551. *Synthesis of Potential Antibacterial Agents. Part I. Some α-Alkylglutaric Acids and Their Derivatives.*

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A number of α-*n*-alkylglutaric acids and their anhydrides have been prepared. By treatment of the anhydrides with sulphanilamide and 2-aminothiazole, substituted glutaramic acids have been obtained. Some of these compounds have been tested for their tuberculostatic activity *in vitro*.

FOR reasons previously stated (Roberts, *Nature*, 1945, **155**, 697) it was thought that agaric acid—obtainable from the Basidiomycete, *Polyporus officinalis*, Vill.—might possess antibacterial activity towards *Mycobacterium tuberculosis*. Although the structure of agaric acid (α-*n*-hexadecylcitric acid) was established many years ago by degradative methods (Thoms and Vogelsang, *Annalen*, 1907, **357**, 145), no really satisfactory synthesis has yet been devised (cf., however, Passerini and Banti, *Chem. Zentr.*, 1931, I, 1432); so we decided to synthesise and investigate a number of compounds related in structure to agaric acid. This decision was strengthened by a consideration of the observations of Franke and Schillinger (*Biochem. Z.*, 1944, **316**, 313) who showed, during an investigation of a wide variety of organic compounds, that aliphatic acids can exert a marked influence on the metabolism of acid-fast bacteria, the nature of the influence depending *inter alia* on the length of the carbon chain. In this paper we describe the preparation and investigation of a number of α-*n*-alkylglutaric acids of varying chain-lengths and also of a number of derivatives containing, in addition to an acidic group, one of two potentially antibacterial groupings, 2-thiazolylcarbamyl or *p*-sulphamylphenylcarbamyl.

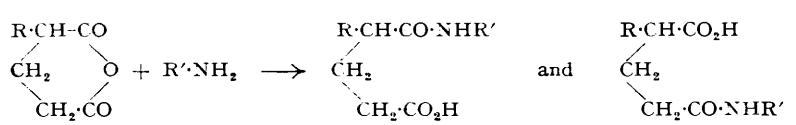
Very few α-alkylglutaric acids have been described. The α-methyl-, α-ethyl-, and α-propyl-acids have been prepared (Auwers and Titherley, *Annalen*, 1896, **292**, 209 *et seq.*; Mellor, *J.*, 1901, **79**, 128) by simultaneous hydrolysis and decarboxylation of the appropriate triethyl alkane-1 : 1 : 3-tricarboxylate by heating it under reflux with strong mineral acid. We found this method to be inapplicable to higher members of the series. α-*n*-Octylglutaric acid has been made by Kögl, Erxleben, *et al.* (*Z. physiol. Chem.*, 1935, **235**, 198) using the acetoacetic ester route, which, however, suffers from the disadvantage that ketones are concomitantly produced. We found the following method to be the most advantageous and to be applicable to the synthesis of an α-alkylglutaric acid of any chain-length. The required alkane-1 : 1 : 3-tricarboxylate—readily prepared from an alkylmalonic ester and β-iodopropionic ester—was hydroysed with an alcoholic solution of potassium hydroxide, and the tricarboxylic acid isolated and then decarboxylated at 180—190°. The product was then heated with aqueous alkali (to hydrolyse some anhydride which was always formed) before the dicarboxylic acid was isolated.

The cyclic anhydrides could not be prepared by heating the α-alkylglutaric acids. Although some water was eliminated by this treatment, analysis showed that the products were not the desired anhydrides but presumably linear anhydrides of possible type

$$R{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}O{\cdot}CO{\cdot}CH_2{\cdot}CH_2{\cdot}CHR{\cdot}CO{\cdot}O-$$

etc. However, the cyclic anhydrides were readily obtained by treatment of the alkylglutaric acids with excess of acetic anhydride.

By treatment of these anhydrides in an organic solvent with primary amines, mixtures of *N*-substituted glutaramic acids were obtained :



(R = *n*-alkyl; R′ = 2-thiazolyl- or *p*-sulphamylphenyl-.)

Separation of the isomers by fractional crystallisation proved very difficult and in some cases only one isomer was isolatable. Unfortunately, it is not possible to allocate unambiguous structures to the compounds obtained. In the case of phenylsuccinic anhydride Anschütz *et al.* (*Annalen*, 1907, **354**, 117) were able to derive a generalisation whereby the structure of the derivatives could be predicted with some certainty, for the amino- or substituted-amino-residue attached itself to the carbonyl group corresponding to the " weaker " carboxyl group in the parent dicarboxylic acid, *i.e.*, to the carboxyl group further removed from the phenyl substituent. We considered this generalisation to be inapplicable to our compounds, first because it was not possible to determine which of the two acid groupings was the weaker, and secondly because in most cases two isomers were concomitantly produced. This problem has remained unsolved.

None of the compounds tested showed notable tuberculostatic activity. We wish to thank Dr. W. C. Tobie (private communication) for informing us that agaric acid, even in a concentration of 1 in 1560, failed to inhibit the growth *in vitro* of a human virulent strain of *M. tuberculosis*. We are greatly indebted to Messrs. Boots Pure Drug Co. for determining the tuberculostatic activities (see below) of some of the synthetic compounds. It is noteworthy that, in the one instance where the two isomers of an *N*-substituted glutaramic acid were tested, no difference in tuberculostatic activity was observed.

Experimental.

[The figures given for tuberculostatic activity represent dilutions, in thousands, at which complete inhibition of the growth of *M. tuberculosis* (human virulent strain) was maintained for 4 weeks in modified Long's medium, the floating pellicle method being used. Figures in parentheses represent dilutions at which partial inhibition occurred. All the tests were carried out in presence of serum.]

Starting Materials.—All the required *n*-alkylmalonic esters were prepared by the usual procedure from ethyl malonate and the appropriate *n*-alkyl halides. The latter, except tetradecyl iodide, were obtained commercially (or prepared from the appropriate alcohols) and, after drying, were fractionally distilled to give compounds with a maximum distillation range of 3°.

n-*Tetradecyl Iodide.*—Ethyl myristate (prepared from " technical " myristic acid) was distilled *in vacuo*, an electrically heated fractionating column being used, and the fraction of b. p. 164—166°/10 mm. was collected (Phillips and Mumford, *J.*, 1932, 902, give b. p. 139°/4 mm.). *n*-Tetradecyl alcohol, obtained by reduction of the ester (Bouveault–Blanc method), was converted into *n*-tetradecyl iodide by treatment with red phosphorus and iodine.

Ethyl β-Iodopropionate.—Ethyl β-chloropropionate, prepared from ethyl acrylate and dry hydrogen chloride (Moureu, Murat, and Tampier, *Ann. Chim.*, 1921, **15**, 239), was converted into ethyl β-iodopropionate by treatment with dry sodium iodide in dry acetone (King and L'Ecuyer, *J.*, 1934, 1903).

General Procedure for the Preparation of 1 : 3 : 3-*Tricarbethoxyalkanes.*—Finely divided sodium (1 atom) was covered with a sufficient quantity of dry ether to act as medium for the reaction. The ethyl *n*-alkylmalonate (1 mol.), dissolved in a little dry ether, was gradually added and the mixture heated under reflux until all the sodium had disappeared. After the mixture had been cooled, ethyl β-iodopropionate (1·02 mols.) was slowly added. A vigorous reaction ensued and sodium iodide separated. The reaction was completed by boiling under reflux until the reaction mixture became neutral to moist litmus-paper. To the cooled mixture, water was added (to dissolve the sodium iodide), and the ethereal layer separated. The aqueous layer was extracted with ether. The ethereal solutions were combined, washed once with water, dried (Na_2SO_4), and the ether evaporated. The residue was distilled *in vacuo* and, after removal of any remaining starting material, the required 1 : 3 : 3-*tricarbethoxyalkane* was collected as a high-boiling fraction. The compounds so obtained (see table) were colourless, viscous oils (or solids of low m. p.) with a faintly rancid odour.

1 : 3 : 3-*Tricarbethoxyalkanes*, $CH_3 \cdot [CH_2]_n \cdot C(CO_2Et)_2 \cdot CH_2 \cdot CH_2 \cdot CO_2Et$.

				Found, %.		Reqd., %.	
n.	Yield, %.	B. p./mm.	Formula.	C.	H.	C.	H.
1	50	170—174°/12 [a]	—	—	—	—	—
2	39	160—166/7 [b]	—	—	—	—	—
3	64	176—180/9	$C_{16}H_{28}O_6$	60·2	9·1	60·7	8·9
4	62	148—153/2	$C_{17}H_{30}O_6$	61·5	9·0	61·8	9·2
5	67	164—167/2	$C_{18}H_{32}O_6$	62·7	9·1	62·8	9·4
6	67	166—169/1·5	$C_{19}H_{34}O_6$	63·7	9·3	63·7	9·6
7	67	180—184/1·5	$C_{20}H_{36}O_6$	64·5	9·7	64·5	9·7
9	49	193—198/1	$C_{22}H_{40}O_6$	65·6	10·2	66·0	10·1
11	50	210—215/1·5	$C_{24}H_{44}O_6$	66·9	9·9	67·3	10·4
13	78	226—230/2 (m. p. 30°)	$C_{26}H_{48}O_6$	68·5	10·6	68·4	10·6
15	54	249—253/1·8 (m. p. 35·5°)	$C_{28}H_{52}O_6$	69·1	11·0	69·4	10·8

[a] Auwers and Titherley, *Annalen*, 1896, **292**, 213, give b. p. 180°/25 mm.
[b] Mellor, *J.*, 1901, **79**, 129, gives b. p. 180—185°/32 mm.

α-n-*Alkylglutaric Acids.*—The α-ethyl- and α-*n*-propyl-glutaric acids were prepared from the corresponding tricarbethoxyalkanes by heating the latter under reflux with concentrated hydrochloric

acid. The remaining acids were prepared by the following technique. The tricarbethoxyalkane (1 mol.) and a 25% solution of potassium hydroxide (3·3 mols.) in ethanol (95%) were boiled under reflux for 3—5 hours. The alcohol was distilled off, the residue dissolved in water, and the solution acidified. The oil which separated was collected by four extractions with ether. The combined extracts were washed with water, dried ($MgSO_4$), and filtered. Evaporation of the ether yielded the tricarboxylic acid (as a light brown gum), which was decarboxylated by heating it at 190° until evolution of carbon dioxide ceased. The crude dicarboxylic acid (1 mol.)—containing some anhydride as impurity—was boiled under reflux with a 20% aqueous solution of potassium hydroxide (*ca.* 2·5 mols.) until a clear solution was obtained (3—4 hours). The solution was cooled, strongly acidified with concentrated hydrochloric acid, and extracted three times with ether. The combined ethereal extracts were washed once with water, and dried ($MgSO_4$), and the ether evaporated *in vacuo*. The residual gums crystallised after treatment by usual methods and the α-*n*-alkylglutaric acids were recrystallised to constant m. p.s from suitable solvents. The *acids* (see table) so obtained are colourless, crystalline solids of low m. p. and of sparing solubility in water. The solubility in water decreases with increasing chain-length (100 g. of water at 20° dissolve 81 mg. of α-*n*-hexadecylglutaric acid).

α-n-*Alkylglutaric acids*, $CO_2H{\cdot}CH_2{\cdot}CH_2{\cdot}CHR{\cdot}CO_2H$.

R.	Solvent for recrystn.*	M. p.	Yield, %.[a]	Formula.	Found. C, %.	Found. H, %.	Found. Equiv.[b]	Required. C, %.	Required. H, %.	Required. Equiv.	Tuberculostatic activity.
Et[c]	A	60·5°	66	—	—	—	—	—	—	—	—
Pr[d]	A	70	72	—	—	—	—	—	—	—	—
Bu	B	41	46	$C_9H_{16}O_4$	57·8	8·3	94·0	57·4	8·6	94·1	0
Am[e]	—	27—28	44	$C_{10}H_{18}O_4$	59·5	9·3	101·8	59·4	9·0	101·1	—
C_6H_{13}	B	37·5	40	$C_{11}H_{20}O_4$	61·4	9·4	109·4	61·1	9·3	108·1	—
C_7H_{15}	C	47	53	$C_{12}H_{22}O_4$	62·3	9·5	116·3	62·6	9·6	115·2	0
C_8H_{17}[f]	C	50·5	45	$C_{13}H_{24}O_4$	63·3	9·8	121·6	63·9	9·9	122·2	0 (1)
$C_{10}H_{21}$	C	57·5	45	$C_{15}H_{28}O_4$	66·0	10·4	—	66·2	10·4	—	—
$C_{12}H_{25}$	B	71·5	44	$C_{17}H_{32}O_4$	67·9	10·7	149·2	68·0	10·7	150·2	—
$C_{14}H_{29}$	D	77·5	63	$C_{19}H_{36}O_4$	69·2	10·9	—	69·5	11·1	—	—
$C_{16}H_{33}$	D	78	45	$C_{21}H_{40}O_4$	70·3	10·9	177·2	70·7	11·3	178·3	—

[a] Calc. on the tricarbethoxyalkane. [b] By the silver salt method. [c] Auwers and Titherley, *loc. cit.*, give m. p. 60·5°. [d] Mellor, *loc. cit.*, gives m. p. 66—68°. [e] Crystallisation was achieved by cooling in alcohol–solid carbon dioxide. [f] Kögl, Erxleben, *et al.*, *loc. cit.*, give m. p. 48°.

* A = Water; B = benzene; C = light petroleum (b. p. 40—60°); D = light petroleum (b. p. 60—80°).

α-n-*Alkylglutaric Anhydrides*.—All attempts to prepare cyclic anhydrides by distillation *in vacuo* of α-*n*-hexyl-, -decyl-, -dodecyl-, -tetradecyl-, or -hexadecyl-glutaric acid were unsuccessful. For example, when α-*n*-tetradecylglutaric acid (4·5 g.) was distilled *in vacuo*, a fraction (3·5 g. of colourless oil) of b. p. 259—262°/15 mm. was obtained which, when cooled, deposited colourless crystals of m. p. 46·5° (Found: C, 71·2; H, 11·1. Calc. for $C_{19}H_{34}O_3$: C, 73·5; H, 11·0. Calc. for the parent acid, $C_{19}H_{36}O_4$: C, 69·5; H, 11·1%). Repeated distillation gave similar results. Fractional crystallisation of the product from light petroleum (b. p. 40—60°) yielded no pure compound.

The following procedure gave pure cyclic anhydrides in good yield. The α-*n*-alkylglutaric acid was heated under reflux for 45 minutes with approximately five times its weight of acetic anhydride. The acetic acid and excess of acetic anhydride were removed at 65° *in vacuo*. In the preparation of α-*n*-butyl- and α-*n*-hexyl-glutaric anhydrides the residues were distilled *in vacuo*; in other cases (see table) the residues, when chilled, deposited crystals of the anhydrides which were recrystallised from light petroleum (b. p. 40—60°). The α-n-*alkylglutaric anhydrides* are colourless viscous oils or colourless solids which separate from light petroleum as pearly lustrous plates.

α-n-*Alkylglutaric anhydrides*, $CH_2\langle{}^{CHR{\cdot}CO}_{CH_2-CO}\rangle O$.

R.	M. p. (or b. p.).	Yield, %.	Formula.	Found, %. C.	Found, %. H.	Req., %. C.	Req., %. H.
C_4H_9	(170—172°/12 mm.)	76	$C_9H_{14}O_3$	62·5	8·1	62·1	8·3
C_6H_{13}	(195—197°/14 mm.)	80	$C_{11}H_{18}O_3$	66·1	9·1	66·7	9·1
C_7H_{15}	36°	67	$C_{12}H_{20}O_3$	67·8	9·3	67·9	9·5
C_8H_{17}	42·5°	49	$C_{13}H_{22}O_3$	69·0	9·6	69·0	9·8
$C_{10}H_{21}$	51°	57	$C_{15}H_{26}O_3$	70·5	10·0	70·8	10·3
$C_{12}H_{25}$	58·5°	70	$C_{17}H_{30}O_3$	71·8	10·7	72·3	10·7
$C_{14}H_{29}$	62·5°	90	$C_{19}H_{34}O_3$	73·7	10·9	73·5	11·0
$C_{16}H_{33}$	69°	85	$C_{21}H_{38}O_3$	74·2	11·1	74·5	11·3

3(*or* 1)-(p-*Sulphamylphenylcarbamyl*)-n-*alkane*-1(*or* 3)-*carboxylic Acids*.—The *n*-alkylglutaric anhydride (1 mol.) and sulphanilamide (1·05 mols.) were dissolved in about twenty times their combined weight of dry acetone and the solvent was expelled by warming on the steam-bath. The residual syrup, when cooled, yielded a light brown solid which was washed with water, with 2N-hydrochloric acid, and finally with water again until the washings were free from chloride ion. The crude *acids* were dried and recrystallised from acetone (see table). By a similar procedure, attempts were made to prepare higher members of this series. Crystalline products were obtained which had correct analyses but melted over a range of about 40°. These products were intractable mixtures of the two possible isomers.

3(*or* 1)-(p-*Sulphamylphenylcarbamyl*)-n-*alkane*-1(*or* 3)-*carboxylic acids*, $NH_2 \cdot SO_2 \cdot C_6H_4 \cdot NH \cdot CO \cdot CHR \cdot CH_2 \cdot CH_2 \cdot CO_2H$ *or* $NH_2 \cdot SO_2 \cdot C_6H_4 \cdot NH \cdot CO \cdot CH_2 \cdot CH_2 \cdot CHR \cdot CO_2H$.

R.	M. p.	Yield, %.	Formula.	Found, %. N.	Found, %. S.	Reqd., %. N.	Reqd., %. S.	Tuberculostatic activity.
C_8H_{17}	176—178°	30	$C_{19}H_{30}O_5N_2S$	7·1	—	7·0	—	0 (5)
$C_{10}H_{21}$	169—175	68	$C_{21}H_{34}O_5N_2S$	6·8	7·7	6·6	7·5	—
$C_{12}H_{25}$	171—174	64	$C_{23}H_{38}O_5N_2S$	6·3	7·3	6·2	7·1	1 or 0 (5)

3(*or* 1)-(2-*Thiazolylcarbamyl*)-n-*alkane*-1(*or* 3)-*carboxylic Acids*.—Equimolecular quantities of the alkylglutaric anhydride and of 2-aminothiazole were dissolved in dry, alcohol-free chloroform, and the solvent was expelled by warming the solution on the steam-bath. An ethereal solution of the cooled, oily residue was twice extracted with 2N-sodium hydroxide, and the ethereal layer was discarded. To the combined alkali extracts was added excess of 2N-hydrochloric acid. The *N*-substituted amic acid was collected in ether (3 extractions); the ethereal solution was washed with water and dried ($MgSO_4$). Evaporation of the ether *in vacuo* yielded a crystalline solid which was recrystallised to constant m. p. In the cases of the derivatives of *n*-heptadecane and *n*-nonadecane the residues obtained by evaporation of the chloroform yielded, on cooling, a light brown gum and a light brown solid, respectively. These residues proved to be mixtures of isomers from which, in both cases, the isomers of lower m. p. were extracted with ether. Data relating to the *acids* are in the following table.

3(*or* 1)-(2-*Thiazolylcarbamyl*)-n-*alkane*-1(*or* 3)-*carboxylic acids*, $(C_3H_2NS)NH \cdot CO \cdot CHR \cdot CH_2 \cdot CH_2 \cdot CO_2H$ or $(C_3H_2NS)NH \cdot CO \cdot CH_2 \cdot CH_2 \cdot CHR \cdot CO_2H$.

R.	Solvent for recrystn.	M. p.	Yield, %.	Formula.	Found, %. N.	Found, %. S.	Reqd., %. N.	Reqd., %. S.	Tuberculostatic activity.
C_4H_9	EtOH [a]	156—157°	14	$C_{12}H_{18}O_3N_2S$	10·4	—	10·4	—	0 (1)
C_6H_{13}	(i) EtOH; (ii) aq. EtOH	154—156	10	$C_{14}H_{22}O_3N_2S$	9·6	—	9·4	—	—
C_7H_{15}	EtOH	158—161	19	$C_{15}H_{24}O_3N_2S$	9·1	—	9·0	—	1 (5)
C_7H_{15} [b]	EtOH	108—111	4·6	$C_{15}H_{24}O_3N_2S$	9·0	—	9·0	—	—
$C_{14}H_{29}$	COMeEt	157—159	13·5	$C_{22}H_{38}O_3N_2S$	6·6	7·7	6·8	7·8	1 (5)
$C_{14}H_{29}$	aq. EtOH	98—100	13·5	$C_{22}H_{38}O_3N_2S$	7·0	—	6·8	—	1 (5)
$C_{16}H_{33}$	$CHCl_3$	154—157	19	$C_{24}H_{42}O_3N_2S$	6·4	—	6·4	—	—
$C_{16}H_{33}$	EtOH	88—89	7·7	$C_{24}H_{42}O_3N_2S$	6·1	—	6·4	—	—

[a] In this table "EtOH" means white industrial methylated spirit.
[b] Isomer obtained as a second fraction in the second recrystallisation.

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