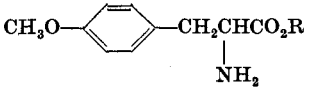


R	Yield, %	m.p., °C[a]	$[\alpha]_D^b$, °	Empirical formula	Analysis					
					Calcd.			Found		
					C	H	N	C	H	N
Ethyl	68	220–222 (d.)		$C_{11}H_{15}NO_3.HCl$	53·77	6·57	5·70	53·65	6·81	5·67
n-Propyl	56	220–222 (d.)	+9·5[c]	$C_{12}H_{17}NO_3.HCl$	55·49	6·99	5·39	55·38	7·17	5·22
n-Butyl	60	231–232 (d.)	−3[d]	$C_{13}H_{19}NO_3.HCl$	57·03	7·36	5·12	57·23	7·48	5·05
n-Hexyl[e]	93	213–214		$C_{15}H_{23}NO_3.HCl$			4·64			4·86

[a] Recrystallized from methanol–ether. [b] *c*, 1·0–2·0; measured at 25–26°. [c] The solvent was ethanol. [d] The solvent was methanol. [e] Satisfactory carbon and hydrogen analyses were never obtained; nevertheless, this crude acid gavc esters in satisfactory yields.

Alteration of the length of the *n*-alkyl chain in *n*-alkyl esters of 4-methoxy-L-phenylalanine (Group A, Table VII) gave rise to progressive changes in the activity of these esters. The methyl, ethyl and propyl members of this series were inactive; activity

Table VII. Group A compounds: *n*-alkyl esters of 4-methoxy-L-phenylalanine



No.	R	Organism[a]								
		Fungi					Bacteria			
		C.a.	*S.c.*	*F.e.*	*H.c.*	*T.m.*	*Myco.*	*Sarc.*	*Subt.*	*Staph.*
4	*n*-Butyl				1000[b]					
6	*n*-Pentyl						500		1000	1000
7	*n*-Hexyl	1000	1000	1000	250	250	125	1000	250	500
8	Cyclohexyl				250P[c]	500P	250	1000	500	500
10	*n*-Heptyl	500	250	500	250	500	125	250	125	125
11	*n*-Octyl[d]	250	125	250	500	125	60	60	60	60
12	*n*-Nonyl[d]	250	125	125	60	60 4P	30	30	30	15
13	*n*-Decyl[d]	250	125	250 30P	30	15 4P	30	30	30	15
15	*n*-Undecyl[d]	1000	500 125P		125P	60	30	30	30	30

[a] The abbreviations used for the micro-organisms in this paper are as follows: *C.a.*, *Candida albicans* Bergen; *S.c.*, *Saccharomyces carlsbergensis* A.T.C.C. 9080; *F.e.*, *Fusarium epispharia* F105; *H.c.*, *Hormodendrum cladosporoides* Z516; *T.m.*, *Trichophyton mentagrophytes* E11; *Myco.*, *Mycobacterium smegmatis* A.T.C.C. 607; *Sarc.*, *Sarcina lutea* A.T.C.C. 10054; *Subt.*, *Bacillus subtilis* A.T.C.C. 6633; *Staph.*, *Staphlococcus aureus* A.T.C.C. 6538P.

[b] The figures are the minimal inhibitory concentration in μg/ml; the 1000 μg/ml level was arbitrarily chosen as the least active level.

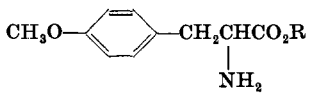
[c] A number followed by the letter P indicates that partial inhibition of the micro-organism was effected by the specified concentration of the ester; this condition is noted only when this level was two or more dilutions below the level of complete inhibition.

[d] The compound was not completely in solution at the highest test level in the antifungal tests. End-points of activity may be inaccurate as dilutions were made from the suspension.

was first suggested by the *n*-butyl and *n*-pentyl members, reached its peak with the *n*-nonyl and *n*-decyl members and was destroyed by the lengthening of the alcohol chain to *n*-octadecyl. One cycloalkyl ester, cyclohexyl (compound 8) was prepared. Although this ester was active, it was less so than the unbranched *n*-hexyl ester (7).

Replacement of methylene groups by isosteric oxygen atoms resulted in greatly lessened activity. Thus, the 2-(2-butyloxy)-ethoxyethyl ester (23) of 4-methoxy-L-phenylalanine had only minimal activity, whereas the *n*-decyl ester (13) was highly active (see Table VII). Introduction of a ring system into the alkyl chain also resulted in, at best, only weakly active structures [benzyl (17), phenethyl (18) and tetrahydrofurfuryl (21).]

Table VIII. Group B compounds: comparison of activity between branched-chain and straight-chain alkyl esters of 4-methoxy-L-phenylalanine



No.	R	Fungi[a]					Bacteria[a]			
		C.a.	*S.c.*	*F.e.*	*H.c.*	*T.m.*	*Myco.*	*Sarc.*	*Subt.*	*Staph.*
4	*n*-Butyl				1000					
5	Isobutyl									
10	*n*-Heptyl	500	250	500	250	500	125	250	125	125
11	5-Methyl-hexyl[b]		250		250		125	125		125
13	*n*-Decyl[c]	250	125	125	60	60 4P	30	30	30	15
14	3,7-Dimethyloctyl[d]		250			125	15	15	15	4

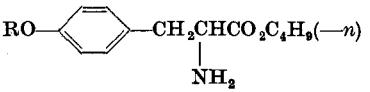
[a] For an explanation of the abbreviations, see footnotes to Table VII.
[b] The compound was not completely in solution at the highest test level in the antifungal and antibacterial tests.
[c] The compound was not completely in solution at the highest test level in the antifungal test.
[d] The compound was not completely in solution at the highest test level in the antibacterial test. In these cases end-points of activity may be inaccurate as dilutions were made from the suspension.

The 2-chloroethyl (19), 4-chlorobutyl (20) and 2-methoxyethyl (22) esters were also prepared. These compounds showed only minimal activity.

In view of the activity of the *n*-alkyl esters, it was pertinent to ascertain the effect on activity caused by branching of the alcohol chain (Group B). This modification resulted in a decrease in antifungal activity, although a slight enhancement in antibacterial activity was observed (see Table VIII). It may be noted, however, that in the latter test the significance of these differences is questionable.

In order to determine the effect on activity caused by the replacement of the 4-methoxy group by other alkoxy groups, certain *n*-butyl 4-*n*-alkoxy-L-phenylalaninates (Group C) were tested. The activity of these esters is summarized in Table IX. It can be seen that the activity progressively increased with increasing length of the carbon chain in the 4-*n*-alkoxy substituent. The most active ester of this group was *n*-butyl 4-*n*-hexyloxy-L-phenylalaninate (31) which has an activity that is

Table IX. Group C compounds: *n*-butyl 4-*n*-alkoxy-L-phenylalaninates

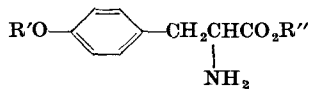


No.	R	Fungi[a]					Bacteria[a]			
		C.a.	*S.c.*	*F.e.*	*H.c.*	*T.m.*	*Myco.*	*Sarc.*	*Subt.*	*Staph.*
4	Methyl				1000					
24	Ethyl						500			1000
25	*n*-Propyl	1000	1000	1000	1000	1000	250	500	500	250
28	*n*-Butyl[b]	1000	500	500 60P	250 60P	250	125	250	250	125
31	*n*-Hexyl[b]	250	125	125	60	15 4P	1000 30P	30	30	30

[a] For an explanation of the abbreviations, see footnotes to Table VII.
[b] The compound was not completely in solution at the highest test level in the antifungal test. End-points of activity may be inaccurate as dilutions were made from the suspension.

approximately the same as that exhibited by the *n*-nonyl (12) and *n*-decyl (13) esters of 4-methoxy-L-phenylalanine (Group A). It is interesting to note that the sum of the carbon atoms in the ester chain and the 4-alkoxy group of these esters is 10–11. Additional *n*-alkyl 4-*n*-alkoxy-L-phenylalaninates (Group D), for which the sum of the carbon atoms in the two chains was in the 7–12 range, were prepared as a result of this observation. As anticipated, increasing values for R_1+R_2 resulted in increasing activity until a plateau was reached at the 10–12 level (Table X). This same phenomenon was observed in those series in which either R_1 or R_2 was held constant.

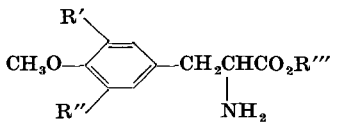
Table X. Group D compounds: *n*-Alkyl (R″) esters of 4-*n*-alkoxy (R′O)-L-phenylalanine in which $R_1+R_2=7-12$



No.	R′	R″	R_1+R_2	Fungi[a]				Bacteria[a]			
				S.c.[b]	*F.e.*	*H.c.*	*T.m.*	*Myco.*	*Sarc.*	*Subt.*	*Staph.*
25	*n*-Propyl	*n*-butyl	7	1000	1000	1000	1000	250	250	500	500
26	*n*-Propyl[c]	*n*-pentyl	8	500	1000	500	500	125	250	250	250
27	*n*-Propyl[c]	*n*-hexyl	9	500	500	500	500	60	125	250	125
28	*n*-Butyl[c]	*n*-butyl	8	500	500 60P	250 60P	250	125	125	250	250
29	*n*-Butyl[c]	*n*-pentyl	9	Active[e]				125	125	125	125
30	*n*-Hexyl[c]	*n*-propyl	9	250	500	500	250	60	125	125	125
31	*n*-Hexyl[d]	*n*-butyl	10	125	125	60	15 4P	1000 30P	30	30	30
32	*n*-Hexyl[d]	*n*-pentyl	11	125	500	500	60	30	30	30	30
33	*n*-Hexyl[c]	*n*-hexyl	12	500	1000	1000	60	30	30	30	30

[a] For an explanation of the abbreviations, see footnotes to Table VII.
[b] As with previous groups the inhibition of *Candida albicans* Bergen growth by these esters was unimpressive.
[c] The compound was not completely soluble at the highest test level in the antifungal and antibacterial tests.
[d] The compound was not completely soluble at the highest test level in the antifungal test. In these cases end-points of activity may be inaccurate as dilutions were made from the suspension.
[e] This ester had activity at 1000 μg/ml against the fungi, but the amount of sample was insufficient to determine its lowest level of activity.

Table XI. Group E compounds: ring-substituted derivatives of *n*-alkyl 4-methoxy-L-phenylalaninates

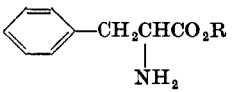


No.	R′	R″	R‴	Fungi[a]					Bacteria[a]			
				C.a.	*S.c.*	*F.e.*	*H.c.*	*T'm.*	*Myco.*	*Sarc.*	*Subt.*	*Staph.*
34	Cl	H	*n*-butyl					1000	500	1000	1000	1000
35	Cl	H	*n*-pentyl[b]	250	250	250	250	250	125	1000	125	250
37	I	I	*n*-butyl[c]	500P	500P	500P		500 125P	60	125	60	60
38	I	I	*n*-pentyl[d]	500	500			250 60P	30	125	60	30
39	I	I	*n*-hexyl[c]	1000	1000				30	125 30P	125 30P	30
40	NO_2	H	*n*-butyl[c]			1000	1000	1000				

[a] For an explanation of the abbreviations, see footnotes to Table VII.
[b] The compound was not completely soluble at the highest test level in the antibacterial test.
[c] The compound was not completely soluble at the highest test level in the antifungal and antibacterial tests.
[d] The compound was not completely soluble at the highest test level in the antifungal test. In all of these cases end-points of activity may be inaccurate as dilutions were made from the suspension.

The effect of ring substitution was also investigated. Thus, esters of 3-chloro, 3,5-diiodo-, 3-nitro-, and 3-amino-4-methoxy-L-phenylalanine (Group E) were prepared and tested. With the exception of butyl 3-amino-4-methoxy-L-phenylalaninate, which had only trace activity, the introduction of these groups resulted in increased activity (Table XI). However, it may be noted that there is little difference in activity between the butyl, pentyl, and hexyl esters of 3,5-diiodo-4-methoxy-L-phenylalanine ($R_1 + R_2 =$

Table XII. Group F compounds: esters of L-phenylalanine



No.	R	Fungi[a]					Bacteria[a]			
		C.a.	*S.c.*	*F.e.*	*H.c.*	*T.m.*	*Myco.*	*Sarc.*	*Subt.*	*Staph.*
43	Allyl	500 125P	500	125 30P						
45	*n*-Butyl						1000			
46	*n*-Pentyl						1000			1000
47	*n*-Hexyl	500P	250P	1000	500	500	500 125P	500	500	250
49	*n*-Nonyl		125	250	250	8	15	30	60	4
48	Phenethyl				1000	1000				

[a] For an explanation of the abbreviations, see footnotes to Table VII.

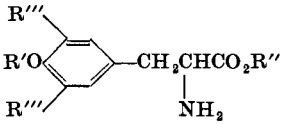
5–7). Thus it would seem that peak activity in this series has already been reached. On a molar basis, the level of antibacterial activity for these compounds is about equal to that of those esters of Groups A–D having $R_1 + R_2 = 10$–12. However, the antifungal activity of the diiodo compounds is considerably less. Esters of the unsubstituted L-phenylalanine (Group F) were also tested. These compounds were about as active as the corresponding 4-methoxy derivatives (Table XII).

It is pertinent to indicate that, since in certain cases (these are noted in the appropriate tables) there was not complete solution of the compound in the test medium, the minimum inhibitory

concentrations for these compounds are probably less than those listed.

All inhibitory concentration values given in Tables VII–XII were determined by a technique whereby heat sterilization of the test medium was carried out after the addition of the candidate com-

Table XIII. The effect of heating upon the activities of certain *n*-alkyl 4-*n*-alkoxy-L-phenylalaninates



No.	R′	R″	R‴	Assay	Bacteria[a]			
					Myco.	*Sarc.*	*Subt.*	*Staph.*
11	Methyl	*n*-octyl	H	H[b]	60	60	60	60
				WH[c]	15	30	30	30
12	Methyl	*n*-nonyl	H	H	30	30	30	15
				WH	8	8	8	8
13	Methyl	*n*-decyl	H	H	30	30	30	15
				WH	8	8	8	8
28	*n*-Butyl	*n*-butyl	H	H	125	250	250	125
				WH	15	60	60	30
31	*n*-Hexyl	*n*-butyl	H	H	1000 30P	30	30	30
				WH	8	8	15	15
37	Methyl	*n*-butyl	I	H	60	125	60	60
				WH	30	60	30	30

[a] For an explanation of the abbreviations, see footnotes to Table VII.
[b] The compound was assayed with heat.
[c] The compound was assayed without heat.

pound. Therefore, it was of interest to determine the minimum inhibitory concentration when the candidate esters were added after sterilization of the test medium. In Table XIII several of the more active esters and their inhibitory concentrations against Gram-positive bacteria when assayed by the two procedures are listed. In all cases the ester was more active when it was added after sterilization of the test medium. This difference was usually two- to four-fold. The lower activity observed when the ester

was sterilized with the test medium may be the result of breakdown of the ester into the inactive alcohol and amino acid. Finally, it may be noted that when the *n*-nonyl and *n*-decyl esters of 4-methoxy-L-phenylalanine were assayed without heat, they demonstrated an activity against Gram-positive bacteria approximately equal to that shown by puromycin (I).

The possibility that the esters functioned as competitive inhibitors of L-phenylalanine or L-tyrosine was considered. However, we have not been able to demonstrate any such effect.

Experimental

The compounds listed in Tables I–IV, with one exception, were prepared using either procedure A or B as noted.* The reactants, when commercially available, were used as received without further purification. When unavailable, they were prepared in this laboratory as described under 'Preparation of Intermediates.'

Procedure A

The amino acid hydrochloride was suspended in the appropriate alcohol (approximately 2 ml per gram of amino acid hydrochloride), and the suspension was treated with acetyl chloride (1 ml per gram of amino acid hydrochloride). The mixture was allowed to reflux until the solid dissolved. In some examples, solution of the suspended solid was facilitated by addition of further acetyl chloride. In a few experiments, solution was not completely effected by this means nor by extended reflux. In these latter experiments the hot mixture was filtered to remove the unreacted amino acid hydrochloride, and the filtrate was worked up as described below to isolate the product.

The reaction filtrate or solution was concentrated under reduced pressure (water-pump) on the steam bath to remove the more volatile material. Only in reactions utilizing the lower-chain alcohols was a solid residue left at this stage. In these examples the residue was recrystallized from the appropriate solvent. In experiments utilizing the higher-chain alcohols, the

* Although optical purity for the various compounds is assumed, the possibility exists that in the course of preparation some degree of racemization may have occurred.

solution remaining after the removal of the volatile material was diluted with ether to precipitate the product which was collected by filtration and purified by recrystallization.

Procedure B

A mixture of the amino acid and the appropriate alcohol which had been saturated with hydrogen chloride (10 ml of alcohol per gram of amino acid) was allowed to reflux for 90 min. Complete solution of the solid was usually attained after 30 min. The excess alcohol was removed under reduced pressure (water-pump) on the steam bath, and the solid residue was purified by recrystallization from the appropriate solvent. In experiments using the higher-chain alcohols the product was isolated by precipitation with ether as described in Procedure A.

n-*Butyl 3-amino-4-methoxy*-L-*phenylalaninate hydrochloride* (*41*). A mixture of *n*-butyl 4-methoxy-3-nitro-L-phenylalaninate hydrochloride (41) (1·5 g, 5·1 mmole) and 10 per cent palladium-on-charcoal catalyst in ethanol (0·140 g) was shaken under an atmosphere of hydrogen in a Parr low-pressure hydrogenation apparatus. After the uptake of hydrogen had ceased, the mixture was filtered. The filtrate was concentrated to a viscous oil under reduced pressure (water-pump) on a steam bath. The residual oil was dissolved in ethanol; this solution was treated with hydrogen chloride and drowned in ether to give the product as a crystalline, grey solid. Further information concerning this ester is given in Table III.

Preparation of Intermediates

The preparation only of new compounds is described.

4-Alkoxy-N-*formyl*-L-*phenylalanines* (*IV*) (*Table V*). The following experiment describes the typical procedure. *N*-Formyl-L-tyrosine (III) (5·0 g, 0·026 mole) was added to a solution of sodium methoxide (2·7 g, 0·050 mole) in methanol (50 ml). After nearly all of the solid was dissolved, ethyl iodide (3·9 g, 0·025 mole, 2·02 ml) was added to the mixture, which was then allowed to reflux for 3 h on the steam bath. The solvent was then removed under reduced pressure (water-pump) on the steam bath. The residue was made acid to litmus paper by the addition of

the required amount of 6 N nitric acid solution; the undissolved solids were collected by filtration, washed thoroughly with ice water, and dried in air. This crude product was recrystallized from dilute methanol to give 2·4 g of *4-ethoxy*-N-*formyl*-L-*phenylalanine* as white crystals, m.p. 170–171°. Further information concerning this product and others prepared in this manner is given in Table V.

4-Alkoxy-L-*phenylalanines* (*V*) (*Table VI*). These compounds were prepared by the following general procedure. The appropriate 4-alkoxy-*N*-formyl-L-phenylalanine (IV) was suspended in 10–20 parts of 3 N hydrochloric acid solution, and the mixture was heated on a steam bath until all suspended solid dissolved. The solution was treated with activated charcoal and filtered. In all examples except 4-ethoxy-L-phenylalanine hydrochloride, the product crystallized upon cooling the filtrate. After removal of the first crop of material by filtration, the filtrate was concentrated under reduced pressure (water-pump) on the steam bath to furnish a second crop. In the case of 4-ethoxy-L-phenylalanine hydrochloride the product was isolated by concentration of the reaction solution.

N-*Formyl-3-nitro*-L-*tyrosine*. A solution of 3-nitro-L-tyrosine[12] (1·60 g, 6·8 mmole) in 90 per cent formic acid (8 ml) was warmed on the steam bath to 50°. The yellow solution was treated with acetic anhydride (2·6 ml), and the temperature rose to 75°. The solution was heated at 85–90° for 1 h and then concentrated to dryness under reduced pressure (water-pump). The residue was recrystallized twice from water to give yellow crystals (1·25 g, 73 per cent), m.p. 180–182°; $[\alpha]_D^{25} + 63°$ (*c*, 1·8 in methanol).

Anal. Calcd. for $C_{10}H_{10}N_2O_6$: C, 47·25; H, 3·97; N, 11·02. Found: C, 47·18; H, 4·08; N, 10·60.

N-*Formyl-4-methoxy-3-nitro*-L-*phenylalanine*. To an ice-chilled, magnetically stirred solution of *N*-formyl-3-nitro-L-tyrosine (73 g, 0·286 mole) in 4 N sodium hydroxide solution (144 ml) was added in seven equal portions 116 ml of 4 N sodium hydroxide solution and 93 g of dimethyl sulphate (0·74 mole, 68·9 ml). Stirring was continued for 2 h after the addition was completed. The solution was acidified by the addition of 6 N nitric acid solution (95 ml), and the solid which separated was collected by filtration to give yellow-green crystals (78·7 g), m.p. 110–114°.

This solid was extracted with several portions of hot ethyl acetate; the total volume of the extracts was 1700 ml. A brown solid (20·2 g) which did not dissolve was discarded. The combined ethyl acetate extracts were treated with decolorizing charcoal, dried over magnesium sulphate and concentrated to a volume of about 300 ml. The crystals which separated on cooling were collected by filtration, giving yellow crystals (38·5 g), m.p. 149–154°. Further concentration of the ethyl acetate solution gave an additional 7·6 g of material (yield, 60 per cent). A sample was again recrystallized from ethyl acetate to give yellow crystals, m.p. 145–148°; $[\alpha]_D^{25} + 5°$ (*c*, 1·0 in dimethylformamide).

Anal. Calcd. for $C_{11}H_{12}N_2O_6$: C, 49·25; H, 4·51; N, 10·45. Found: C, 48·96; H, 4·38; N, 10·59.

4-Methoxy-3-nitro-L-*phenylalanine hydrochloride.* A mixture of *N*-formyl-4-methoxy-3-nitro-L-phenylalanine (0·186 g, 0·7 mmole) and 5 per cent hydrochloric acid solution (5 ml) was heated on a steam bath for 3 h. The resulting solution was taken to dryness under reduced pressure (water-pump) on the steam bath. The residual solid was recrystallized from water (2 ml) to give yellow needles (0·157 g, 82 per cent), m.p. 228–230° (d.) after darkening from 220°. The analyses for this material indicated that it might contain some 3-nitro-L-tyrosine hydrochloride; the material was used as obtained above for conversion to its *n*-butyl ester (40).

Anal. Calcd. for $C_{10}H_{12}N_2O_5.HCl$: C, 43·41; H, 4·74; N, 10·12. Found: C, 41·60; H, 4·74; N, 10·55.

N-*Formyl-3,5-diiodo*-L-*tyrosine.* 3,5-Diiodo-L-tyrosine dihydrate (10·0 g, 0·021 mole) was dissolved in 90 per cent formic acid (60 ml) by heating to about 50°; 20 ml of acetic anhydride was added portionwise. The temperature rose to 70° and was held at 70–80° for 15 min. The clear solution was taken to dryness under reduced pressure (water-pump) on a steam bath. Toluene was added to the residual white solid, and the solvent was removed in the same manner. After trituration of the solid with ether, the material was collected by filtration to give 9·6 g of crystals (99 per cent yield), m.p. 199–201° (d.) with previous shrinking at 188°. Two recrystallizations from aqueous methanol gave colourless plates of *N*-formyl-3,5-diiodo-L-tyrosine, m.p. 208–209° (d.)

Anal. Calcd. for $C_{10}H_9I_2NO_4$: C, 26·1; H, 1·97; N, 3·05. Found: C, 26·9, 27·0; H, 2·18, 2·22; N, 2·95.

3,5-Diiodo-N-formyl-4-methoxy-L-phenylalanine. This material was obtained in the same manner as *N*-formyl-4-methoxy-3-nitro-L-phenylalanine (see above). The yield of crystalline material, m.p. 182–183° (d.), was 68 per cent. One recrystallization from dilute methanol did not raise the melting range.

Anal. Calcd. for $C_{11}H_{11}I_2NO_4$: C, 27·8; H, 2·34; N, 2·95. Found: C, 28·6; H, 2·56; N, 2·96.

3,5-Diiodo-4-methoxy-L-phenylalanine hydrochloride. This compound was prepared by acid hydrolysis of the *N*-formyl derivative as described above for the preparation of the 4-alkoxy-L-phenylalanines. The product was obtained as a hydrochloride, m.p. 218–220°, in 71 per cent yield. Conversion to the free base gave material melting at 216–218° (d.) (m.p. varied with the rate of heating) which depressed the melting range of the hydrochloride. A melting point of 204–206° has been reported.[13]

Anal. Calcd. for $C_{10}H_{11}I_2NO_3$: C, 26·9; H, 2·49; I, 56·8; N, 3·14. Found: C, 26·8; H, 2·68; I, 54·4, 54·7; N, 3·40.

3-Chloro-4-methoxy-L-phenylalanine hydrochloride. To a solution of *N*-acetyl-4-methoxy-L-phenylalanine (5·0 g)[1] in acetic acid (25 ml) at 50°, sulphuryl chloride (1·8 ml) was added during 3 min with swirling. The temperature was maintained at 50–55° by occasional cooling. Ten minutes later the solution was taken to dryness under reduced pressure (water-pump) on the steam bath. The residual *N*-acetyl-3-chloro-4-methoxy-L-phenylalanine could not be crystallized. The residue was dissolved in hot 6 N hydrochloric acid solution (25 ml) and heated on the steam bath for 2 h. Crystals began to separate, and the mixture was chilled in an ice bath. The product was collected by filtration and washed with ice-cold 6 N hydrochloric acid solution until the washings were colourless; yield, 3·2 g (57 per cent) of rose-coloured solid, m.p. 225–230° (d.) with sintering at 215°. The product was dissolved in hot water (20 ml) and treated with activated charcoal. 12 N hydrochloric acid solution (20 ml) was added to the reheated filtrate. Cooling of the solution gave colourless crystals, m.p. 235° (d.) with sintering at 215°; $[\alpha]_D^{25} - 7·7°$ (*c*, 2·0 in H_2O).

Anal. Calcd. for $C_{10}H_{12}ClNO_3.HCl$: C, 45·2; H, 4·93; N, 5·27. Found: C, 45·2; H, 5·18; N, 5·40.

Assay Procedure

Two 8 to 12 mg portions of the compound to be tested were accurately weighed into 18 × 150 mm Pyrex test tubes. The portion to be tested for antifugal activity was suspended in sufficient melted yeast-malt agar to result in a concentration of 1000 μg/ml. The portion to be tested for antibacterial activity was suspended in Trypticase-Soy agar B.B.L. Both tubes were autoclaved at 15 lb/in^2 for 15 min. Upon removal from the autoclave the tubes were placed in a water bath and cooled to approximately 50°. The contents of each tube were shaken and poured into sterile Petri dishes. Special care was taken to suspend undissolved compounds prior to the pouring operation. After the agar had solidified, the surfaces were streaked with the test cultures. The plates with the bacteria were incubated at about 35°. The fungal tests were incubated at 25°. The plates with the bacteria were examined after 16 h incubation. Growth was recorded on an arbitrary −, +, + +, + + + scale to indicate the range from complete inhibition to normal growth. The plates were re-examined after an additional 24 h, and the growth of the mycobacterium was similarly recorded. The yeast cultures were read after 16 h, the hormodendrum and fusarium streaks after 40 h, and the trichophyton after 88 h of incubation. Those compounds which completely inhibited growth of any of the four bacteria or five fungi at the 1000 μg/ml level were reinvestigated to determine the minimal effective concentrations.

Summary. The preparation and *in vitro* antifungal and antibacterial activity of certain esters of phenylalanine and substituted phenylalanines are described. The relationship of structure to antifungal and antibacterial activity is discussed.

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