THE SYNTHESIS OF SOME POTENTIAL ANTIMETABOLITES OF PHENYLALANINE

PART I. THE SYNTHESIS OF SOME $\gamma\gamma$-DIALKYL-α-AMINOBUTYRIC ACIDS

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Received April 22, 1959

Seven new dialkylaminobutyric acids have been synthesised as potential antiviral and antibacterial agents. None showed any significant activity against the Newcastle disease virus *in vitro*.

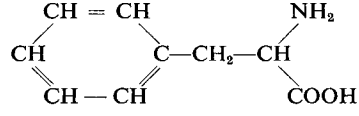
It is now a well established fact, that the natural amino acids are essential metabolites for many micro-organisms, but until recently, little work had been done with viruses. Many structural analogues of the natural amino acids have been synthesised, and a number of compounds with antibacterial activity discovered, but there is little systematic work of this nature with viruses.

The work of Dickinson and Thompson[1] indicated that phenylalanine was probably an essential metabolite for the Influenza A virus and further work by Dickinson (personal communication) suggests that this is also true for the Newcastle disease virus. This provides a possible point of attack in the search for compounds chemotherapeutically active against the smaller or so-called, true viruses. This suggestion is further supported by the evidence of antiviral acitivity in (*a*) the *ortho*, *meta* and *para* fluorophenylalanines[2], (*b*) the 2-substituted-3,4-dihydroxyphenylalanines[3], (*c*) α-amino-β-phenyl-ethane-sulphonic acid[4] and (*d*) β-(1-naphthyl) alanine[5].

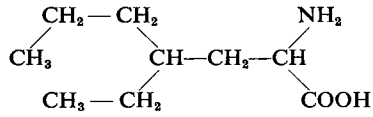
Dickinson[1] found the activity of β-phenylserine against the Influenza A virus to be reduced by modification of the amino, hydroxyl, or carboxyl groups, except in the methyl and ethyl esters, thus indicating that gross alterations of the polar parts of the molecule reduce activity. The increase in activity of the esters may be caused by an increase in the penetration of the compound into the cells owing to a decrease in the polarity of the molecule.

With these facts in mind, it seemed that the most reasonable point at which to modify the phenylalanine molecule, was the aromatic ring.

A variety of substitutions in the aromatic ring have been carried out by many workers in the past, but those compounds corresponding to an opened phenyl ring do not appear to have been reported in the literature and such compounds might fulfil the requirements of an antiviral antagonist of phenylalanine.



β-Phenylalanine



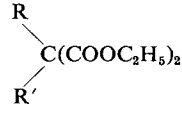
γ-Ethyl-γ-propyl-α-aminobutyric acid

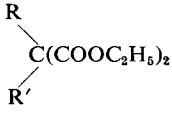
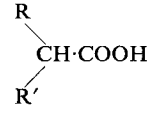
Compounds equivalent to opened 5, 6, 7, 8 and 9 membered rings have been synthesised and tested for activity against the Newcastle disease virus.

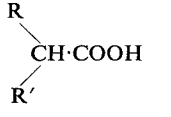
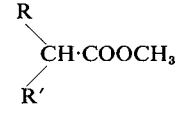
Preparation

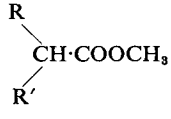
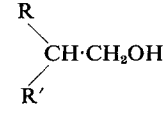
Acetamidomalonic ester and related acylamino esters have found extensive use in the synthesis of the natural[6–13] and also the synthetic amino acids containing an aromatic or heterocyclic nucleus[14–17] whereas new aliphatic amino acids have received little attention. The parent of the series, the γγ-dimethyl compound is the naturally occurring amino acid leucine and this has been prepared by the condensation of β-methyl allyl chloride with acetamidomalonic ester in 50 per cent yield[12], and its acetyl derivative from *iso*butyl bromide, the latter in 30 per cent yield[13]. The method seemed applicable to the synthesis of this series of compounds, which has now been accomplished by the condensation of the appropriate alkyl bromides with acetamidomalonic ester involving the following reaction sequence.

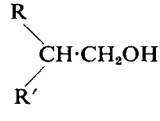
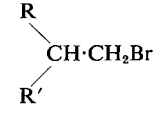
1. $RBr + CH_2(COOC_2H_5)_2 \xrightarrow{NaOC_2H_5} R{\cdot}CH(COOC_2H_5)_2$

2. $R'Br + R{\cdot}CH(COOC_2H_5)_2 \xrightarrow{NaOC_2H_5}$ 

3.  $\xrightarrow[\text{Heat}]{\text{Hydrolyse}}$ 

4.  $\xrightarrow{CH_2N_2}$ 

5.  $\xrightarrow{LiAlH_4}$ 

6.  $\xrightarrow{PBr_3}$ 

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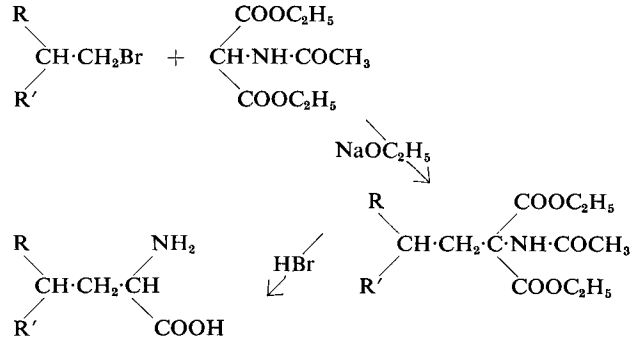
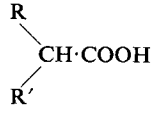


The synthesis of the αα-dialkyl acetic acids was by the standard procedure, preliminary work indicating, that there was no detriment to the yield, if the dialkyl malonic ester was not purified before hydrolysis and decarboxylation.

The methyl esters of the αα-dialkyl acetic acids were conveniently prepared by treating the acid the an excess of freshly prepared ethereal diazomethane[18].

TABLE I

αα-DIALKYL ACETIC ACIDS



R	R′	B.p. found	B.p. reported	n_D^{25}	Yield per cent	M.W. found	M.W. required	Reference
CH_3	C_3H_7	192–3°	192–3°	1·4110	66	117·2	116	24
CH_3	C_4H_9	209–10°	209°	1·4180	64	126·5	130	25
C_2H_5	C_3H_7	208–10°	210°	1·4158	68	129·0	130	26
C_3H_7	C_3H_7	221–2°	219–22°	1·4205	50	143·9	144	27
C_3H_7	C_4H_9	238–40°	238–40°	1·4210	66	158·8	158	28
C_4H_9	C_4H_9	252–55°	255°	1·4213	70	173·5	172	29, 30

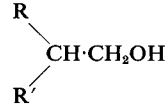
The primary alcohols were obtained by the reduction of the appropriately substituted acetic acid, or its methyl ester with lithium aluminium hydride. Brown[19], in his review of the applications of lithium aluminium hydride, states that the reduction of the carboxylic acid generally give a much lower yield of alcohol, than the reduction of the ester, but Sarel and Newman[20] have shown that with some acids, the reverse is true. Our work with αα-dipropyl acetic acid and α-ethyl-α-propyl acetic acid has shown that, in these instances, the yields by the two routes are comparable, being 82 and 84 per cent from the ester and 94 and 81 per cent from the acid respectively. Consequently, the overall yield from the acid is much greater if the ester stage is omitted, the yields from αα-dipropyl acetic acid via the methyl ester being 66 per cent and similarly from α-ethyl-α-propyl acetic acid, 59 per cent. Therefore, in the synthesis of the other alcohols, the substituted acetic acid methyl ester was not prepared.

Another synthesis of these $\beta\beta$-dialkyl ethanols according to Gilmore and Catlin[21], involving the action of gaseous formaldehyde on the Grignard reagent formed from the appropriate secondary bromide was attempted. It was found that the Grignard compound formed only with great difficulty, and the yield of alcohol obtained was very low.

The action of phosphorous tribromide on the appropriate alcohols under anhydrous conditions as described by Noller and Dinsmore[22] to form the required $\beta\beta$-dialkyl-α-bromo-ethanes was found to be the most convenient for their synthesis. The modification of Tseng, Hsu and Hu[23] whereby the reaction mixture is heated on a steam bath for one to two hours after the completion of addition produced a slight increase in the yield of bromide.

TABLE II

$\beta\beta$-DIALKYL ETHANOLS



R	R′	Compd. reduced by $LiAlH_4$	Yield per cent	B.p. found	B.p. reported	n_D^{25}	Reference
CH_3	C_3H_7	Acid	82	148°	148°	1·4153	31
CH_3	C_4H_9	Acid	90	163–4°	162–4°	1·4205	32
C_2H_5	C_3H_7	Acid	81	165–7°	164–6°	1·4222	33
		Methyl ester	84				
C_3H_7	C_3H_7	Acid	94	92° at 23 mm.	179°	1·4272	34, 35
		Methyl ester	81	177–8°			
*C_3H_7	C_4H_9	Acid	92	103–4° at 22 mm.	—	1·4295	—
C_4H_9	C_4H_9	Acid	98	140° at 54 mm. 219–220°	218–19°	1·4285	36

* β-Propylhexanol has not been reported in the literature to date. Analysis gives C, 74·3 per cent and H, 13·8 per cent, $C_9H_{20}O$ requires C, 74·9 per cent and H, 13·9 per cent.

Five of the seven bromides do not appear to have been reported in the literature (see Table III). The substituted acetamidomalonic esters were prepared in the manner described by Snyder and others[7,10,13], refluxing being continued until the reaction mixture was neutral to moist litmus. Often only a small amount of sodium bromide was precipitated from the reaction mixture, and the compounds themselves could not be obtained crystalline. In each instance, a viscid brown oil was obtained which did not solidify, even on prolonged vacuum desiccation over phosphorus pentoxide, nor could it be recrystallised from a variety of solvents.

The crude α-alkyl-α-acetamidodiethyl malonates were hydrolysed by refluxing with 48 per cent hydrobromic acid for 24 hours and the resulting amino acids obtained by precipitation at their isoelectric points (pH 5–6) with ammonia.

Biological Results

The seven amino acids were tested against the Newcastle disease virus in tissue culture, using monolayers of chick embryo. The results showed

that none of the compounds possessed any significant activity at concentrations of 0·3 and 1·4 mg. per ml.

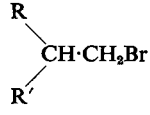
No severely toxic effects were exhibited towards the host cells at the higher concentration.

EXPERIMENTAL

The details of individual compounds are given in Tables I, II, III and IV. Typical syntheses are described.

TABLE III

ββ-DIALKYL-α-BROMO-ETHANES



R	R′	Yield per cent	B.p. found	B.p. reported	n_D^{25}	Analysis found C	Analysis found H	Analysis required C	Analysis required H	Reference
CH_3	C_3H_7	63	141–2°	142°	1·4475	—	—	—	—	37, 38, 39
CH_3	C_4H_9	50	75–6° at 8 mm.	—	1·4461	46·7	8·5	46·9	8·4	—
C_2H_5	C_3H_7	57	63–4° at 25 mm.	—	1·4421	46·6	8·1	46·9	8·4	—
C_2H_5	C_4H_9	73	75–7° at 16 mm.	80° at 18 mm.	1·4465	—	—	—	—	40, 41
C_3H_7	C_3H_7	65	80–2° at 18 mm.	—	1·4461	50·0	9·1	49·8	8·8	—
C_3H_7	C_4H_9	45	83° at 19 mm.	—	1·4515	52·8	9·3	52·2	9·2	—
C_4H_9	C_4H_9	51	130–2° at 60 mm.	—	1·4490	53·9	9·3	54·3	9·5	—

n-*Propylmalonic acid diethyl ester.* Sodium (46 g., 2·0 mol.) in dry ethanol (1·5 l.) was treated with malonic ester (320 g., 2·0 mol.) the mixture heated to reflux and *n*-propyl bromide (246 g., 2·0 mol.) added slowly over one hour. Refluxing was continued for six hours, until the reaction mixture was neutral to moist litmus, and the majority of the solvent then removed by distillation. The residue was cooled, diluted with water (200 ml.) the upper layer separated, and the aqueous layer extracted with ether (50 ml.). The organic liquors were dried over anhydrous sodium sulphate, the ether removed and the residue distilled to yield *n*-propyl malonic acid diethyl ester (370 g. 91 per cent) b.p. 117–118° at 22 mm.

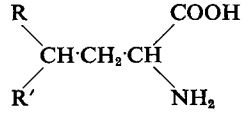
α-*Ethyl*-α-n-*propylacetic acid.* Sodium (23 g., 1·0 mol.) in dry ethanol (1·0 l.) was treated as above with *n*-propylmalonic acid, diethyl ester (202 g., 1·0 mol.) followed by ethyl bromide (109 g., 1·0 mol.), and the mixture refluxed for 19 hours until neutral. The reaction mixture was worked up to yield the crude ester, which was hydrolysed by dropping slowly into a stirred, refluxing solution of potassium hydroxide (140 g., 2·5 mol.) in ethanol (1·0 l.). After refluxing overnight, the solvent was removed over a water bath, the soapy residue dissolved in the minimum

quantity of water, cooled in ice and acidified with concentrated hydrochloric acid. The upper layer was separated and the aqueous residue extracted successively with ether (50 ml. portions) until no residue remained on evaporating a portion on a steam bath. The combined organic liquors were evaporated on a steam bath and the sticky brown residue was heated on a metal bath at 180° for 6 hours. The organic layer was separated from the small amount of water which had formed, dried over anhydrous sodium sulphate and fractionated to yield α-ethyl-α-*n*-propylacetic acid (94·5 g., 70 per cent) b.p. 208–210°.

*α-Ethyl-α-*n*-propylacetic acid methyl ester.* α-Ethyl-α-*n*-propylacetic acid (23·6 g.) was treated with an excess of an ethereal solution of diazomethane, from *p*-tolylsulphonyl-methyl-nitrosoamide, and the mixture stood overnight. The ether was removed and the residue shaken with 10 per cent aqueous sodium hydroxide (25 ml.) and twice with water (25 ml.). After drying over anhydrous sodium sulphate the residue was distilled to yield α-ethyl-α-*n*-propyl-acetic acid methyl ester (18·3 g., 71 per cent) b.p. 157°.

TABLE IV

γγ-DIALKYL-α-AMINOBUTYRIC ACIDS



R	R′	Reflux* time hours	Yield† per cent	M.p.‡	Analyses found C	found H	found N	required C	required H	required N
CH_3	C_3H_7	48	40	211–2°	60·2	10·7	8·6	60·3	10·7	8·8
CH_3	C_4H_9	48	50	226°	62·6	11·1	8·4	62·4	10·9	8·1
C_2H_5	C_3H_7	48	49	220–3°	61·5	10·9	8·1	62·4	10·9	8·1
C_2H_5	C_4H_9	60	48	227–8°	64·2	11·4	7·7	64·2	11·3	7·5
C_3H_7	C_3H_7	72	33	221–2°	64·5	11·1	7·3	64·2	11·3	7·5
C_3H_7	C_4H_9	72	46	229–31°	65·6	11·4	7·0	65·7	11·5	7·0
C_4H_9	C_4H_9	72	40	229–30°	66·7	11·6	6·4	66·7	11·6	6·5

* The reflux time is for the condensation between acetamidodiethyl malonate and the appropriate alkyl bromide.
† The yield is calculated from the appropriate alkyl bromide
‡ Melting points all occurred with decomposition.

β-Ethylpentanol. Method A. Lithium aluminium hydride (4·0 g., 0·1 mol.) was stirred into a slurry with ether (200 ml.), previously dried over lithium aluminium hydride, and α-ethyl-α-*n*-propyl-acetic acid methyl ester (18·3 g., 0·125 mol.), in an equal volume of dry ether, was added at a rate which just maintained reflux. After the completion of addition, the mixture was refluxed for a further 30 minutes, and the complex decomposed by the addition of wet ether, and then iced dilute hydrochloric acid. The ethereal layer was separated, washed with 10 per cent aqueous sodium bicarbonate (20 ml.), water (20 ml.) and dried over anhydrous sodium sulphate. After removal of the ether, the residue was distilled to yield β-ethylpentanol (12·5 g., 83 per cent), b.p. 165–167°.

β-Ethylpentanol. Method B. Lithium aluminium hydride (20 g., 0·5 mol.) in dry ether (500 ml.) was treated as above with α-ethyl-α-*n*-propylacetic acid (65 g., 0·5 mol.). Isolation of the product yielded β-ethylpentanol (47·1 g., 81 per cent), b.p. 165–167°.

α-Bromo-β-ethylpentane. β-Ethylpentanol (34·8 g., 0·3 mol.) was cooled to −10° and phosphorous tribromide (32·5 g., 0·13 mol.) was slowly added maintaining the temperature below 0°. After the completion of addition, the reaction mixture was stirred overnight and then heated on a steam bath for 2 hours. The mixture was poured into iced water (250 ml.), the lower layer separated and shaken with concentrated sulphuric acid (d. 1·84, 25 ml.). The organic layer was then washed successively with 25 ml. portions of water, aqueous 10 per cent sodium carbonate, and water, dried over anhydrous sodium sulphate, and distilled to yield *α-bromo-β-ethylpentane* (35 g., 65 per cent), b.p. 63–64° at 25 mm.

α-Acetamido-α-(β-ethylpentyl)malonic acid diethyl ester. Sodium (2·3 g., 0·1 mol.) in dry ethanol (250 ml.) was treated with α-acetamidomalonic acid diethyl ester (21·7 g., 0·1 mol.) the mixture heated to reflux, and α-bromo-β-ethylpentane (17·9 g., 0·1 mol.) was added over 30 minutes. The mixture was refluxed for 48 hours, until neutral to moist litmus, and the bulk of the ethanol removed over a steam bath. The residue was cooled, diluted with iced water (250 ml.) the upper layer separated and the aqueous residue extracted with ether (50 ml.). The combined organic liquors were dried over anhydrous sodium sulphate and the ether removed to yield a viscid brown oil (20·7 g.), which did not solidify on prolonged vacuum desiccation. All attempts to recrystallise the oil proved unsuccessful. The yield of oil was equivalent to 65 per cent of *α-acetamido-α-(β-ethylpentyl)malonic acid diethyl ester.*

γ-Ethyl-γ-propyl-α-aminobutyric acid. The whole of the crude α-acetamido-α-(β-ethylpentyl)malonic acid diethyl ester was refluxed with 48 per cent of hydrobromic acid (100 ml.) for 48 hours. After cooling, the resultant brown crystals were filtered off, and the residue evaporated under reduced pressure. Water was added and the mixture re-evaporated. This was repeated until the distillate was only faintly acidic.

The final concentrate and the crystals were dissolved in water (200 ml.) treated with decolourising charcoal, filtered and the volume reduced to half. The resulting colourless solution was adjusted to pH 5–6 with dilute ammonia, the bulky precipitate filtered off and recrystallised twice from water to yield *γ-ethyl-γ-propyl-α-aminobutyric acid* (8·5 g., 59 per cent) as colourless plates, m.p. 220–222° with decomposition.

Acknowledgements. The authors are indebted to the Pharmaceutical Society of Great Britain for the award of the Wellcome Research Scholarship (to B.J.M.), and to Dr. L. Dickinson, Mr. P. Oxley and the Boots Pure Drug Co., for biological evaluation and chemical analyses. We would also like to thank Professor W. H. Linnell for encouragement.

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