THE PREPARATION AND RESOLUTION OF ACRIDYL(5)-N.ALANINE ETHYL ESTER

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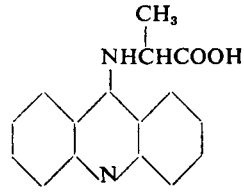
IT HAS BEEN REPORTED that D amino-acids are present in the molecules of various antibiotic polypeptides such as gramicidin[1,2], tyrocidin[3], gramicidin S[4], aerosporin[5] and bacitracin[6]. The penicillins on degradation give D-penicillamine (β:β-dimethylcysteine) and du Vigneaud and his collaborators[7] have shown that an antibiotic penicillin may be synthesised from D-penicillamine, whereas the isomer from L-penicillamine is biologically inactive.

Several workers have prepared substances containing D amino-acids for study as antibacterial agents. These substances either represented possible fragments of the antibiotic molecules, or were of closely related structure. Harris and Work[8] prepared two open chain pentapeptides containing the five amino-acids of gramicidin S in the sequence suggested for the amino-acids in the antibiotic molecule[9]. In one of the pentapeptides the phenylalanine possessed the L configuration; in the other it had the D configuration as in the antibiotic. No significant difference in antibacterial action was found between the two pentapeptides. Fruton[10] synthesised the diketopiperazine from D-leucyl-L-tryptophane because gramicidin had an unusually high content of these two amino-acids; the compound possessed no antibacterial activity. D-leucine and D-valine have been isolated from gramicidin hydrolysates and Fling, Minard and Fox[11] synthesised the prolyl derivatives of both D- and L-valine and D- and L-leucine. Proline was chosen as the second component of the dipeptides because gramicidin contained no free amino groups. No antipodal specificity was observed in the growth inhibitory actions of the dipeptides and a similar result was observed for the corresponding phthalyl derivatives. The four diastereoisomeric leucylleucines were prepared by Fox, Kobayashi, Melvin and Minard[12], who considered D-leucyl-D-leucine of especial interest because of its relationship to D-valyl-D-valine, which had been isolated from partial hydrolysates of gramicidin. No appreciable activity was found in any of the dipeptides. Linnell and Smith[13] synthesised DL-Nβ-hydroxyethylalanine which combined the essential structural features of both alanine, the simplest amino-acid exhibiting optical isomerism, and also ethanolamine which had been isolated from gramicidin hydrolysates. The racemic compound possessed no growth inhibitory properties.

These results indicate that growth inhibitory properties are not concomitant with the presence of D amino-acids in a molecule. The available evidence supports the opinion of Work[14] that antibiotics containing D amino-acids are active, not because they have this character in common, but rather in virtue of their individual structures, of which the D amino-

acid must be regarded as an integral structural part. The toxicity of these antibiotics may be due to the possession of cyclic structures, such as have been suggested for gramicidin S and tyrocidin[14], or to some hitherto undiscovered features of the molecules. The mere presence of a D amino-acid in a molecule does not seem to be sufficient for the production of antibacterial properties in that molecule and some other structural feature or features appear to be necessary. An illustration of this consideration is the contrast between the thiazolidine-β-lactam system of the penicillins and the structure of D-penicillamine which has no antibacterial properties.

It therefore appeared of interest to prepare the optical isomers of a substance which combined the structures of an amino-acid and an established antibacterial nucleus. Comparison of the antibacterial activities of the two isomers would afford information relating to the importance of configuration of the amino-acid residue in such compounds. 5-Aminoacridine was chosen as the antibacterial nucleus and the compound DL-acridyl(5)-N.alanine was prepared in 96 per cent. yield, as a yellow powder, m.pt., 214°C., by the condensation of 5-chloracridine with DL-alanine in phenol solution.



Bacteriological testing of this compound was not possible because of its insolubility and the instability of its salts in aqueous media. A similar result had been reported for acridyl(5)-N.glycine by Dupré and Robinson[15]. Esterification with ethyl alcohol in the presence of dry hydrochloric acid gas gave an 80 per cent. yield of the racemic ethyl ester as small yellow prisms, m.pt. 75°C. The ester gave well defined crystalline compounds with picric and picrolonic acids and its diacetate and monohydrochloride were quite stable in aqueous solutions. A 0·1 per cent. aqueous solution of the diacetate possessed growth inhibitory activity against *Staphylococcus aureus* and *Streptococcus pyogenes.*

The racemic ester was resolved by the use of (+) tartaric acid. Hot absolute alcoholic solutions, containing equivalent quantities of the ester and (+) tartaric acid, on mixing gave an 87 per cent. yield of the racemic ester (+) tartrate. This salt was a deep yellow powder, m.pt., 165° C. $[\alpha]_D^{20°C.}$ + 18° (water, c = 4·0). Fractional crystallisation from alcohol (80 per cent.) gave the (+) ester (+) tartrate as long yellow needles, m.pt., 118° C. $[\alpha]_D^{20°C.}$ + 54·5° (water, c = 4·0). Evaporation of the mother liquors and repeated crystallisation of the residue from absolute alcohol gave the (−) ester (+) tartrate as a yellow powder, $[\alpha]_D^{20°C.}$ − 19·5° (water, c = 4·0).

The ester isomers were isolated by making aqueous solutions of the respective (+) tartrates alkaline with ammonia and extracting with benzene. They formed viscous yellow oils, (+) acridyl(5)-N.alanine theyl ester, $[\alpha]_D^{20°C.}$ + 128° (alcohol (96 per cent.), c = 2); (−) acridyl (5)-N.alanine ethyl ester, $[\alpha]_D^{20°C.}$ − 122° (alcohol (96 per cent.), c = 2).

Preliminary bacteriological tests on aqueous solutions of the (+) tartrates showed that these salts caused inhibition of growth in the dilutions shown in Table I.

TABLE I

ANTIBACTERIAL ACTIVITIES OF ISOMERS OF ACRIDYL(5)-N.ALANINE ETHYL ESTER

Organism	Racemic ester (+) tartrate	(±) ester (+) tartrate	(−) ester (+) tartrate
(1) *Staphylococcus aureus* ...	1/6000	1/6000	1/3000
(2) *Streptococcus pyogenes* ...	1/4000	1/4000	< 1/2000

Two conclusions may be drawn from these results. In the first instance, a small but significant difference does exist between the antibacterial activities of the two isomers and secondly, the introduction of the amino-acid residue has considerably reduced the activity of the parent amino-acridine. In order to provide a more definite answer to the query involved in this research, it will be necessary to find a compound in which no reduction of activity is caused by the introduction of the amino-acid residue. In fact, it would be an advantage if an enhancement of activity could be achieved. To this end it is intended to prepare β-acridyl-(5)-alanine and, if promising results are obtained, it is intended to extend the work to other amino-acids such as leucine, phenylalanine, etc.

EXPERIMENTAL

(1) *5-Chloroacridine.*—N.phenylanthranilic acid, prepared in 80 per cent. yield by the method of Allen and McKee,[16] was converted to 5-chloroacridine in 75 per cent. yield by heating with phosphorus oxychloride according to the directions of Magidson and Grigorowski.[17] Purification of the crude reaction product was effected by Soxhlet extraction with light petroleum (boiling-range 80° to 100° C.), the 5-chloracridine being obtained as flat yellow plates m.pt., 118° C.

(2) DL-*Acridyl(5)-N.alanine.*—10·7 g. of 5-chloracridine (1/20 mole) was mixed with 40 g. of phenol in a 250-ml. flask and the mixture heated in an oil bath to 80° C., when a yellow solution was formed. 8·9 g. (1/10 mole) of finely powdered alanine was added and the mechanically stirred mixture heated at 120° to 125° C. for 2 hours. It was then allowed to cool and poured, with stirring, into 500-ml. of ether. A brownish yellow oil was formed which quickly solidified to a hard yellow solid. This was filtered, powdered and triturated with successive quantities of 10 per cent. aqueous ammonia until the washings gave a negative test for chloride ion. It was insoluble in most organic solvents, with the except of glacial acetic acid, but could be recrystallised from

a large volume of alcohol (96 per cent.), being soluble about 1 in 500 in the boiling alcohol. The recrystallised material formed a yellow granular powder, m.pt., 214°C. (decomposes with effervescence). Yield 12·7 g. (95 per cent.). Found: C, 70·7 ; H, 5·48 ; N, 10·7 per cent. $C_{16}H_{14}N_2O_2$ requires C, 72·1 ; H, 5·26 ; N, 10·52 per cent.

(*a*) *Monoacetate.*—0·5 g. was dissolved in 5 ml. of cold glacial acetic acid and 20 ml. of ether was added. A yellow precipitate was obtained which on filtration and drying formed a light yellow powder. Yield: 0·42 g., m.pt., 226°C. (decomposes with effervescence). Found: C. 66·8 ; N, 8·7 per cent. $C_{18}H_{18}N_2O_4$ requires C, 66·3 ; N, 8·6 per cent.

(*b*) *Monohydrochloride.*—0·5 g. was dissolved in 10 ml. of hot absolute alcoholic hydrochloric acid and the solution filtered. Addition of 30 ml. of dry ether to the filtrate gave a yellow precipitate. This on drying formed a yellow powder which was easily soluble in water and gave a positive test for chloride ion. Yield: 0·3 g.; m.pt., 170°C. (decomposes with effervescence). Found: C, 62·8 ; N, 9·5 ; Cl, 12·0 per cent. $C_{16}H_{15}N_2O_2Cl$ requires C, 63·6 ; N, 9·24 ; Cl, 11·73 per cent.

Both the acetate and hydrochloride were easily water-soluble, but deposition of the free acid, which was practically insoluble in water, commenced after 24 hours and was almost complete after 96 hours.

3. DL-*Acridyl*(5)-*N.alanine ethyl ester.*—15 g. of DL-acridyl(5)-N. alanine were mixed with 300 ml. of absolute alcohol and the mixture refluxed for 90 minutes, while a stream of dry hydrogen chloride gas was passed. It was then poured into a mixture of 100 ml. of 10 per cent. aqueous ammonia and 100 g. of ice, when a yellowish brown oil was precipitated. This was extracted with successive quantities of chloroform, which were separated and dried over anhydrous sodium sulphate. Removal of the solvent gave a brown oily residue which crystallised from 70 per cent. aqueous alcohol as small light yellow prisms, m.pt., 75°C. Yield: 13·1 g. (80 per cent). Found: C, 71·4 ; H, 6·26 ; N, 9·25 per cent. $C_{18}H_{18}N_2O_2$ requires C, 73·4 ; H, 6·12 ; N, 9·5 per cent.

The substance was practically insoluble in water, but easily soluble in organic solvents and a dilute aqueous alcoholic solution gave a strong greenish fluorescence in visible and ultra-violet light.

(*a*) *Diacetate.*—1 g. of the ester was heated with 10 ml. of glacial acetic acid for 5 minutes on a boiling water-bath. Addition of 200 ml. of ether to the cold solution gave an almost immediate deposition of clusters of yellow needle crystals which were recrystallised from benzene. Yield: 1·1 g. m.pt., 117°C. Found: C, 63·75; H, 6·32; N, 6·97 per cent.; $C_{22}H_{26}N_2O_6$ requires C, 63·77 ; H, 6·28 ; N, 6·76 per cent.

(*b*) *Monohydrochloride.*—Cooling of the esterification reaction mixture caused the appearance of yellow needles of the ester hydrochloride which contained one molecule of alcohol crystallisation, m.p.t., 193°C. Found: C, 61·3 ; H, 6·5 ; N, 7·85 per cent.; $C_{18}H_{19}N_2O_2Cl$, C_2H_5OH requires C, 62·1 ; H, 6·46 ; N, 7·6 per cent.

(*c*) *Picrate.*—This, and the two succeeding derivatives, were made by dissolving 0·2 g. of the ester in 10 ml. of alcohol (90 per cent.) and mixing the boiling solution with a solution of 0·3 g. of the respective nitro

compound in 10 ml. of alcohol. Yellow powder. Yield: 0·25 g. m.pt., 194°C. Found: C, 55·0; H, 4·07; N, 13·0 per cent. $C_{24}H_{21}N_5O_9$ requires C, 55·1; H, 4·0; N, 13·37 per cent.

(*d*) *Styphnate.*—Light yellow powder. Yield: 0·2 g. m.pt., 178°C. Found C, 53·1; H, 3·8; N, 13·2 per cent. $C_{24}H_{21}N_5O_{10}$ requires C, 53·4; H, 3·9; N, 13·0 per cent.

(*e*) *Picrolonate.*—Yellow powder. Yield: 0·25 g. m.pt., 227°C. (decomposed). Found: C, 59·4; H, 4·5; N, 14·2 per cent. $C_{27}H_{26}N_6O_8$ requires C, 57·5; H, 4·6; N, 14·9 per cent.

4. *Resolution of* DL-*Acridyl*(5)-*N.alanine ethyl ester.*

(*a*) DL-*Acridyl*(5)-*N.alanine ethyl ester* (+) *tartrate.*—8 g. of the racemic ester and 4 g. of (+) tartaric acid were dissolved in 100 ml. of hot absolute alcohol. On cooling small yellow crystals appeared. Yield: 10·5 g. (87 per cent.), m.pt., 165°C. (decomposes with effervescence). $[\alpha]_D^{20°C.}$ + 18° (4 per cent. solution in distilled water). Found: C, 57·7; H, 5·65; N, 6·2 per cent.; $C_{22}H_{24}N_2O_8$ requires C, 59·4; H, 5·4; N, 6·3 per cent. The optical rotation and melting-point remained unchanged after several recrystallisations from alcohol, but as the substance was easily soluble in water, fractional recrystallisation was attempted from a series of aqueous alcohols. It was found that alcohol (80 per cent.) caused the preferential separation of the (+) ester (+) tartrate.

(*b*) (+) *Acridyl*(5)-*N.alanine ethyl ester* (+) *tartrate.*—20 g. of the racemic ester (+) tartrate were dissolved in 400 ml. of hot aqueous alcohol (80 per cent.). Long yellow needle crystals slowly formed and these were filtered after 48 hours' standing. Yield: 7·1 g. m.pt., 118°C. $[\alpha]_D^{20°C.}$ + 54·5° (4 per cent. solution in distilled water). The melting-point and optical rotation remained constant after several recrystallisations from the same solvent and it was considered that this material was the (+) ester (+) tartrate.

(*c*) (+) *Acridyl*(5)-*N.alanine ethyl ester.*—2 g. of the (+) tartrate salt were dissolved in 50 ml. of distilled water, 30 ml. of benzene was added and then 1 per cent. aqueous ammonia drop by drop. After each addition of the ammonia the yellow precipitate formed was shaken into the benzene. When no further precipitation occurred the benzene layer was separated and dried for 24 hours over anhydrous sodium sulphate. Removal of the solvent under reduced pressure left a yellow viscous oily residue. Yield: 1·05 $[\alpha]_D^{20°C.}$ + 128° (2 per cent. solution in alcohol (96 per cent.). Found: C, 74·4; H, 6·5; N, 9·02 per cent. $C_{18}H_{18}N_2O_2$ requires C, 73·4; H, 6·12; N, 9·5 per cent. The (+) ester gave a crystalline picrate, m.pt., 175°C. (m.pt. of racemic ester picrate, 194°C.) Found: C, 54·5; H, 3·65; N, 13·3; $C_{24}H_{21}N_5O_9$ requires C, 55·1; H, 4·0; N, 13·37 per cent.

The use of chloroform instead of benzene in the preparation caused complete racemisation of the ester.

(*d*) (−) *Acridyl*(5)-*N.alanine ethyl ester* (+) *tartrate.*—The mother liquid from the crystallisation of the (+) ester (+) tartrate was allowed

to remain at room temperature for a further 48 hours and 1·5 g. of a yellow powder, m.pt., 111° to 113°C., $[\alpha]_D^{2\ °C.}$ + 30·5°, was obtained by filtration. The solvent was removed from the filtrate and the yellow viscous residue repeatedly crystallised from absolute alcohol until a constant value for the optical rotation was obtained. The material formed a yellow powder which did not possess a sharp melting-point, the substance softened gradually from 80°C. onwards. $[\alpha]_D^{20°C.}$ - 19·5° (4 per cent. solution in distilled water.)

(*e*) (−) *Acridyl*(5)-*N.alanine ethyl ester.*—This was isolated from the (−) ester (+) tartrate by a similar procedure to that used for the (+) ester. It was a yellow viscous oil, $[\alpha]_D^{20°C.}$ − 122° (2 per cent. solution in alcohol (96 per cent.)).

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