# 286. *Creatine and Creatinine. Part I. Acyl Derivatives of Creatinine.*

By H. R. Ing.

*With Notes on their Electrometric Titration.*

By R. A. Kekwick and G. M. Richardson.

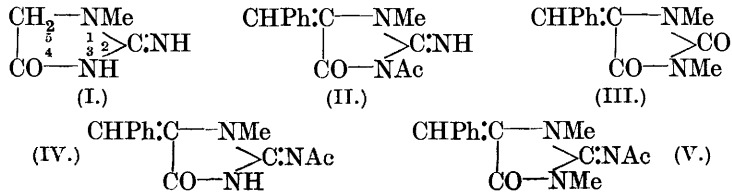
The commonly accepted formula for creatinine (I) admits of three types of tautomeric system, *viz.*, the imino-amino, the lactam-lactim, and the keto-enolic. By assuming an enolic form of creatinine Anslow and King (J., 1929, 1210) have given a convincing explanation of the formation of a red creatinine picrate. Greenwald (*J. Amer. Chem. Soc.*, 1925, **47**, 1440) had previously shown that only creatinine derivatives which retained the possibility of keto-enolic change gave a red colour with picric acid and caustic alkalis (Jaffé's reaction; *Z. physiol. Chem.*, 1886, **10**, 399). The possibility of an enolic phase in the creatinine molecule is also suggested by the red colour which creatinine solutions give with ferric chloride and by the formation of a tribenzoyl derivative (Greenwald, *loc. cit.*).

Less evidence is available for the imino-amino system, but the existence of a true amino-phase in the molecule is suggested by the elimination of one atom of nitrogen in the reactions with nitrous acid (Wilson, *J. Biol. Chem.*, 1923, **56**, 183; Plimmer, J., 1925, **127**, 2651), and hypobromite (Cordier, *Monatsh.*, 1926, **47**, 327).

There appears to be no evidence in the literature for the lactam-lactim system. The formation of a silver derivative $C_4H_6ON_3Ag$ (Schmidt, *Arch. Pharm.*, 1913, **250**, 330) may be accounted for in terms of either an enolic or a lactim structure.

A study of some acyl derivatives of creatinine has provided new evidence in favour of all three types of tautomerism in creatinine derivatives.

Benzylideneacetylcreatinine (Erlenmeyer, *Annalen*, 1895, **284**, 49) contains no possibility of keto-enolic tautomerism and does not give a red colour with picric acid and caustic alkalis. It has now been found that benzylideneacetylcreatinine dissolves readily in caustic alkalis and a *potassium* salt was isolated. These facts are difficult to interpret in terms of Erlenmeyer's formula (II).



The potassium salt of benzylideneacetylcreatinine with methyl iodide gave *benzylideneacetylmethylcreatinine*, which was hydrolysed by acid to benzylidenedimethylhydantoin (III). The constitution of the last substance was proved by its oxidation with permanganate in acid solution to dimethylparabanic acid and benzaldehyde. Consequently benzylideneacetylcreatinine must be represented by (IV) and its methyl derivative by (V).

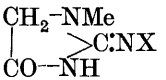
Benzylideneacetylcreatinine has only weakly acidic properties. It is precipitated from its solutions in alkali by carbon dioxide and its potassium salt is considerably hydrolysed in aqueous solution with separation of crystalline benzylideneacetylcreatinine. These facts are readily explained by assuming lactim formation, of which formula (IV) admits. Moreover, the methyl derivative (V), in which lactimisation cannot occur, is insoluble in cold alkalis. The system ·CO·NH·C:NAc may be regarded as analogous to the imido-system ·CO·NH·CO·, the acetyl group, by decreasing the basic character of the amidine residue (·NH·C:NH), conferring acidic character upon the imido-hydrogen. Removal of the acetyl group leads to a big decrease in acidic properties. Thus benzylidene-creatinine is only very little soluble in cold alkalis but, although insoluble in hot water, it dissolves in hot 2*N*-potassium hydroxide and crystallises unchanged from the cooled solution. Benzylidene-acetylcreatinine appears to provide the first clear-cut example of lactam-lactim tautomerism in a creatinine derivative.

The ease with which benzylideneacetylmethylcreatinine (V) is hydrolysed by dilute acid to a hydantoin (III) is remarkable. This change also occurs in methylating benzylideneacetylcreatinine in alkaline solution with methyl sulphate. It does not occur in the absence of the 3-methyl group, *e.g.*, benzylideneacetylcreatinine is rapidly hydrolysed by dilute acid to benzylidenecreatinine. The hydrolysis of the imino-group by acid when the hydrogen atom in position 3 is replaced by methyl, and consequently imino-amino tautomerism is impossible, suggests that creatinine derivatives with hydrogen in position 3 exist in acid solution as the amino-phase.

Benzylideneacetylcreatinine and acetylcreatinine oxime are the only acetyl derivatives of creatinine which have been previously described. Erlenmeyer (*loc. cit.*) obtained diacetylcreatine by the acetylation of creatine, but the acetylation of creatinine does not appear to have been previously studied. With acetic anhydride at 60—65° creatinine yields *acetylcreatinine* as the main product. A second product is also formed (but could not be isolated), since the mother-liquors on treatment with water yield diacetylcreatine. At 100° creatinine and acetic anhydride yielded a mixture of products, from which *diacetylcreatinine* and *triacetylcreatinine* were eventually

obtained, and a residual gum which yielded diacetylcreatine by evaporation of its aqueous solution. The structure of diacetylcreatine will be considered in a later communication.

Acetylcreatinine is readily converted into benzylideneacetylcreatinine by heating it with benzaldehyde and acetic anhydride, and consequently it must be represented by formula (VI; X = Ac).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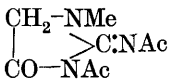
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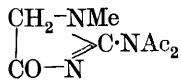
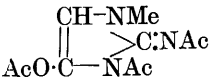

(VIII.)


(IX.)

It possesses both acidic and basic properties. A *hydrochloride* and *picrate* were prepared and also a *potassium* salt. Diacetylcreatinine gives a positive Jaffé reaction and a red colour with ferric chloride and therefore the most probable formula is (VII), although (VIII) is also possible. Triacetylcreatinine gives a red colour in the Jaffé reaction (compare tribenzoylcreatinine, which does not), but this may be due to rapid hydrolysis of the third acetyl group. It is probably represented by (IX).

Benzoylcreatinine was first described by Urano (*Beitr. Chem. Physiol. Path.*, 1907, **9**, 183). A supposed isomeride obtained by Greenwald (*loc. cit.*) has proved to be a mixture of Urano's compound and tribenzoylcreatinine. Benzoylcreatinine is readily soluble in dilute alkalis, including ammonia, and is reprecipitated by carbon dioxide. A *potassium* salt, $C_{11}H_{10}O_2N_3K$, was prepared. By analogy with acetylcreatinine, the benzoyl group is probably attached to the extra-nuclear nitrogen atom (VI; X = Bz).

*Diphenoxyphosphorylcreatinine* [VI; X = $(PhO)_2PO$] also exhibits phenolic properties, and a *sodium* salt was isolated.

## *Electrometric Titration of Acylcreatinines.*

### (A) *Monoacylcreatinines.*

(By R. A. Kekwick, University College, London.

Electrometric titrations were carried out at 25° by the method of Cannan and Knight (*Biochem. J.*, 1927, **21**, 1384) and the constants recorded are uncorrected apparent dissociation constants.

*Benzoylcreatinine.*—The curve of this substance revealed a group dissociating with a $p_K$, 8·79. The substance begins to be pptd. at $p_H$ 9·0 and below this $p_H$ is very sparingly sol. Consequently the dissociating group is probably acidic. Benzoylcreatinine possesses weakly basic properties, but its insol. in $H_2O$ made it impossible to measure a basic constant.

*Acetylcreatinine.*—A complete curve was obtained showing two dissociating groups having constants $p_{K_1}$, 3·51 and $p_{K_2}$, 8·35 respectively. The former constant refers to a basic group. The constant $p_K$, indicates that the acidic

group in acetylcreatinine is somewhat stronger than that in benzoylcreatinine. No appreciable hydrolysis occurred during the titration.

*Benzylideneacetylcreatinine.*—This showed some base-binding power in very alkaline solutions ($p_H$ about 11·0), but it began to be pptd. from solution at $p_H$ 10·8. It had no measurable acid-binding power.

## (B) *Diacetylcreatinine.*

(By G. M. RICHARDSON, Imperial College, London.)

Diacetylcreatinine undergoes hydrolysis in both acid and alkaline solution. Velocities were not directly examined, but the following incidental observations may indicate their extent. In one hour, $p_H$ 2·3 to 5·5, 10% hydrolysis occurred; in 3·5 hours, $p_H$ 4·2 to 2·0 to 5·5, 40%; and in 4 hours, $p_H$ 4·2 to 1·3 to 5·5, 80%. In alkaline solution, rates increased with percentage neutralisation. With a full equivalent of sodium hydroxide ($p_H > 10$), 40% hydrolysis occurred in 2 minutes.

Thus, even though titration was as rapid as possible, little significance would attach to calculated dissociation constants. Direct interpolations from the diagram, after due correction for the activity of hydrogen or hydroxyl ions, have therefore been made. If, however, extensive hydrolysis was permitted, the resultant solution was stable over the observed $p_H$ range, permitting a moderately satisfactory calculation of constants. Such deviations as occurred suggest that a small percentage of diacetylcreatinine, either inefficiently hydrolysed (after 3 hours at $p_H$ 9 to 10) or else in equilibrium with its hydrolytic products, still remained.

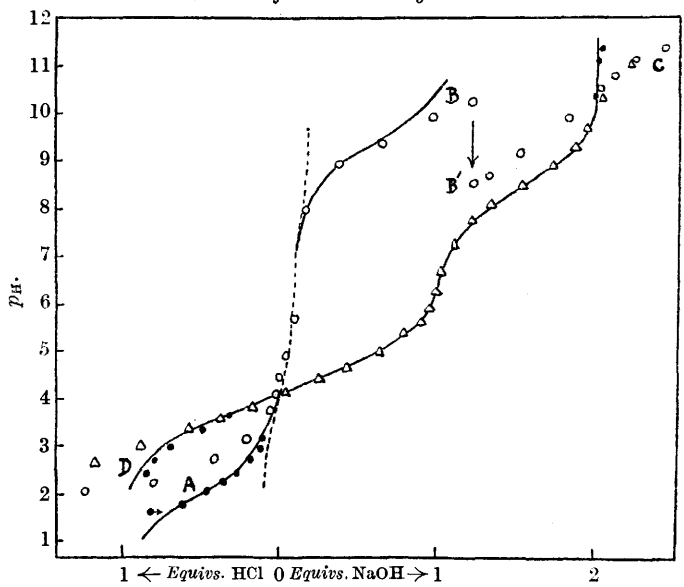
Constants interpolated for diacetylcreatinine are, $p_{K_1}$ 1·9, $p_{K_2}$ 9·5. Those calculated after hydrolysis are $p_{K_1}$ 3·44, $p_{K_2}$ 4·72, $p_{K_3}$ 8·45. There can be little doubt, having regard to probable differences of ionic strength during measurement, that the first and third of the latter constants refer to acetylcreatinine, and the second to acetic acid ($p_K$ 4·73). This evidence precludes the possibility of ring scission during hydrolysis (creatine, $p_{K_1}$ 2·62, $p_{K_2}$ *ca.* 14; Cannan and Shore, *Biochem. J.*, 1928, **22**, 920).

The diagram depicts a complete titration. Starting at A with a slightly acid solution of diacetylcreatinine, titration was carried through to B, the five alkaline points being estimated in 2·5 mins. At B, the progressive hydrolysis obvious in the readings was allowed to proceed to B′ during 80 mins., and afterwards continued for 70 mins., with titration to C. The return titration to D yielded very satisfactory duplicate potentials, which agreed within 0·3 mv., and re-established themselves rapidly after each addition. Measurements were carried out in 0·01*M*-solution at 25° by a technique similar to that of Mr. Kekwick. The calc. curve AB assumes a 10% preliminary hydrolysis to acetylcreatinine and AcOH during potential establishment (broken line), and accepts constants 1·9 and 9·5 for diacetylcreatinine. Curve

CD assumes a $p_K$ of 4·7 for AcOH, and Kekwick's constants for acetylcreatinine.

If the first and the second constant of diacetylcreatinine are basic and acidic, respectively, the second acetyl substituent has slightly weakened both these dissociations, though to an extent not comparable with the weakened acidity of the benzylidene derivative.

*Titration of* 0·01M-*diacetylcreatinine.*




AB *Calculated curve* — · · · · ; *experimental* ○ ; *corrected* ●.
CD *Hydrolytic products, calculated curve* — — ; *experimental* △ ; *corrected* ▲.

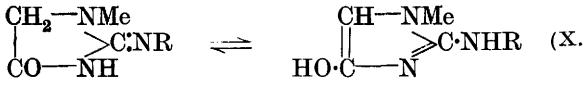
## *Discussion.*

The acidic character of the monoacylcreatinines is not due to their conversion into acylcreatines, as is shown by (1) the analysis of their unhydrated alkali-metal salts and (2) their dissociation constants, which are comparable with those of phenols. An acidic group in formula (VI, X = Acyl) can obviously be derived from either the keto-enolic or the lactam-lactim system. It is, however, improbable that the lactam-lactim system is responsible for the acidic properties of monoacylcreatinines, since benzylideneacetylcreatinine, which contains only this system, is shown by its chemical properties and electrometric titration to be a much weaker acid than the monoacylcreatinines.

The view that monoacylcreatinines owe their acidic character to the keto-enolic system is also supported by the data for diacetyl-

creatinine. The rapid hydrolysis in acid and in alkaline solution of one acetyl group in this compound is consistent with either of the formulæ (VII) and (VIII), in which the second acetyl group is attached to a nitrogen atom already linked to carbonyl, and whichever formula be accepted, the acidic group ($p_K$ 9·5) can only be derived from the keto-enolic system. The similarity in $p_K$ values for the acidic groups of diacetylcreatinine and monoacylcreatinines makes it highly probable that the latter compounds also owe their acidic properties to the keto-enolic system. Such a view, moreover, is capable of supplying a simple explanation of the emergence of acidic (phenolic) properties in acylcreatinines when creatinine itself does not show them.

The combined possibilities of the imino-amino and keto-enolic systems in creatinine can theoretically give rise to a structure in which the ring becomes aromatic, *viz.*, 2-amino-4-hydroxy-1-methylglyoxaline (X; R = H), and in which the hydroxy-group should be



(X.)

phenolic. In view of the general stability of conjugated ring systems it seems probable that conditions which stabilise the amino-phase of the molecule will also stabilise the enolic group and vice versa. In creatinine itself the carbonyl group may be expected to stabilise the imino-structure by attracting the mobile proton of the imino-amino system to the nuclear nitrogen (3). Acylation of the extranuclear nitrogen will tend to neutralise this effect and increase the probability of the amino-structure and, by inference, of the enolic structure. On this view the emergence of acidic properties in acylcreatinines is due to the stabilisation of the conjugated glyoxaline ring (X; R = Acyl), in which the 4-hydroxy-group will be phenolic. The stability of acylcreatinines in alkaline solution, for they show no tendency to form creatine derivatives, is also accounted for by their formulation as derivatives of the conjugated ring (X).

Formula (VIII) for diacetylcreatinine retains the possibility of conjugated ring formation by enolisation, but (VII) does not, and consequently (VII) should be a weaker acid than (VIII) on the view suggested above. Since the introduction of a second acetyl group into acetylcreatinine diminishes the acidic properties, (VII) is regarded as the more satisfactory representation for diacetylcreatinine.

## EXPERIMENTAL.

*Benzylideneacetylcreatinine.*—This was obtained as golden-yellow rods (from EtOH or $C_7H_8$), m. p. 210—211°, by the method of Erlenmeyer who gives

m. p. 213° (Found: N, 17·5. Calc. for $C_{13}H_{13}O_2N_3$: N, 17·3%). The *potassium* salt, obtained from excess of 20% KOH aq., formed pale yellow, woolly needles (from $Me_2CO$), sol. in $H_2O$, EtOH, and hot AcOEt. The aq. solution slowly deposits cryst. benzylideneacetylcreatinine (Found after drying at 110°: K, 12·8, 12·9. $C_{13}H_{12}O_2N_3K$ requires K, 13·9%). The low K figure is attributed to the liberation of free benzylideneacetylcreatinine during recryst.

A solution of the K salt in $Me_2CO$ was boiled with a slight excess of MeI until neutral. The solid left after evaporation of the solvent was recryst. several times from hot $H_2O$, *benzylideneacetylmethylcreatinine* being obtained in yellow needles, m. p. 129—130° (Found: C, 65·7; H, 5·9; N, 16·4. $C_{14}H_{15}O_2N_3$ requires C, 65·4; H, 5·8; N, 16·3%), insol. in cold alkalis but readily sol. in dil. HCl. This solution when warmed gave benzylidene-*NN'*-dimethylhydantoin as a solidifying oil; needles, m. p. 93—94°, from MeOH (Found: C, 66·8; H, 5·4; N, 13·1. Calc. for $C_{12}H_{12}O_2N_2$: C, 66·7; H, 5·5; N, 13·0%) (Nicolet and Campbell, *J. Amer. Chem. Soc.*, 1928, **50**, 1155, record m. p. 92°).

*Benzylidenecreatinine.*—A solution of benzylideneacetylcreatinine in dil. HCl was boiled for a few mins., cooled, and treated with $NH_3$ aq.; the pptd. benzylidenecreatinine crystallised from EtOH in colourless rectangular prisms, m. p. 247° after sintering (Found: N, 20·6. Calc. for $C_{11}H_{11}ON_3$: N, 20·9%). Nicolet and Campbell (*loc. cit.*) describe it as yellow flakes, m. p. 244°.

*Acetylation of Creatinine.*—Creatinine (10 g.) was warmed at 60—65° with $Ac_2O$ (20 c.c.), and the clear solution poured into $Et_2O$ (250 c.c.). The oil which separated crystallised rapidly and after standing at 0° for an hour the solid was collected and recrystallised three times from EtOH or AcOEt. *Acetylcreatinine* was obtained in colourless prisms, m. p. 124—125° (Found: N, 27·0. $C_6H_9O_2N_3$ requires N, 27·4%), readily sol. in $H_2O$. *Hydrochloride*, fine needles, m. p. 185—186° (decomp.), from EtOH (Found: Cl, 18·5. $C_6H_9O_2N_3$,HCl requires Cl, 18·5%). *Picrate*, prisms, m. p. 170—172° (decomp.), from hot $H_2O$ (Found: picric acid, 60·0. $C_6H_9O_2N_3$,$C_6H_3O_7N_3$ requires picric acid, 59·6%). The *potassium* salt, prepared from excess of conc. alc. KOH and a warm solution of acetylcreatinine in EtOH, separated on rapid cooling in plates, which were washed with EtOH and dried at 100° (Found: K, 20·2. $C_6H_8O_2N_3K$ requires K, 20·2%).

The yield of acetylcreatinine decreases rapidly if the acetylation is performed above 65°; *e.g.*, 70% at 65°, 36% at 100°, and nil at 140°.

The ethereal mother-liquor from the prep. of acetylcreatinine deposited a second cryst. product on standing or more rapidly on addition of a few c.c. of $H_2O$. This diacetylcreatine, woolly needles, m. p. 177—178°, from EtOH, was identical with that obtained by the acetylation of creatine. Erlenmeyer (*loc. cit.*) recorded m. p. 165° (Found: N, 19·5. Calc. for $C_8H_{13}O_4N_3$: N, 19·5%).

When creatinine and excess of $Ac_2O$ were heated together on the steam-bath for 1 hr. or boiled for 15 mins., and the latter evaporated, a red gum remained, which was repeatedly extracted with boiling abs. $Et_2O$. The residue was dissolved in the minimum of dry AcOEt and left in the ice-room. The ethereal extract was allowed to evaporate at room temp. and a cryst. product separated, which was again extracted with dry $Et_2O$, in which a part was now insol. This part was used to seed the AcOEt solution of the

$Et_2O$-insol. gum, and more crystals were obtained. Repeated crystn. of the $Et_2O$-insol. crystals from AcOEt gave *diacetylcreatinine* in well-formed rectangular rods with blunt ends, m. p. 164—165° (Found: C, 48·9; H, 5·6; N, 21·2. $C_8H_{11}O_3N_3$ requires C, 48·7; H, 5·6; N, 21·3%), readily sol. in $H_2O$ and EtOH, and recovered unchanged by evaporation of its aq. solution. The *picrate*, prepared in alc. solution, is rather sol. in $H_2O$ but crystallises from EtOH in needles, m. p. 139—140° (Found: picric acid, 54·1.

$$C_8H_{11}O_3N_3,C_6H_3O_7N_3$$

requires picric acid, 53·8%).

The united ethereal mother-liquors after removal of diacetylcreatinine were evaporated and the residual gum, which crystallised slowly, was distilled in vac. The fraction, b. p. 160—170°/5 mm., solidified and then gave large diamond-shaped crystals, m. p. 63—65°, of *triacetylcreatinine* after repeated crystn. from dry $Et_2O$ (Found: C, 50·5; H, 5·5; N, 17·5. $C_{10}H_{13}O_4N_3$ requires C, 50·2; H, 5·4; N, 17·6%). Triacetylcreatinine is readily sol. in $H_2O$, EtOH, $C_6H_6$, $CHCl_3$, and AcOEt, moderately easily in boiling $Et_2O$ and $CCl_4$, and insol. in ligroin.

*Benzoylcreatinine.*—Urano (*loc. cit.*) described this compound as forming yellow needles, m. p. 187°. We have obtained it by his method as colourless needles (from EtOH; charcoal), m. p. 193—194° after sintering (Found: N, 19·4. Calc. for $C_{11}H_{11}O_2N_3$; N, 19·4%).

Benzoylation of creatinine in pyridine with PhCOCl (compare Greenwald, *loc. cit.*) yielded a mixture which was separated by fractional crystn. from EtOH into long flat needles, m. p. 193—194°, of benzoylcreatinine and clumps of short thin needles, m. p. 245—246° (decomp.), identical with tribenzoylcreatinine obtained by benzoylating creatinine in pyridine with excess of PhCOCl.

The *potassium* salt, obtained from benzoylcreatinine in 20% KOH aq., formed colourless plates (from EtOH), readily sol. in $H_2O$ and MeOH, sparingly in conc. KOH aq. and cold EtOH (Found after drying at 110°: K, 15·4. $C_{11}H_{10}O_2N_3K$ requires K, 15·3%).

*Diphenoxyphosphorylcreatinine.*—Dry creatinine (13 g.) and diphenoxyphosphoryl chloride (15 g.) were refluxed in dry $Me_2CO$ for 24 hrs. The cooled liquid was filtered, the solid washed with $Me_2CO$, stirred with cold $H_2O$ to remove unchanged creatinine and its hydrochloride, dried (yield, including that obtained by evaporation of the $Me_2CO$ mother-liquor, 10 g.), and thrice recrystallised from EtOH, *diphenoxyphosphorylcreatinine* being obtained in flat prisms, m. p. 127—128° (Found: C, 55·6; H, 4·6; N, 12·2; P, 9·0. $C_{16}H_{16}O_4N_3P$ requires C, 55·6; H, 4·6; H, 12·2; P, 9·0%). If the experimental conditions were not anhydrous, a second product with a higher m. p. and smaller solubility in EtOH was obtained. It crystallised from EtOH in flat needles, m. p. 158—159°, and was proved to be *creatinine diphenoxyphosphate* by direct synthesis from creatinine and diphenoxyphosphoric acid in alc. solution (Found: C, 52·9; H, 5·0; N, 11·4; P, 8·7. $C_4H_7ON_3,C_{12}H_{11}O_4P$ requires C, 52·9; H, 5·0; N, 11·6; P, 8·5%).

*Sodium diphenoxyphosphorylcreatinine*, deposited from a solution of diphenoxyphosphorylcreatinine in 20% NaOH aq., was washed with ice-cold $H_2O$ and dried over $P_2O_5$ in vac. (Found: Na, 6·4. $C_{16}H_{15}O_4N_3PNa$ requires Na, 6·3%). It dissolves in dil. acids, but is rapidly hydrolysed with formation of diphenoxyphosphoric acid. It is more stable in alkaline solution,

from which it can be repptd. by $CO_2$, but conditions which remove the phenol groups also hydrolyse the phosphoric amide group.

The author is indebted to Mr. R. A. Kekwick and Dr. G. M. Richardson for their co-operation and to Dr. P. Eggleton for the phosphorus estimations.

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