Journal of Medicinal and Pharmaceutical Chemistry

VOL. 2, No. 4 (1960)

The Synthesis of Certain 9-(3-Acylamino-3-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurines, Analogues of the Antibiotic Puromycin

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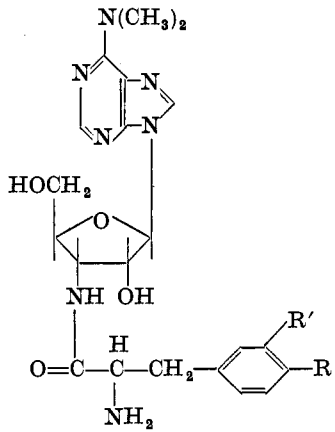
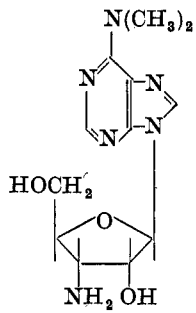
The antibiotic puromycin has been shown to have the structure, 6-dimethylamino-9-(3-deoxy-3-*p*-methoxyphenylalanylamino-β-D-ribofuranosyl)-purine (I).[1,2] Cleavage of the *p*-methoxy-L-phenylalanyl moiety from the antibiotic[3] gave the aminonucleoside (II) which still possessed interesting biological properties. Although the complete antibiotic was active against certain bacteria (*in vitro*),[4] amoebae,[5] certain animal tumours[6] and *Trypanosoma equiperdum*,[7] the aminonucleoside (II) showed activity only against tumours[8] and trypanosomiasis[9] but did so to a greater extent than the parent antibiotic. Therefore, it seemed apparent that the antibacterial and antiamoebic activities of puromycin were in some manner connected with the presence of the *p*-methoxy-L-phenylalanyl moiety and, consequently, one approach towards the synthesis of puromycin analogues consisted in replacing this moiety with other amino acids. Baker, Joseph and Williams[3] have described a series of analogues of (I) in which certain naturally occurring amino acids such as glycine, L-leucine, L-tryptophan, L-tyrosine and L-phenylalanine were linked to the aminonucleoside (II) through an amide bond. Antibacterial testing showed that these synthetic compounds were at best no more active than the parent antibiotic, the L-phenylalanyl derivative being the most active of the series. This compound also had an activity against trypanosomiasis, equal to that of puromycin. This activity picture seemed to suggest that additional analogues prepared for this programme should contain amino acids closely related to phenylalanine and *p*-methoxy-L-phenylalanine. The preparation of such analogues received additional stimulus from

the observation[10] that simple *p*-alkoxy-L-phenylalanine esters had antibacterial activity and that this activity increased with the size of the *p*-alkoxy chain.

Among the analogues synthesized in the present study were those in which the *p*-methoxy group was replaced by various other alkoxy groups (III) or by fluorine (V) and those in which the *p*-methoxy-L-phenyealanyl moiety was substituted in the *meta* position by chlorine or the nitro group (IV). The preparation of





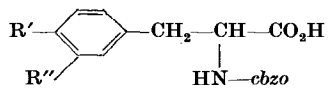
II

(I); R = OCH_3, R′ = H
(III); R = O-alkyl, R′ = H
(IV); R = OCH_3, R′ = NO_2 or Cl
(V); R = F, R′ = H

most of the *p*-alkoxy-L-phenylalanine derivatives, required as starting materials for this work, is described in an accompanying paper by Allen and co-workers.[10] One additional L-phenylalanine derivative, *p*-isopropoxy-L-phenylalanine, was synthesized by the same procedure.[10] *p*-Fluoro-DL-phenylalanine was commercially available.

For condensation with the aminonucleoside (II), all of the amino acids, with the exception of *p*-methoxy-*m*-nitro-L-phenylalanine, were first converted to their carbobenzyloxy (*cbzo*) derivatives (cf. Table I); coupling with (II) was then carried out by the mixed carbonic anhydride procedure as described by Baker and co-workers.[3] The resulting *cbzo*-blocked puromycin analogues are listed in Table II. In the case of *p*-methoxy-*m*-nitro-L-phenylalanine,

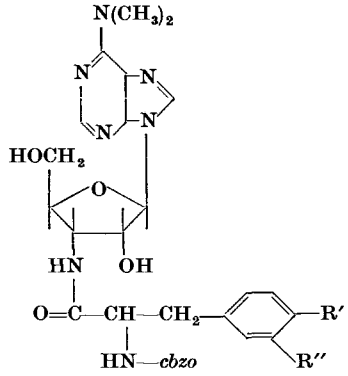
Table I. *cbzo*—Amino acids



R′	R″	Empirical formula	Yield %	m.p., °C[a]	Optical rotation °		Analysis, %					
							Calcd.			Found		
					$[\alpha]_D^{25}$	c^b	C	H	N	C	H	N
OCH_2CH_3	H	$C_{19}H_{21}NO_5$	71	81–83	+13·2	2·20	66·46	6·16	4·08	66·17	6·03	4·13
$O(CH_2)_2CH_3$	H	$C_{20}H_{23}NO_5$	92	83–85[c]	+11·4	2·03	67·21	6·49	3·92	67·39	6·55	4·17
$OCH(CH_3)_2$	H	$C_{20}H_{23}NO_5$	60	79–81	+11·9	1·77	67·21	6·49	3·92	67·41	6·73	4·21
$O(CH_2)_3CH_3$	H	$C_{21}H_{25}NO_5$	83	88–90	+10·0	2·00	67·90	6·78	3·77	68·08	7·12	3·52
$O(CH_2)_5CH_3$	H	$C_{23}H_{29}NO_5$	83	79–81[d]	+35·9	2·03[e]	69·15	7·32	3·51	69·18	7·66	3·67
F[f]	H	$C_{17}H_{16}FNO_4$	51	124–126	− 5·7	1·05[g]	64·35	5·09	4·42	64·05	5·08	4·52
OCH_3	Cl	$C_{18}H_{18}ClNO_5$	87	103–105[a]	+0·93	2·15[e]	59·44	4·89	3·85	59·19	5·16	4·00

[a] Recrystallized from ether–hexane unless stated otherwise. [b] In ethanol unless otherwise stated. [c] Recrystallized from ethanol–petroleum ether (90–100°). [d] Recrystallized from petroleum ether (90–100°). [e] In chloroform. [f] DL-amino acid. [g] In 2-methoxyethanol.

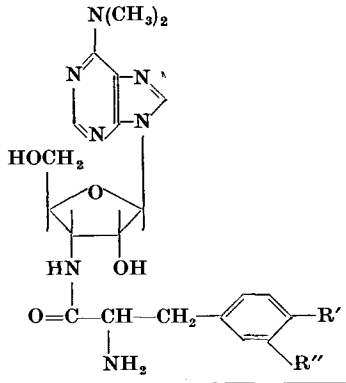
Table II. *cbzo*—Aminoacyl aminonucleosides



R′	R″	Empirical formula	Method of preparation	Yield %	m.p., °C[a]	Optical rotation [°]		Analysis, %					
								Calcd.			Found		
						$[\alpha]_D^{25}$	c^b	C	H	N	C	H	N
OCH_2CH_3	H	$C_{31}H_{37}N_7O_7$	B	78	204–206	−29·8	2·04	60·08	6·08	15·82	59·98	6·15	15·53
$O(CH_2)_2CH_3$	H	$C_{32}H_{39}N_7O_7$	B	98	208–209	−32·4	1·02[c]	60·64	6·20	15·53	60·40	6·33	15·39
$OCH(CH_3)_2$	H	$C_{32}H_{39}N_7O_7$	B	74	215–217	−28·9	2·07	60·64	6·20	15·53	60·43	6·36	15·60
$O(CH_2)_3CH_3$	H	$C_{33}H_{41}N_7O_7$	A	70	212–214	−30·2	2·05	61·19	6·38	15·17	61·29	6·72	15·46
$O(CH_2)_5CH_3$	H	$C_{35}H_{45}N_7O_7$	B	–[d]	202–203	−28·2	1·84[c]	62·20	6·71	14·51	61·91	7·04	14·56
F	H	$C_{29}H_{32}FN_7O_6$	A	63	212–216[e]	−29·5	0·98	58·67	5·43	16·52	58·44	5·63	16·34
OCH_3	Cl	$C_{30}H_{34}ClN_7O_7$	A	46	214–217	−31·2	1·15	56·21	5·35	15·29	56·31	5·69	15·70

[a] Recrystallized from ethanol unless stated otherwise. [b] In 2-methoxyethanol unless stated otherwise. [c] In pyridine. [d] No accurate yield could be determined because this compound was always solvated. [e] Recrystallized from ethanol–ethyl acetate.

Table III. Aminoacyl aminonucleosides

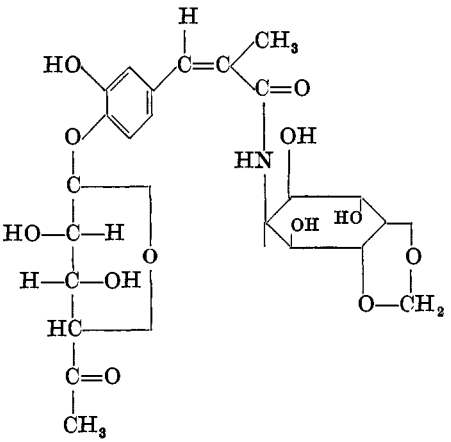


R′	R″	Empirical formula	Yield %	m.p., °C[a]	Optical rotation °		Analysis, %					
							Calcd.			Found		
					$[\alpha]_D^{25}$	c^b	C	H	N	C	H	N
OCH_2CH_3	H	$C_{23}H_{31}N_7O_5$	50	163–166	−27·6	1·09	56·89	6·43	20·20	56·52	6·84	19·97
$O(CH_2)_2CH_3$	H	$C_{24}H_{33}N_7O_5$ C_2H_5OH	87[c]	175–177	−51·0	1·72[d]	57·23	7·20	17·97	57·11	7·23	18·04
$OCH(CH_3)_2$	H	$C_{24}H_{33}N_7O_5$	50	185–188	−23·2	1·04	57·70	6·66	19·63	57·54	6·67	19·77
$O(CH_2)_3CH_3$	H	$C_{25}H_{35}N_7O_5$	72	186–188	−26·0	0·31[e]	58·46	6·87	19·09	58·52	7·04	19·34
$O(CH_2)_5CH_3$	H	$C_{27}H_{39}N_7O_5$ C_2H_5OH	75	164–166	−28·2	1·10	59·27	7·72	16·69	59·41	7·89	17·10
F	H	$C_{21}H_{26}FN_7O_4$	63	208–210	−12·8	1·02[f]	54·89	5·70	21·34	54·80	6·01	21·67
OCH_3	NO_2	$C_{22}H_{28}N_8O_7 \cdot H_2O$	49	128–131[g]	−25·5	0·98	49·43	5·65	20·97	49·50	5·41	20·83
OCH_3	Cl	$C_{22}H_{28}ClN_7O_5 \cdot H_2O$	87[c]	203–205[h]	−21·7	2·16[f]	50·42	5·77	18·71	50·33	5·90	18·92

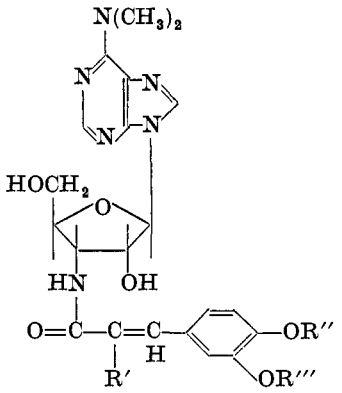
[a] Recrystallized from ethanol unless stated otherwise. [b] In 2-methoxyethanol unless stated otherwise. [c] Crude yield. [d] In pyridine. [e] In ethanol. [f] In methanol. [g] Recrystallized from water. [h] Recrystallized from ethanol–ethyl acetate.

the amino function was blocked with the phthaloyl group and condensation of the resulting phthalimido derivative with (II) was effected *via* the corresponding acid chloride in dimethylformamide with triethylamine as the acid acceptor. Deblocking to give the final products (Table III) was accomplished by hydrogenolysis of the *cbzo*-derivatives and by hydrazinolysis of the phthaloyl derivative.

At the time the work under discussion was being carried out, J. B. Patrick and co-workers[11] of these laboratories were investi-



(VI)



(VII); R′ = H, R″ + R‴ = >CH$_2$
(VIII); R′ = R‴ = CH$_3$, R″ = COC$_2$H$_5$
(IX); R′ = R‴ = CH$_3$, R″ = H

gating the antibiotic Hygromycin[12]* which eventually was shown to have structure (VI). Since the 3,4-dihydroxy-α-methylcinnamoylinosamine portion of this antibiotic has a certain formal resemblance to the *p*-methoxy-L-phenylalanyl-3-aminoribofuranosyl portion of puromycin,† it seemed worth while to prepare a few puromycin analogues containing 3,4-dihydroxycinnamoyl derivatives connected to the aminonucleoside (II) through amide linkages. Thus, the *N*-3,4-methylenedioxycinnamoyl-(VII), *N*-4-methoxy-3-propionoxy-α-methylcinnamoyl-(VIII) and *N*-3-hydroxy-4-meth-

* Hygromycin is also known as Homomycin.[12b]
† This was suggested to us by Dr. J. B. Patrick.

oxy-α-methylcinnamoyl-(IX) derivatives of the aminonucleoside were prepared. For the syntheses of (VII) and (VIII), the corresponding cinnamic acids were converted to the acid chlorides with thionyl chloride, and these were then coupled with the aminonucleoside in dimethylformamide with triethylamine as acid acceptor. Compound (IX) was prepared from (VIII) by a sodium methoxide catalyzed deacylation.

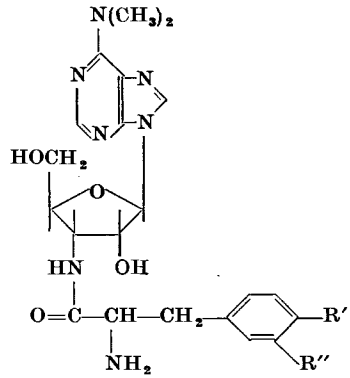
Biological Activities

None of the analogues prepared in the course of this investigation surpassed puromycin when tested *in vitro* against several bacterial strains, in mice against *Trypanosoma equiperdum*, *in vitro* against *Endamoeba histolytica*, and against the transplanted mammary adenocarcinoma in C_3H mice.* The results of antitrypanosomiasis and antibacterial testing are detailed in Table IV. It may be noted that varying the length of the alkoxyl chain did not cause any substantial change in antibacterial activity. This is in contrast to the structure–activity relationship observed with the *p*-alkoxy-L-phenylalanine ester series described in the accompanying paper.[10] Although certain of the *p*-alkoxy-L-phenylalanine esters showed substantial *in vitro* antifungal activity, none of the compounds in the present series had this activity. Against the mammary adenocarcinoma, only the *p*-ethoxy-, *p*-propoxy- and *p*-fluoro derivatives were considered to be active. When tested against Sarcoma 180, all of the compounds were inactive (as were I and II). The *p*-propoxy derivative was the only compound in this series which was active against the $6C_3HED$ lymphosarcoma. This was of special interest since neither puromycin nor the aminonucleoside (II) possesses this activity.

The 3,4-dihydroxycinnamoyl aminonucleoside derivatives (VII), (VIII) and (IX) were inactive against tumours and *Trypanosoma equiperdum* and were inferior to puromycin as antibacterial agents.

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

Table IV. Biological activities



R′	R″	Antitrypanosomiasis activity[a]	Antibacterial activity,[b,c] minimal inhibitory concentration in μg per ml							
			Myco.	*Staph.*	*Sarc.*	*Subt.*	*Pseud.*	*Prot.*	*Coli*	*Salm.*
OCH_2CH_3	H	<0·25	31	8	4	2	250	–	–	–
$O(CH_2)_2CH_3$	H	<0·25	8	8	4	8	500	500	500	500
$OCH(CH_3)_2$	H	–[d]	31	62	15	31	–	1000	1000	1000
$O(CH_2)_3CH_3$	H	–[d]	31	4	1	–	–	–	–	–
$O(CH_2)_5CH_3$	H	<0·25	15	15	4	15	–	–	–	–
F	H	0·25	15	31	2	15	500	500	500	500
OCH_3	NO_2	<0·25	62	–	–	–	–	–	–	–
OCH_3	Cl	–[d]	250	31	4	15	–	1000	–	1000

[a] The activity of the aminonucleoside (II) is taken as 1.

[b] Dash marks indicate that the compound was inactive at the highest test level of 1000 μg/ml.

[c] The abbreviations used for the micro-organisms in this paper are as follows: *Myco.*, *Mycobacterium smegmatis* A.T.C.C. 9080; *Staph.*, *Staphylococcus aureus* A.T.C.C. 6538P; *Sarc.*, *Sarcina lutea* A.T.C.C. 10054; *Subt.*, *Bacillus subtilis* A.T.C.C. 6633; *Pseud.*, *Pseudomonas aeruginosa*; *Prot.*, *Proteus vulgaris*; *Coli*, *Escherichia coli*; *Salm.*, *Salmonella gallinarum*.

[d] Inactive at 100 mg/kg oral dose.

Experimental*

N-*Formyl*-p-*isopropoxy*-L-*phenylalanine.* Isopropyl iodide (4 ml) was added to a solution of *N*-formyl-L-tyrosine[13] (8·36 g, 0·04 mole) and sodium methoxide (4·32 g, 0·08 mole) in anhydrous methanol (40 ml) and the solution was allowed to reflux for 7 h. The reaction mixture was acidified with 6 N nitric acid and the resulting precipitate was collected, washed with cold water and dried *in vacuo* to yield 6·9 g (69 per cent); m.p. 124–132°. For analysis the material obtained in a similar experiment was recrystallized twice from water; m.p. 132–133°; $[\alpha]_D^{25} + 91·4°$ (*c*, 0·64 in chloroform).

Anal. Calcd. for $C_{13}H_{17}NO_4$: C, 62·13; H, 6·82; N, 5·58. Found: C, 62·28; H, 6·71; N, 5·77.

p-*Isopropoxy*-L-*phenylalanine hydrochloride.* A mixture of *N*-formyl-*p*-isopropoxy-L-phenylalanine (2·6 g, 0·01 mole) and 3 N hydrochloric acid (40 ml) was heated on the steam bath for 1 h and then treated with activated charcoal and filtered. The filtrate was chilled and the white crystalline precipitate which formed was collected, washed with ice water and dried *in vacuo* to yield 1·5 g (58 per cent); m.p. 209–219° (d.). Recrystallized from ethanol–ether it showed m.p. 205–208°; $[\alpha]_D^{25} - 6·4°$ (*c*, 0·94 in methanol).

Anal.† Calcd. for $C_{12}H_{17}NO_3.HCl$: C, 55·50; H, 7·00; N, 5·39. Found: C, 56·52; H, 6·92; N, 5·87.

Cbzo-*amino acids.* The *cbzo*-derivatives shown in Table I were prepared by adding a toluene solution containing 15 mmole of carbobenzyloxy chloride to which had been added 5 ml of chloroform, dropwise to a chilled solution of the amino acid (10 mmole) and sodium hydroxide (1·5 g) in water (50 ml). The mixture was stirred in an ice bath for 1–2 h and then extracted with several portions of ether. The aqueous phase was acidified with 3 N hydrochloric acid and the resulting precipitate was collected by filtration and washed with water. The filtrate and washings were combined and extracted with chloroform. The filtered precipitate was also dissolved in chloroform and the combined chloroform

* Melting points were taken on a Kofler micro hot-stage and are corrected. Ultraviolet absorption spectra were determined on a Cary recording spectrophotometer.

† Analytical values became worse after repeated crystallizations.

solution was washed with water, dried and partially decolorized with activated charcoal, filtered and freed from solvent *in vacuo*. The residual solids were crystallized as indicated in Table I.

Condensation of cbzo-*amino acids with the aminonucleoside (II).* To a chilled solution of the *cbzo*-amino acid (2 mmole) in dimethylformamide (6 ml) containing triethylamine (0·3 ml) was added ethyl chlorocarbonate (0·028 ml, 2·2 mmole), and the mixture was stirred for 10 min. The suspension was then added dropwise to a chilled mixture of the aminonucleoside (II) (2·2 mmole) and triethylamine (0·52 ml) in dimethylformamide (8 ml). The mixture was stirred in an ice bath for 30 min and was then stored at 7° overnight. The solvent was removed *in vacuo* and the residue was worked up by one of the following two methods.

Method A. The residue was treated with chloroform–water and the solid which formed was collected, washed with chloroform and dried *in vacuo*. For recovery of additional material, the filtrate and washings were combined, and the organic phase was separated, washed with water, dried over magnesium sulphate and evaporated *in vacuo*. The residue was triturated with ethanol to give additional product.

Method B. The residue was triturated with aqueous ethanol and the resulting solid was collected, washed with water and ethanol, and dried *in vacuo*. The products so obtained are described in Table II.

Aminoacyl aminonucleosides. Palladium–carbon catalyst (0·5 g, 10 per cent) was added to a solution of the *cbzo*-aminoacyl aminonucleoside (2 mmole) in dimethylformamide (75 ml). Hydrogen was bubbled through the mixture at 60–70° until no more carbon dioxide was evolved. The suspension was filtered through diatomaceous earth and the solvent was removed under reduced pressure. The residue was triturated with ethanol and the resulting crystalline solid was further purified by recrystallization from ethanol unless stated otherwise. The compounds so obtained are described in Table III.

p-*Methoxy*-m-*nitro*-N-*phthaloyl*-L-*phenylalanine.* A mixture of *p*-methoxy-*m*-nitro-L-phenylalanine[10] (2·4 g, 10 mmole), phthalic anhydride (1·46 g) and dimethylformamide (75 ml) was heated under reflux for 3 h. Evaporation *in vacuo* afforded an orange residue which was dissolved in chloroform (50 ml). The solution

was washed with 3 N hydrochloric acid (20 ml) and water (20 ml). The yellow solid which precipitated during the water wash was collected and was washed with water and chloroform. The dried solid weighed 1·62 g and had m.p. 210–214°. For the recovery of additional material, the chloroform phase of the combined filtrate and washings was dried over magnesium sulphate and was evaporated *in vacuo*. The residual gum was crystallized from ethanol–ether to yield an additional 0·91 g, m.p. 202–210° for a combined crude yield of 2·53 g, (68 per cent). Recrystallization from ethanol–ether gave m.p. 212–214°; $[\alpha]_D^{25} - 3 \cdot 1°$ (*c*, 1·95 in dimethylformamide).

Anal. Calcd. for $C_{18}H_{14}N_2O_7$: C, 58·38; H, 3·81; N, 7·57. Found: C, 58·30; H, 3·72; N, 7·27.

6-Dimethylamino-9-[3-deoxy-3-(p-*methoxy*-m-*nitro*-N-*phthaloyl*-L-*phenylalanylamino*)-*β*-D-*ribofuranosyl*] *purine*. A solution of phosphorus pentachloride (0·507 g) in benzene (5 ml) was added to a suspension of *p*-methoxy-*m*-nitro-*N*-phthaloyl-L-phenylalanine (0·855 g, 2·3 mmole) in anhydrous benzene (40 ml) and the mixture was allowed to reflux for 3 h. Evaporation under reduced pressure afforded a gum which was further evaporated three times with small portions of toluene. This left a yellow gummy product (0·997 g) which was dissolved in dimethylformamide (15 ml). The solution was added dropwise to a mixture of the aminonucleoside (II) (0·75 g, 2·55 mmole), triethylamine (0·75 ml) and dimethylformamide (50 ml). The mixture was stirred at room temperature for 75 min and was then evaporated *in vacuo*. The residue was triturated with water and the resulting solid was collected, washed with water and dried to afford a yellow solid (1·29 g, 87 per cent), m.p. 142–150°. Three recrystallizations from ethanol gave m.p. 152–155°.

Anal. Calcd. for $C_{30}H_{30}N_8O_9.H_2O$: C, 54·21; H, 4·85; N, 16·86; H_2O, 2·71. Found: C, 54·12; H, 4·66; N, 16·74; H_2O, 2·78.

6-Dimethylamino-9-[3-deoxy-3-(p-*methoxy*-m-*nitro*-L-*phenylalanylamino*)-*β*-D-*ribofuranosyl*] *purine*. A mixture of 6-dimethylamino-9-[3-deoxy-3-(*p*-methoxy-*m*-nitro-*N*-phthaloyl-L-phenylalanylamino)-*β*-D-ribofuranosyl] purine (0·495 g, 0·76 mmole), 100 per cent hydrazine hydrate (0·05 ml) and 2-methoxyethanol (2·4 ml) was heated on a steam bath for 7 min, evaporated under

reduced pressure and glacial acetic acid (0·48 ml) in 2-methoxyethanol (2·3 ml) was added. The mixture was heated on the steam bath for 10 min and was then again evaporated *in vacuo* to afford a residue which was dissolved in ethanol (20 ml) containing triethylamine (0·26 ml). The solution was stored at 7° for 20 h and was then filtered. The orange precipitate was washed with a little cold ethanol and dried; yield, 0·2 g (49 per cent); m.p. 125–130°. The analytical sample was recrystallized twice from water (see Table IV).

*6-Dimethylamino-9-[3-deoxy-3-(3,4-methylenedioxycinnamoylamino)-β-*D*-ribofuranosyl] purine (VII).* A solution of 3,4-methylenedioxycinnamic acid (2·0 g, 10·5 mmole) in thionyl chloride (20 ml) was allowed to reflux for 1 h and was then evaporated under reduced pressure. The residue was evaporated several times with small portions of benzene and was then dissolved in dimethylformamide (15 ml). This solution was added slowly to a chilled mixture of the aminonucleoside (II) (2·94 g, 10 mmole), triethylamine (3·5 ml) and dimethylformamide (50 ml). The internal temperature was 10–12° during the addition (6 min). The stirred mixture was kept at room temperature for 30 min, and was poured into water (270 ml). The resulting solid was collected and was washed thoroughly with water. The air-dried material was dissolved in boiling ethanol (600 ml), the solution clarified with Norit activated carbon and concentrated to a small volume. The solid which crystallized was collected, washed with a little ethanol and dried; it weighed 2·1 g (45 per cent), m.p. 242–244°. For analysis the material was recrystallized from hot 2-methoxyethanol; m.p. 244–245°; $[\alpha]_D^{25} - 89·5°$ (*c*, 1·27 in pyridine).

Anal. Calcd. for $C_{22}H_{24}N_6O_6$: C, 56·40; H, 5·16; N, 17·94. Found: C, 56·26; H, 5·48; N, 17·66.

*4-Methoxy-3-propionoxy-α-methylcinnamic acid.** A mixture of isovanillin (55 g, 0·36 mole), fused sodium propionate (55 g) and propionic anhydride (150 ml) was allowed to reflux for 5 h and was then drowned in 2·5 l. of water. The oil which separated was washed with water and then mixed with ether. The ethereal solution was washed with sodium bisulphite solution and then with

* This preparation was carried out by the late Mr. W. Klein and by Mr. R. Williams.

saturated sodium bicarbonate solution. The bicarbonate extracts were combined and filtered from a small amount of solid material. The filtrate was acidified with hydrochloric acid (Congo red) and the precipitate which formed was collected and dried in air (16 g). For purification, the solid was dissolved in bicarbonate solution and this was extracted several times with benzene. The aqueous phase was acidified and the solid was collected and recrystallized from dilute ethanol; yield, 9·1 g (9·5 per cent), m.p. 112–122°. Recrystallization from ether–hexane with Norit activated carbon gave material of m.p. 130–135°.

Anal. Calcd. for $C_{14}H_{16}O_5 \cdot \frac{1}{2}H_2O$: C, 61·52; H, 6·28. Found: C, 61·87; H, 5·98.

6-Dimethylamino-9-[3-deoxy-(4-methoxy-3-propionoxy-α-methylcinnamoylamino)-β-D-ribofuranosyl] purine (VIII). Conversion of 4-methoxy-3-propionoxy-α-methylcinnamic acid (2·77 g, 10·5 mmole) to the acid chloride was carried out as described in the preparation of (VII). The crude acid chloride in dimethylformamide (15 ml) was added to a stirred, chilled mixture of the aminonucleoside (II) (2·94 g, 10 mmole), triethylamine (3·5 ml) and dimethylformamide (50 ml). The reaction was worked up as before for (VII); however, since the product did not crystallize when the reaction mixture was poured into water, the mixture was extracted with ten 50 ml portions of chloroform and the combined extracts were washed with a little water. The dried (magnesium sulphate) chloroform solution was evaporated *in vacuo* and the residue was crystallized with ether. The solid was collected, washed with ether and dried; yield, 3·2 g (59 per cent), m.p. 193–197°. A small amount of material was recrystallized twice from ethanol; m.p. 203–204°; $[\alpha]_D^{25} - 77·8°$ (*c*, 0·67 in 2-methoxyethanol).

Anal. Calcd. for $C_{26}H_{32}N_6O_7$: C, 57·77; H, 5·97; N, 15·55. Found: C, 57·55; H, 5·96; N, 16·14.

6-Dimethylamino-9-[3-deoxy-3-(3-hydroxy-4-methoxy-α-methylcinnamoylamino)-β-D-ribofuranosyl] purine (IX). The propionyl compound (VIII) (540 mg, 1 mmole) obtained above was added to methanol (15 ml) containing sodium methoxide (108 mg). The solution was heated to boiling for a few minutes and was then mixed with water (20 ml). A small amount of solid separated. This was collected and washed with two 5 ml portions of 4 N

sodium hydroxide. The filtrate was neutralized with hydrochloric acid and extracted with several portions of ethyl acetate. The combined extracts (85 ml) were washed with water, heated to boiling and filtered from traces of undissolved material. Concentration of the filtrate to 15–20 ml and cooling resulted in the precipitation of a white solid which was collected, washed with ether and dried; it weighed 327 mg (68 per cent); m.p. 208–213°. For analysis the substance was recrystallized from methanol with activated charcoal, m.p. 203–205°; $[\alpha]_D^{25} - 103°$ (*c*, 0·766 in dimethylformamide).

Anal. Calcd. for $C_{23}H_{28}N_6O_6$: C, 57·01; H, 5·83; N, 17·35. Found: C, 57·25; H, 6·17; N, 17·01.

Summary. The preparation of certain substituted phenylalanyl and cinnamoyl derivatives of 9-(3-amino-3-deoxy-β-D-ribofuranosyl)-6-dimethylaminopurine is described. These compounds are analogues of the antibiotic puromycin. The *in vitro* antibacterial and antifungal activities and the *in vivo* antitrypanosomiasis and antitumour activities are reported.

Acknowledgement. We wish to thank Mr. L. Brancone and staff for microanalyses, Mr. W. Fulmor and staff for polarimetric and spectroscopic work, and Dr. J. Clark and staff for the large-scale preparation of certain intermediates.

(*Received 15 February, 1960*)

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