306.—*Creatine and Creatinine. Part II. Alleged Acyl Derivatives of Creatine.*

By H. R. Ing.

Two alleged acylcreatines have been described in the literature, *viz.*, " diacetylcreatine " (Erlenmeyer, *Annalen*, 1895, **284**, 49) and " phthalyldicreatine " (Urano, *Beitr. chem. Physiol. Path.*, 1907, **9**, 183) : it is now shown that neither is a creatine derivative.

" Diacetylcreatine," $C_8H_{13}O_4N_3$, was obtained by Erlenmeyer by acetylating creatine with acetic anhydride. It is a neutral substance and Erlenmeyer suggested the formula

$$NHAc\cdot C(:NH)\cdot NMe\cdot CH_2\cdot CO\cdot OAc \quad (I).$$

This formula is improbable on several grounds. Acetylguanidine forms salts with acids (Korndörfer, *Arch. Pharm.*, 1903, **241**, 467) and consequently a substance of formula (I) should be basic. Moreover, " diacetylcreatine " is recovered unchanged by evaporation of its aqueous solution and it also crystallises from hot alcohol, facts which are inconsistent with an open-chain anhydride formula.

The clue to the structure of " diacetylcreatine " was provided by its reaction with ammonia. It dissolved readily in concentrated aqueous ammonia and a crystalline product, $C_3H_6O_2N_2$(A), separated slowly. A second crystalline product, $C_5H_{10}O_2N_2$(B), was isolated from the ammoniacal mother-liquor. These two substances can obviously be derived from diacetylcreatine by the addition of the elements of ammonia :

$$C_8H_{13}O_4N_3 + NH_3 = C_3H_6O_2N_2 + C_5H_{10}O_2N_2.$$

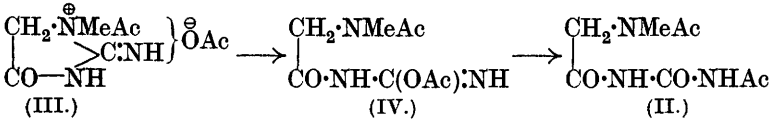
The substance A was found to be acetylurea by comparison with a synthetic specimen. Acetylurea was also obtained by dissolving diacetylcreatine in aqueous methylamine solution and consequently both its nitrogen atoms must be derived from diacetylcreatine. The formula of diacetylcreatine can therefore be extended to $C_4H_8ON\cdot CO\cdot NH\cdot CO\cdot NHAc$, in which scission by ammonia is assumed to occur at the dotted line.

The substance (B) will on this view be an amide and it must contain the second acetyl group. The simplest formula is that of the amide of *N*-acetylsarcosine, $NMeAc\cdot CH_2\cdot CO\cdot NH_2$. *N-Acetylsarcosine amide* was prepared from *N*-acetylsarcosine through the methyl ester and was found to be identical with (B).

" Diacetylcreatine " must consequently be *s.*-(*N*-acetylsarcosyl)-acetylurea (II).

The only other compound which could be isolated from the acetylation products of creatine was 1-methylhydantoin. No acetyl derivatives of creatinine were obtained. The formation of the

curious urea derivative (II) seems to imply the attack of acetic anhydride upon the tertiary nitrogen atom of creatine and it is suggested that the first stage of the reaction is the formation of the unstable quaternary salt (III), which isomerises to (IV) :



(III.) (IV.) (II.)

The latter substance is an imido-acid anhydride and consequently migration of acetyl from oxygen to nitrogen with production of (II) may be expected to occur readily. Hydrolysis of (IV), if it occur, will lead to a urea derivative, $NMeAc{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CO{\cdot}NH_2$, which could lose acetamide to form 1-methylhydantoin.

Diacetylcreatine (II) is also formed as a by-product in the acetylation of creatinine (compare Part I; this vol., p. 2047). It probably arises from acetylcreatinine, acetic anhydride attacking the tertiary nitrogen as an alternative to the formation of diacetylcreatinine. By analogy with the reactions suggested for creatine the substance $NMeAc{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}C(OAc){:}NAc$ will be formed, which will be readily hydrolysed to diacetylcreatine (II). It was shown in Part I that diacetylcreatine could only be isolated from the acetylation products of creatinine after the addition of water.

The opening of the creatinine ring in the formation of diacetylcreatinine resembles somewhat the ring scission observed by Bamberger and Berle (*Annalen*, 1893, **273**, 351) in the benzoylation of glyoxaline. A closer analogy is provided by the acetylation of *s.*-diphenylguanidine, which was found by Busch, Blume, and Punge (*J. pr. Chem.*, 1909, **79**, 537) to yield acetanilide and *N*-acetyl-*N'*-phenylurea. The formation of dialkylacetamides and cyanuric acid in the acetylation of *as.*-dialkylureas (van der Zande, *Rec. trav. chim.*, 1889, **8**, 233) is also of interest in this connexion and may be explained by the loss of proton from the unstable kation $NH_2{\cdot}CO{\cdot}\overset{\oplus}{N}R_2Ac$. It is hoped to study further the acylation of guanidine derivatives.

" Phthalyldicreatine " was obtained by Urano (*loc. cit.*) by heating creatine or creatinine with phthalic anydride. He described it as forming hair-like needles, m. p. 212°, and gave it the formula $C_6H_4[CO{\cdot}NH{\cdot}C(:NH){\cdot}NMe{\cdot}CH_2{\cdot}CO_2H]_2$. By repeating his procedure a substance probably identical with Urano's compound was obtained. It had the same composition and crystalline form and similar solubilities but melted at 223°. It was proved to be *dicreatinine phthalate* by comparison with the neutral salt prepared from creatinine and phthalic acid.

EXPERIMENTAL.

Acetylation of Creatine.—Diacetylcreatine was prepared by Erlenmeyer's method, but its crystalln. was facilitated by addition of EtOH to the gummy reaction product; woolly needles, m. p. 177—178° (Erlenmeyer gave m. p. 165°) (Found: C, 45·0; H, 6·3; N, 19·5. Calc. for $C_8H_{13}O_4N_3$: C, 44·7; H, 6·0; N, 19·5%).

The final mother-liquor from the separation of diacetylcreatine left on evaporation a mixture of diacetylcreatine and a substance sparingly sol. in cold EtOH. The latter substance was separated, by fractional crystn. from EtOH, in long prisms, m. p. and mixed m. p. with authentic 1-methylhydantoin 158—159° (Found: C, 42·5; H, 5·1; N, 24·7. Calc. for $C_4H_6O_2N_2$: C, 42·1; H, 5·3; N, 24·6%).

N-Acetylsarcosine Amide.—Sarcosine (Cocker and Lapworth, J., 1931, 1894) was acetylated by Paulmann's method (*Arch. Pharm.*, 1894, **232**, 601). Acetylsarcosine (m. p. 133—135° after recrystn. from Me_2CO) was added to ethereal CH_2N_2 (slight excess). The oily methyl ester obtained by evaporation of the Et_2O was dissolved in NH_3 aq. (*d* 0·880) and left over-night: evaporation in vac. left a gum, which was crystallised from EtOH over H_2SO_4 in vac. Recryst. twice from EtOH, *N-acetylsarcosine amide* formed prisms, m. p. 140—141°. It melts slowly and begins to soften at 137° (Found: N, 21·5. $C_5H_{10}O_2N_2$ requires N, 21·5%).

Action of Ammonia on Diacetylcreatine.—When a solution of diacetylcreatine in a little cold NH_3 aq. (*d* 0·880) was stirred, a crystalline product separated, m. p. 205—208°. Recrystn. from EtOH and from hot H_2O gave needles of acetylurea, m. p. and mixed m. p. 220—221° (Found: C, 35·4; H, 6·0; N, 27·1. Calc. for $C_3H_6O_2N_2$: C, 35·3; H, 5·9; N, 27·4%).

The ammoniacal solution of diacetylcreatine after removal of acetylurea was evaporated, and the residue dissolved in the minimum of hot EtOH; on cooling, acetylurea crystallised. The alc. mother-liquor was evaporated: the residual gum crystallised when stirred and was repeatedly crystallised from EtOH or AcOEt; prisms, m. p. and mixed m. p. with *N*-acetylsarcosine amide 140—141° after softening at 137° (Found: C, 46·6; H, 7·8; N, 22·0. $C_5H_{10}O_2N_2$ requires C, 46·2; H, 7·7; N, 21·5%).

Dicreatinine phthalate crystallised, when a mixture of a sat. aq. solution of creatinine (1 mol.) and a hot alc. solution of phthalic acid (0·5 mol.) cooled, in white woolly needles, m. p. 223° (decomp.) (Found: N, 21·6. $2C_4H_7ON_3,C_8H_6O_4$ requires N, 21·4%).

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