CCXXXIX. EXPERIMENTS ON AMINO-ACIDS

IV. THE METHYL ETHERS OF SOME *N*-ACETYL-HYDROXYAMINO-ACIDS

By RICHARD LAURENCE MILLINGTON SYNGE

From the Biochemical Laboratory, Cambridge

(*Received 5 October 1939*)

In Paper III of this series the isolation of a "hydroxyamino-acid fraction" from protein hydrolysates is described, and it is shown that the components of this fraction are not readily separable from one another by direct crystallization. In view of the possibility of extractional fractionation of amino-acids in the form of their *N*-acyl derivatives outlined in Papers I and II of this series, it seemed desirable to search for a series of derivatives of the hydroxyamino-acids which would show a similar "spread" in their partition coefficients between a suitable pair of immiscible solvents.

The possibility of using the *N*-benzoyl derivatives was first investigated, although there are theoretical objections to introducing so heavy and fat-soluble a group. These objections were confirmed by experiment.

The ratio of the solubilities of *N*-benzoyl-*dl*-serine [Sørensen & Andersen, 1908] in water and ethyl acetate was determined. This may be taken as a measure of the partition, and can be compared with the same figures obtained for *N*-benzoyl-*dl*-threonine and *N*-benzoyl-*dl-allo*threonine by West & Carter [1937]. This is done in Table I. It seems unlikely from this that the *N*-benzoyl derivatives would be suitable for the extractional fractionation of hydroxyamino-acids.

Table I. *Solubilities of* N-*benzoyl-hydroxyamino-acids in ethyl acetate and water*

Compound	Temp. ° C.	Solubility in EtOAc in mg./ml. A	Solubility in H_2O in mg./ml. B	B/A
N-Benzoyl-*dl*-serine	19	20·9	25·5	1·2
N-Benzoyl-*dl*-threonine	25	10·2	20·6	2·0
N-Benzoyl-*dl-allo*threonine	25	2·4	8·5	3·5

The partition of the *N*-acetyl derivatives between ethyl acetate and water phases was next investigated. In this case the determination was carried out as in Paper I of this series, and the symbols *P* and *c* have the same meaning here as there. The "*N*-acetylthreonine" was prepared by acetylation in solution without working up. A mixture of *dl*-threonine and *dl-allo*threonine (the gift of Prof. A. C. Chibnall, to whom I express my thanks) was used as starting material. The aqueous phase for this particular determination was 0·3*N* NaCl and 0·1*N* acetic acid. The results are shown in Table II.

From this, the *N*-acetyl series seemed as unsuitable for the purpose as the *N*-benzoyl series. It seemed likely that the properties of the molecule were dominated by the presence of a free hydroxyl group, and it was therefore decided to investigate the possibilities of masking this group. Methylation, as introducing only a small group into the molecule, seemed the most hopeful line of approach. It seemed likely that the original amino-acid could be regenerated from its

Table II. *Partition of* N-*acetyl-hydroxyamino-acids between ethyl acetate and water*

Compound	Temp. ° C.	*P*	*c*
N-Acetyl-*dl*-serine	20	75	6
"*N*-Acetylthreonine"	20	45	4
N-Acetyl-*l*-hydroxyproline	20	50	5

N-acetyl-*O*-methyl derivative by refluxing with HBr, as in the preparation by West & Carter [1937] of very good yields of threonine isomerides in one step from their *N*-formyl-*O*-methyl derivatives.

N-Acetyl-*O*-methyl-*l*-tyrosine has been prepared from *N*-acetyl-*l*-tyrosine by methylation with aqueous NaOH and dimethyl sulphate [Karrer *et al.* 1922; Behr & Clarke, 1932]. If this method were applied to the methylation of a mixture of *N*-acetyl-hydroxyamino-acids, obvious difficulties would arise in freeing the product from excess reagents and salt. A possible alternative seemed to be the use of Purdie's reagents (silver oxide and methyl iodide). This would be expected to yield the methyl esters of the *N*-acetyl-*O*-methyl compounds, and, in view of the work of Cherbuliez *et al.* [1929; 1930] on the distillation of the ethyl esters of acetamino-acids, these should be distillable from any non-volatile reaction products. The possibility of *N*-methylation by the reagents had to be borne in mind, but since I had in the past observed that *N*-acetyl-*O*-trimethyl-β-methylglucosaminide [Cutler *et al.* 1937] results from the direct action of Purdie's reagents on *N*-acetyl-glucosamine, it seemed worth while to ascertain their effect on *N*-acetyl-hydroxyamino-acids.

It was found that, on treating *N*-acetyl-*l*-tyrosine, *N*-acetyl-*l*-hydroxyproline and *N*-acetyl-*dl*-serine with Purdie's reagents, in each case a crystalline, distillable methyl ester of the *N*-acetyl-*O*-methyl-hydroxyamino-acid resulted in fair yield. In each case this could be converted quantitatively by saponification into the free acid.

N-Acetyl-*O*-methyl-*dl*-serine was also prepared from *O*-methyl-*dl*-serine. *N*-Acetyl-*O*-methyl-*dl-allo*threonine was prepared from *O*-methyl-*dl*-allothreonine. Both these amino-acids were the gift of Dr Herbert E. Carter, to whom I express my thanks.

The partition of the *N*-acetyl-*O*-methyl-hydroxyamino-acids between chloroform and water was determined (see Paper I of this series). The results are shown in Table III.

Table III. *The partition of* N-*acetyl*-O-*methyl-hydroxyamino-acids between chloroform and water*

Compound	Temp. ° C.	*P*	*c*
N-Acetyl-*O*-methyl-*dl*-serine	20	450	9
N-Acetyl-*O*-methyl-*dl-allo*threonine	20	160	7
N-Acetyl-*O*-methyl-*l*-hydroxyproline	20	23	6
N-Acetyl-*O*-methyl-*l*-tyrosine	20	2·1	2·4

It will be seen that this series of derivatives is capable of providing the basis for extractional fractionation of the hydroxyamino-acids.

No experiments have yet been carried out on mixtures; in order to prepare the "hydroxyamino-acid fraction" (see Paper III of this series) of a protein hydrolysate for such a fractionation, a possible line of approach would be to remove $SO_4^{=}$ exactly with Ba from the aqueous layer after the second chloroform extraction had been carried out. The resulting solution would be evaporated to

dryness, and methylated with Purdie's reagents. The product would be distilled. The distillate would be saponified by $Ba(OH)_2$, and after exact removal of Ba by H_2SO_4, a solution of *N*-acetyl-*O*-methyl-hydroxyamino-acids suitable for extractional fractionation should result.

Experimental

N-*Acetyl*-O-*methyl*-l-*tyrosine methyl ester* (*I*)

1 g. of *N*-acetyl-*l*-tyrosine was dissolved in 6 ml. of dry acetone and treated for 2 hr. at 37° with 6 ml. of methyl iodide and 6 g. Ag_2O. The mixture was filtered, the insoluble material was well washed with acetone and the combined filtrate and washings were evaporated to dryness *in vacuo*. The residue was again treated with 6 ml. of methyl iodide and 6 g. Ag_2O at 37°. The mixture was filtered and evaporated to dryness as before. On distillation, 0·9 g. of material distilled at 180–200°/0·05 mm. The *product* crystallized in the receiver, and recrystallisation from ether yielded 0·6 g. M.P. 106–107°. $[\alpha]_D^{20°}$ +26·3° (alcohol, $l=2$, $c=4·2$). (Found: C, 61·9; H, 7·10; N, 5·64; OMe, 24·3 %. $C_{13}H_{17}O_4N$ requires C, 62·1; H, 6·77; N, 5·58; OMe, 24·7 %.)

N-*Acetyl*-O-*methyl*-l-*tyrosine* (II)

(I) was dissolved in excess of *N* NaOH and kept for 4 hr. at room temperature. On acidifying with HCl, crystallization occurred, and the product on recrystallization from water had M.P. 151° (not depressed on admixture with a sample of *N*-acetyl-*O*-methyl-*l*-tyrosine provided by Prof. H. T. Clarke, to whom I express my thanks).

$[\alpha]_D^{20°}$ +54·3°, +54·4°; $[\alpha]_{5461}^{20°}$ +65·9° (alcohol, $l=2$, $c=1·3$). (Behr & Clarke [1932] record M.P. 150–151°; $[\alpha]_{5461}^{20·5°}$ +67·6° (alcohol, $c=5$).) (Found: C, 60·5; H, 6·21; N, 5·84; OMe, 13·4 %. Calc. for $C_{12}H_{15}O_4N$: C, 60·8; H, 6·33; N, 5·90; OMe, 13·1 %.) Acid equiv. wt. Found: 231. Calc.: 237.

N-*Acetyl*-O-*methyl*-l-*hydroxyproline methyl ester* (III)

1·4 g. of *N*-acetyl-*l*-hydroxyproline (see Paper III of this series) were methylated as in the preparation of (I), using double the quantities of reagents. It was advisable to grind the starting material to a fine powder, as it is not very soluble in acetone. 1·24 g. of distillate (125–150°/0·05 mm.) were obtained, which crystallized immediately. Recrystallization from ether yielded a *product* with M.P. 76–77°. $[\alpha]_D^{18°}$ −81·0° (alcohol, $l=2$, $c=4·5$). (Found: C, 54·1; H, 7·44; N, 6·91; OMe, 31·2 %. $C_9H_{15}O_4N$ requires C, 53·8; H, 7·46; N, 6·96; OMe, 30·8 %.)

N-*Acetyl*-O-*methyl*-l-*hydroxyproline* (IV)

1·0 g. of (III) was dissolved in 50 ml. *N*/3 $Ba(OH)_2$ and was kept at room temperature for 3 hr. Ba was then removed exactly with H_2SO_4, and the filtrate from $BaSO_4$ was evaporated to dryness *in vacuo*. The *product* was crystallized from chloroform by addition of ether, and was recrystallized from a minimum of water, in which it is very soluble. M.P. 152–153°; $[\alpha]_D^{20°}$ −104·3° (water, $l=2$ $c=3$). (Found: C, 51·5; H, 7·03; N, 7·47; OMe, 16·9 %. $C_8H_{13}O_4N$ requires C, 51·3; H, 6·95; N, 7·48; OMe, 16·6 %.) Acid equiv. wt. Found: 184. Calc.: 187.

N-*Acetyl*-O-*methyl*-dl-*serine methyl ester* (V)

1·18 g. of *N*-acetyl-*dl*-serine (see Paper III of this series) were methylated with the same amounts of reagents as in the preparation of (III). As the starting material is a glass of low solubility in acetone, it was obtained in a finely divided

state by evaporating a strong aqueous solution with kieselguhr in a mortar in a vacuum desiccator. When dry, the product was thoroughly ground. The resulting powder was transferred to the reaction vessel and thoroughly dried in the desiccator. After methylation, distillation (100–140°/0·05 mm. Hg) yielded 0·9 g. of a *product* which crystallized in the receiver on keeping overnight. Recrystallization from ether (in which the compound was rather soluble) yielded 0·4 g. M.P. 70–71°. (Found: C, 48·3; H, 7·25; N, 7·95; OMe, 35·0 %. $C_7H_{13}O_4N$ requires C, 48·0; H, 7·43; N, 8·00; OMe, 35·4 %.)

N-*Acetyl*-O-*methyl*-dl-*serine* (VI)

This compound could be made by saponification of (V) but it was found that the crude distillate containing (V) on saponification yielded a contaminant which rendered the crystallization of (VI) rather difficult. This contaminant could be removed by prolonged extraction of an aqueous solution of the saponification product with chloroform. I am grateful to Mrs R. V. Pitt Rivers for carrying out this extraction. The compound could be more conveniently prepared by direct acetylation of *O*-methyl-*dl*-serine, using the procedure described in the Appendix to Paper I of this series. The *compound* crystallized very slowly from ethyl acetate. M.P. 108–109°. (Found: C, 43·8; H, 6·95; N, 8·42; OMe, 18·1 %. $C_6H_{11}O_4N$ requires C, 44·7; H, 6·82; N, 8·69; OMe, 19·2 %.) Acid equiv. wt. Found: 165. Calc.: 161.

N-*Acetyl*-O-*methyl*-dl-allo*threonine* (VII)

This *compound* was prepared in the same way as (VI) by direct acetylation of *O*-methyl-*dl*-*allo*threonine [West & Carter, 1937]. It was recrystallized from acetone. M.P. 151°. (Found: C, 47·9; H, 7·77; N, 7·69; OMe, 18·1 %. $C_7H_{13}O_4N$ requires C, 48·0; H, 7·43; N, 8·00; OMe, 17·7 %.) Acid equiv. wt. Found: 170. Calc.: 175.

SUMMARY

The preparation and properties of some *N*-acetyl-*O*-methyl-hydroxyamino-acids are described.

The partition coefficients of these compounds between chloroform and water have been measured.

It is suggested that, by means of these derivatives, extractional fractionation of the hydroxyamino-acids from protein hydrolysates might be accomplished.

I wish in particular to thank Dr D. J. Bell, Prof. A. C. Chibnall, Prof. C. R. Harington, Prof. Sir F. G. Hopkins, Dr H. R. Marston, Dr A. J. P. Martin, Sir C. J. Martin, Dr A. Neuberger, Mr N. W. Pirie and Dr J. G. Sharp for their advice, interest, and encouragement in respect of the work described in these four papers.

I am also particularly grateful to the International Wool Secretariat, who have maintained me with a Studentship, and have generously provided me with apparatus and materials.

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