THE PREPARATION OF ALPHA-DIKETONES AND THEIR COLORIMETRIC REACTIONS WITH CREATINE IN ALKALINE SOLUTION

Presented to the Faculty of the Virginia Agricultural and Mechanical College and Polytechnic Institute as a partial fulfillment of the requirements for the degree of Master of Science in Chemistry

by

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TABLE OF CONTENTS

HISTORICAL

PART I

ORGANIC ESTERS OF ORTHOSILICIC ACID

Prior to 1917 about five hundred compounds of silicon were known, exclusive of the silicic acids and silicates. The chemistry of these compounds has been reviewed by Stock[1] and discussed under the linkings Si-H, Si-C, Si-halogen, Si-N, Si-Si, Si=Si, SiO, and Si-O-S. Bygden[2] later gave an historical summary of the chemistry of silicon compounds and their relation to carbon compounds[3]. An anonymous summary also appeared in 1931. The chief reason why it has been possible to extend carbon chemistry so enormously is the fact that the affinity of C is about equally strong for positive and negative non metallic elements such as H, O, S, N, Cl, or C. Although the maximum valency of silicon is also four with regard to both hydrogen and oxygen, yet there is an enormous difference between the positive and negative affinities. The affinity for oxygen is so predominant that practically all other silicon linkages are broken down by the action of cold water. Also the four linkages are symmetrically distributed in carbon, not in silicon[4]. For

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1. Alfred Stock. Ber 50, 171 - 82 (1917)
   Alfred Stock. Ber 54 A, 142 - 58 (1921)

2. Arthur Bygden. Inang. Disc. Uppsala (1916) cf. C. A. 12, 1774

3. Kaiser Wilhelm Institute for silicate research
   Annon. Tonind-Ztg. 55, 1418 - 21 (1931)

4. Barlow and Pope. C. A. 3, 298 (1909)

these and other reasons very little chemical similarity exists between silicon and carbon compounds of the same structure[1]. The nomenclature of silicon compounds is very confusing: in some cases they are named according to the analogous carbon compounds; in others by special names. Various attempts have been made to systematize the nomenclature[2].

The compounds of silicon are usually highly volatile and are decomposed by cold water and air. To avoid these difficulties and others special apparatus has been devised for exact chemical and physical work with substances having a boiling point below 200°A, particularly when only small amounts of the material are available. This special apparatus is described by Stock[3] and directions given for operation.

The lower members of the silicon series corresponding to the methane or CH_4 (CnH_2n) series are, like the carbon compounds, gasses at ordinary temperatures and pressures. Mono silane or mono silicane, SiH_4 is a gas with a characteristic odor, m.p. -185°C, d -185 = 0.68. It is decomposed

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1. A. Stock. Ber 50, 171 - 182 (1917)

2. see (2); also A. Stock. Ber 50, 169 - 170; J. Chem. Soc. 112, II, 204 (1917)

3. A. Stock. Ber 51, 983 - 9 (1918); Ber 54 A, 142 - 58 (1921)

by water forming H_2 and SiO_2, does not react visibly with most reagents, and is slightly soluble in organic solvents. Disilane or disilicane Si_2H_6 is a gas, m.p. -132.5°, density as liquid at -25° = 0.686, other properties similar to mono silane. Tri silane is a liquid at ordinary temperatures. These silicon hydrides as well as the tetra, penta, and hexa silanes have been prepared by Stock and Somieski[1] by the action of hydrochloric acid on magnesium silicide. The mechanism of this reaction has been studied in detail (2). The hydrides thus prepared may be used as the starting point for making other silicon derivatives. Kaufmann[3] prepared organic silicon compounds by the inter-action of hydroxy carboxylic acid esters with silicic acid alky lesters, and by treatment of hydroxy carboxylic acids or their esters or salts with silicon halides.

Stock and Somieski[4] found that the reaction between bromine and pure silane SiH_4 is very violent, but that by controlling conditions the compounds bromo mono silane SiH_3Br and dibromo mono silane SiH_2Br_2 could be prepared in pure state. They have also prepared chlorosilane SiH_3Cl by the action of HCl and SiH_4 with $Al Cl_3$ as a catalyst[5]. SiH_3Br on shaking with water is transformed into disiloxane $(SiH_3)_2O$ and compounds of the type $(SiR_3)_2O$ and $[Si(OR-)_3]_2O$ have frequently been prepared[6].

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1. A. Stock and K. Somieski. Ber 49, 111 - 57 (1916)
2. Schwarz and Konrad. Ber 55 B, 3242 - 52 (1922)
3. H. P. Kaufmann. Brit A 343, 165 (Nov 16, 1928)
4. Stock and somieski. Ber. 50, 1939 - 54. (1917) J. Chem. Soc. 114, II, 110 (1917)
5. Stock and Somieski. Kaiser Wilhelm Inst fur Chemie. Ber 52 B, 1696 (1919)
6. Stock, Somieski, and Wintgen. Ber 50, 1754 - 64 (1917)

Dibromo-mono-silaxane reacts with water to form HBr and a volatile compound oxomonosilane SiH_2O[1], isolated in gaseous monomeric form. Like formaldehyde, the corresponding carbon compound, it polymerizes very easily. This polymerization is characteristic of practically all silicon compounds, particularly of the esters $Si(OR)_4$. These will be taken up later.

The decomposition of silicon hydrides by water is increased by the alkaline content of the water. In some cases the alkaline content of the glass containers had an appreciable effect. Reaction with NaOH solutions furnished a means of analysis of gaseous silicon hydrides to Stock and Somieski[2].

The methylated derivatives have been prepared by the action of $ZnMe_2$ on SiH_3Cl and SiH_2Cl_2[3].

Disilane is very similar in its reactions to mono silane[4]. It reacts with HX in the presence of AlX_3 even more readily than monosilane. The chief product is $Si_2H_4X_2$ although other halogenated products are formed. Isomers are indicated, as in the carbon compounds. The hydrolysis of these compounds proceeded exactly as did those of mono silane, but the decomposition by alkali was somewhat slower due to the more stable Si-Si

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1. Stock, Somieske, and Wintgen. Ber 50, 1754 - 64 (1917)
   Stock and Somieski. Ber 52, 1851 - 60 (1919)
   A. Stock. Chem. Ztg. 43, 594 (1919)

2. Stock and Somieski. Ber 51, 989 - 96 (1918)

3. Stock and Somieski. Kaiser Wilhelm Inst. fur Chemie, Ber 53 B, 695 - 724 (1920)

4. Stock and Somieski. Ber 53, 759 - 69 (1920)

linkage. Kipping[1] has attempted to separate the d,l, and i. forms of disubstituted Si_2H_6.

An attempt has been made to apply the Wertz synthesis to the silicon compounds[2] by the use of SiH_3X with Na-K alloys and Na-amalgams. In some cases complete decomposition of the hydride took place with formation of hydrogen gas and solid products; in others, gaseous products such as Si_2H_6 and Si_4H_{12} were isolated also.

The action of NH_3 on SiH_3Cl and SiH_2Cl_2 has been studied by Stock and Somieski[3], who report that nitrogen derivatives of monosilane are formed which contain the groups SiH_3 and SiH_2, many of which are volatile, simple molecular substances. Another basis for comparison of carbon and silicon is furnished by these compounds, and their reactions are studied with this in view. Polymerization of the silicon compounds was very marked. R. Schwarz[4] worked with Si-N compounds in an attempt to form ammonium silicate. He reports the formation of compounds of the type $(NR_4)_2Si_3O$.

H. Kautzky[5] repeated the work of Wöhler[6] and Hönigschmidt[7], who reported the formation of a compound, "silicon" by the action of HCl on Ca Si_2.

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1. F. S. Kipping. Univ. College. J. Chem. Soc. 119, 647 - 53 (1921)

2. Stock and Somieski. Kaiser Wilhelm Inst. fur Chemie, Berlin Dahlem Ber 54 B 524 - 31 (1921)

3. Stock and Somieski. Ber 54, 740 - 58 (1921)

4. R. Schwarz. Freiberg i/B Ber 49, 2358 - 64 (1916)
   R. Schwarz. Ber 52, 601 - 6 (1919)
   R. Schwarz and R. Sonard. Ber 53, 1 - 17 (1920)

5. H. Kautzky. Z. anorg. allgem. Chem. 117, 209 - 42 (1921)

6. Wohler. Aun. 127,264 (1863)

7. Honigschmidt. C. A. 4, 30 (1910)

Kautzky reports the formation of a mixture of three substance. Oxydisilin Si_2H_2O is a white solid. From this is obtained the silical derivatives $(Si_2OH)Br$ and $(Si_2OH)OH$. The third product is silicic acid. No satisfactory explanation is given for the unsaturation of these compounds. Kipping and Sands[1] report the formation of saturated and unsaturated silicon hydrides of the formula Si_4H_8, by the action of sodium on diphenyldichloromonosilane, Ph_2SiCl_2. No formulas are suggested. They also have worked out a method of analysis[2] by the use of piperidine as the basic agent to liberate hydrogen from the silicon compound.

Heterocyclic compounds containing silicon have been prepared by Zappi[3]. Bygden[4] prepared certain derivatives of benzyl trimethyl silicane, such as the sulfonic acid.

The atomic refraction and atomic dispersion of quadrivalent silicon have been deduced from saturated silicon tetra-alkyls[5]. Sugden has also applied his parachor to silicon derivatives such as $Si(OR)_4$ and SiR_4[6].

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1. F. S. Kipping and J. E. Sands. Univ. Coll. J. Chem. Soc. 119, 830 - 47 (1921)

2. Ibid. 848 - 50 (1921)

3. E. V. Zappi. Anales soc. quim. Argentina 8, 55 - 66 (1920)

4. Arthur Bygden. J. Prkt. Chem. 96, 86 - 104; J. Chem. Soc 114, I, 134 (1918)

5. Gerhard Gruttner and Erich Krause. Ann. 415, 338 - 62 (1918), J. Chem. Soc. 114, 11, 382 - 3 (1918)

6. Samuel Sugden and Henry Wilkins. J. Chem. Soc. (1931), 126 - 8

Wintgen studied the vapor pressure and heat of vaporization of silicon hydrides and their simple derivatives[1] and calculated values from equations such as the Clausius Clapeyron equation and that of Nernst.

In general, the esters of orthosilicic acid[2], $Si(OR)_4$ are prepared by the action of the alcohol on silicon tetrachloride in the absence of water. An inert diluent such as benzene may be used. This general method has been used by a number of workers[3]. The $SiCl_4$ is added dropwise to the alcohol, and the hydrogen chloride gas formed is expelled by heating or by passing through a current of dry air. Last traces of HCl may be removed by treatment with mercury, or HgO or PbO. The metallic chlorides formed are insoluble and can be separated by decantation.

Esters of orthosilicic acid are very sensitive to traces of moisture and are quickly decomposed by cold water. They also polymerize very readily, thus showing in a marked degree the two most characteristic properties of organic silicon compounds.

Orthosilicic acid, $Si(OH)_4$[4] and higher members of the series have been made and their properties determined. The structure of $Si(OCH_3)_4$ has been studied by Eulitz[5], who says that it consists of a tetrahedral

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1. Otis Hutchins. Trans. Am. Electrochem. Soc. 35 (1919)

2. T. H. Farbenind. A. G. Brit. 298, 788, Oct. 24, 1927
Tunnetaro Sakani and Kansai Paint Kabuslieki Kaiska, Japan 5, 90,227. Feb. 10, 1931
Huckel, Mennhoffer, Gerke, and Franck. Ann. 477, 99 - 160 (1929)
Muller. Cunradi and Lechner (to T. H. Farbenind A. G.) U. S. 1, 725, 620. Aug. 20, 1929
Helfavich and Hausen. Ber 57 B, 795 - 9 (1924)

3. L. Kalenburg and R. Koenig. J. Phys. Chem. 12, 290 - 2 (1908)

4. Werner Eulitz. Z. Krist. 80, 204 - 37 (1931)

arrangement of four complex groups, each containing a silicon atom surrounded tetrahedrally by four oxygen atoms and four methyl groups. Signer and Weiler[1] studied the Raman effect in methyl silicates. Sugden[2] has also applied his parachor to methyl orthosilicate.

The preparation and properties of ethylorthosilicate are given in detail by Solana and Moles[3]. Dearing and Reid[4] worked with the ethyl ester and also prepared the butyl, amyl, heptyl, and octyl esters, and attempted to prepare esters from such compounds as $PhCH_2CH_2OH$ and C_2H_5SH. The reaction of $SiCl_4$ and RSH is very slow. The reaction of $Si(OC_2H_5)_4$ with anhydrides such as $C_6H_4(CO)_2O$ and $(CH_3CO)_2O$ was studied. Also the Friedel Crafts synthesis, using C_6H_6, $AlCl_3$ and $Si(OC_2H_5)_4$. Derivatives up to $C_6(C_2H_5)_6$ were obtained.

Jorg and Stetter[5] report that phenol reacts with silicon tetrachloride to give a mixture of diphenoxysilicondichloride, triphenoxysiliconchloride, and phenylorthosilicic ester, $Si(OC_6H_5)_4$. The p-brom derivatives were also prepared, and the reaction with the Grignard studied.

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1. Helo. Chem. Acta 16, 115 - 21 (1933)
2. Sugden and Wilkies. J. Chem. Soc. 1931, 126 - 8
3. L. Solana and E. Moles. Anales soc. espau fis quim 30, 886 (1932)
4. A. W. Dearing and E. Emmett Reid. Johns Hopkins Univ. J. Chem. Soc. 50, 3058 - 62 (1928)
5. Heinrich Jorg and J. Stetter. J. prakt. Chem. 117, 305 - 10 (1927)

The complex pyrocatecholates of silicon have been prepared by Rosenheim, Raibmann, and Schendel[1]. Gruttner and Cauer[2] have prepared nucleus halogen, substituted silicon hydrocarbons and applied them to various syntheses. Esters of the type $BrC_6H_4Si(OR)_3$ were prepared by adding $BrC_6H_4SiCl_3$ to about three times the calculated amount of the cold alcohol, distilling off the excess alcohol under atmospheric pressure and fractionating the residue in vacuo; 50% yield. The p-triethylsilylphenyl alcohols were obtained by slowly adding twice the calculated amount of aldehyde to the magnesium compound from $p\text{-}BrC_6H_4SiE_3$. Also compounds of the type R_3SiOH were prepared, and others such as dioxyphenyl p-bromphenyl silane, $C_6H_5(BrC_6H_4)Si(OC_2H_5)_2$.

Uchida and Kondo synthesized silicon compounds of menthol[3].

Helferich and Hausen[4] made use of orthosilicates in the preparation of acetals. Dry HCl is passed into a mixture of the aldehyde or ketone, 2 - 3 mols of the alcohol and 1.1 mols of the orthosilicate. The particular conditions necessary must be determined for each reaction, but in many cases good results were obtained.

Gilman and Vernon[5] studied the reaction between organomagnesium

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1. Rosenheim, Raibmann, and Schendel. Z. anorg. allgemein. Chem., 196, 160 - 76 (1927)

2. Gerhard Gruttner and Mariaune Cauer. Techn. Hochschnle, Berlin. Ber 51, 1283 - 92 (1918)

3. So Uchida and Toshio Kondo. J. Soc. Chem. Ind. Japan 36, supple binding 190 - 0 (1933)

4. Burckhardt Helferich and Josef Hausen. Ber. 57 B, 795 - 9 (1924)

5. Henry Gilman and C. C. Vernon. J. Chem. Soc. 48, 1063 - 6 (1926)

halides and the aryl esters of orthosilicic acid. The alcosol of SiO_2 is easily prepared[1] from $Si(OC_2H_5)_4$ by hydrolyzing it with the calculated amount of water in the presence of small quantities of HCl or H_2SO_4. The stability of the alcosol is greater than that of the hydrosol.

Tetra-a-thienyl silicon $(C_4H_3S)_4Si$ is prepared from iodothiophene, magnesium and silicon tetrachloride[2].

Esters of orthosilicic acid are easily hydrolyzed by water, and various attempts to prepare the silicic acids by this method have been made. Thiessen and Koerner[3] report the formation of orthosilicic acid ($SiO_2.2H_2O$); pyrosilicic acid ($2SiO_2.3H_2O$); and metasilicic acid ($SiO_2.H_2O$). But in an attempt to repeat this work Solana and Moles[4] were unable to obtain the same results.

In their study on the influence of form and polarity of molecules on the X-ray spectrum of liquids, Katz and Selman[5] found that the two amorphous rings found in case of methyl and ethyl orthosilicate indicated that the inner ring was coused by the central molecule, the outer ring by the OR groups surrounding the central atom.

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1. Kenkyo Inaba. Bull. Inst. Phys. Chem. Research (Tokyo) 7, 948 - 56 English Edition 1, 92 - 3 (1928)

2. Erich Krause and Gerhard Renwarz. Ber 62 B, 1710 - 6 (1929)

3. P. A. Thiessen and O. Koerner. Z. anorg. allgem. Chem. 182, 343 - 50 (1929)

4. Solana and Moles. Anales soc. espan. fis. quim. 28, 171 - 6 (1930)

5. J. R. Katz and J. Selman. Z. Physik. 46, 392 - 405 (1928)

In the comparison of the action of the esters of inorganic acids and the action of acids themselves[1], the action of various esters of inorganic acids on NaOH, $NaOC_2H_5$, and sodium salts of ester acids the strength of which approaches that of alcohol or water was examined in anhydrous solution. Among others, the action of $Si(OC_2H_5)_4$ on $NaOC_2H_5$.

In the reaction whereby alkyl orthosilicates are obtained from $SiCl_4$ and the appropriate alcohol, variable quantities of high-boiling viscous products are obtained. According to Konrad, Bachle and Signer[2], these result from the presence of water in the alcohols. They found that treatment of one mol of $Si(OCH_3)_4$ with 0.5 mol H_2O formed almost exclusively the disilicic ester $[Si(OCH_3)_3]_2$. Use of one mol of water gives a practically quantitative yield of a polymer having a molecular weight of approximately 10,000. They assumed that the action of water is purely hydrolytic and leads to an infinite series of consecutive reactions, ultimately producing homogeneous molecules of the type $(CH_3O)_3SiO[Si(OCH_3)_2O]Si(OCH_3)_3$, and derived an expression for the degree of polymerization. Calculated values agreed well with those obtained by experiment. The polymerization process is not an association of molecules, but is a chemical phenomenon, depending on hydrolysis and condensation. In the large silicon ester molecules, atomic union occurs by normal co-valent linkings between definite atoms.

The cyclohexanol esters have been prepared by Signer and Gross[3], and

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1. Marga Janczak. Roczniki Chem. 10, 115, 55 (155 - 7 in French) (1930)

2. Konrad, Bachle, and Signer. C. A. 24, 3755 (1930) Ann. 474, 276 - 295

3. R. Signer and H. Gross. Ann. 488, 56 - 73 (1931)

polymerized by means of silver carbonate (Ag_2CO_3). Signer and Weiler [1] have also studied the Raman effect for the mono, di, tri, and decameric esters of methyl ortho silicate, and have worked out a curve showing the relationship between the degree of polymerization and Raman Frequency.

The monosilane ortho esters decompose on heating under pressure with an atmosphere of hydrogen, as shown by Dolgov and Volnov[2]. They assumed that silicon could be set free from the esters of monosilane in which the bond between silicon and carbon is weakened by the interposed oxygen atom[3]. $(C_2H_5O)_4Si$ at 250°C is decomposed into $Si(OH)_4$ and C_2H_4 and Si is liberated at 380°. The reaction with $(C_6H_5O)_4Si$ is complete.

The chief commercial use for the orthosilicate esters is in the paint industry, where they are used in two ways: as a stone preservative, and as a paint medium. King[4] has given an excellent summary of the work along this line and the uses made of silicate esters in paints. When alcoholic solutions of silicate esters are mixed with water, they are hydrolyzed and the hydrolysis is accompanied by a rise in temperature and increased viscosity. The intermediate products of the reaction are internally condensed silicates; the final product is colloidally dispersed SiO_2

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1. R. Signer and J. Weiler. Helv. Chem. Acta. 16, 115 - 21 (1931)

2. B. N. Dolgov and Yu. N. Volnov. J. Gen. Chem. (U.S.S.R.) 1, 330 - 9 (1931)

3. Hertkova. Ber 18, 1679 (1885)

4. Geo. King. J. Oil and Color Chem. Assocn. 13, 28 - 55 (1930)

bearing a negative charge. The colloidal solution sets to a gel at a rate which depends upon the amount of water present. Solutions containing water in the ratio $H_2O:(-OC_2H_5) \geq 2:1$ set in 160 days, according to King[1]. But those containing water in the ratio $H_2O:(-OC_2H_5) \leq 1:2$ showed no tendency to set unless the water was allowed to evaporate. Liquid silicon esters not dispersed in any solvent are slowly hydrolyzed by contact with water, but the product is granular SiO_2 which is not suitable for protective coatings.

Solutions of partially hydrolyzed esters of silicic acid are used for preserving stone, painting plaster[2], and the like. The stone preservative contains about 9% SiO_2. This binder preserves stone by cementing together loose particles and filling voids with a SiO_2 gel. Stone decays because its natural binder is destroyed by acidified rain water; the ester binder penetrates decayed stone and sets to a hard silica gel. This setting takes place in two stages: the first consists of the formation of silica gel plasticized with silicic acid ester, which prevents setting up sudden stresses in the film; the second stage is the conversion of the ester to silica, SiO_2. Silicic acid ester medium is coagulated by acid or alkali, and must be stabilized for use on plaster. On surfaces of low porosity it must be thinned with

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1. Geo. King. J. Oil and Color Chem. Assocn. 13, 28 - 55 (1930)
2. G. King. Paint Manuf. 1, 16 - 20, 52 - 5 (1931)

toluene or xylene. Neutral pigments easily wet with alcohol are suitable for use in this binder.

The paint medium is prepared by stirring 315 liters of ester into a solution of 50.7 liters of water in 135 liters of 94% alcohol until a uniform solution is obtained, then adding 250 liters of silicon ester. It is claimed the solution may be preserved in sealed containers. Pigments such as TiO_2, earth colors, and iron oxide black may be used.

The advantages claimed for silicon ester paints are: compatability with wet plaster, heat resistance, non-blistering, easy cleaning, porosity permitting the breathing of a surface, easy working, and economy. On the other hand the disadvantages are: porosity, non-adhesion to distempers and oil paints, and non-storageability. Examples of silicic acid ester paint used on asbestos sheet, engine exhaust manifolds, fire proofed fabric, and plaster are given by King.[1] Further work along this line has been done by Frydlender[2] and Stericker[3].

This review covers the literature on Organic Esters of Orthosilicic Acid given in"Chemical Abstracts" from 1912 to 1935. Judging from the amount of work that has been done along this line, it was thought best not to undertake any research work in this field at present.

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1. King. J. Oil and Color Chem. Assocn. 13, 28 - 55 (1930)

2. J. H. Frydlender. Rev. Prod. Chim. 33, 720 - 3 (1930)

3. W. Stericher. Can. Chem. Met. 15, 135 (1931)

PART II

THE PREPARATION OF ALPHA-DIKETONES AND THEIR COLORIMETRIC REACTIONS WITH CREATINE IN ALKALINE SOLUTION

Creatine was discovered in meat extract by Chevreul in 1832.[1] Liebig[2] in 1847 showed that creatine could be obtained from the flesh of a number of animals, and concluded that it is regular constituent of the muscles of all higher animals. He also established its empirical formula $C_4H_9O_2N_3.H_2O$, and in the decomposition products of creatine by heating with hydrochloric acid he discovered creatinine. Pettenkofer[3] found creatinine in the urine of man. This was confirmed by Heintz[4] in 1847.

In 1868 Volhard[5] synthesized creatine by bringing cyanamide and sarcosine together in alcoholic solution and holding the mixture for several hours at the temperature of boiling water:

$$C_3H_7NO_2 + CN.NH_2 = C_4H_9O_2N_3$$

The yield was poor, and the structural formula was not known. This was given later by Strecker[6] and Erlenmeyer[7] who also gave the formula for

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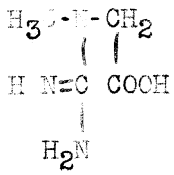
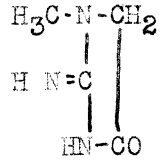
1. Chevreul. J. pharm. 21, 231 - 242; J. Prakt. Chem. 6, 120 - 139 (1835)
2. Liebig. C. R. Acad. Sci. 24, 69 - 73; 195 - 198 (1847)
3. Hunter's Monograph. Longmans, Green and Co. Ltd. (1928)
4. Heintz. Poggendorff's Ann. Phys. Chem. 70, 466 - 480
5. Hunter's Monograph; CREATINE AND CREATININE (LONGMANS, GREEN, AND CO. LTD. 1928)
6. Strecker. Lehrbuck der Organischen Chemie, 5te aufl. p. 586 - 588 (1867)
7. Erlenmeyer. Ann. Chem. Pharm. 146, 259 - 260 (1868)

creatinine.



Creatine



Creatinine

According to the present day knowledge, these formulas seem to represent the facts best. Chemically named, creatine is methylguanidine-acetic acid, and creatinine is glycolyl-methylguanidine or 2-Imido-5-Keto-3-methyl-Tetrahydro-Imidazole. Regarded as a glycocoll derivative, creatine is linked to the amino acids and proteins. Its guanidine group connects it with the complex amino acid arginine. Also it may possibly be related to the betaines and choline. Creatinine is formed from creatine by ring closure, and is connected with such imidazole derivatives as histidine, the purines and allantoin.

Hunter[1] gives an excellent discussion of the physical and chemical characters of creatine and creatinine. One of the most important properties of the two is the readiness of each to transform itself into the other under appropriate conditions. In the presence of sufficient mineral acid the transformation of creatine to creatinine is for all practical purposes, irreversible. The conversion of creatinine to creatine is not so easily done but under proper conditions proceeds practically quantitatively.

The syntheses of creatine and creatinine are of importance in determining the structure, but are too expensive to be used as methods of pre-

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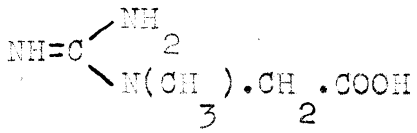
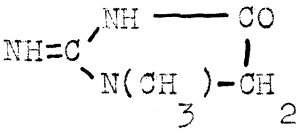
1. Hunter's monograph, page 13



Creatine



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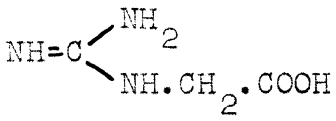
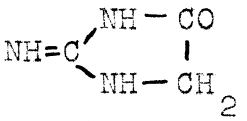
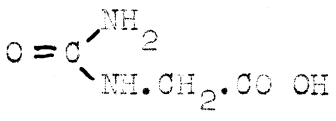


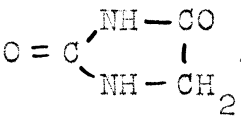
Glycocyamine



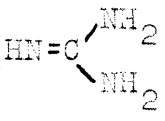
Glycocyamidine



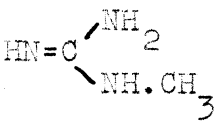
Hydantoic acid



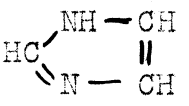
Hydantoin



Guanidine



Methylguanidine



Imidazole

$NH_2CH_2.COOH$

Glycocoll

$NH(CH_3).CH_2.COOH$

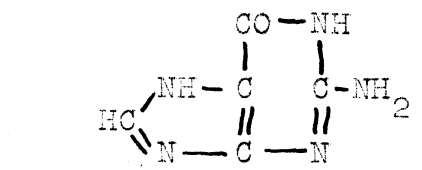
Sarcosine

$HO.N(CH_3)_3.CH_2.COOH$

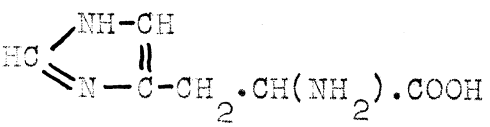
Glycocoll-betaine

$HO.N(CH_3)_3.CH_2.CH_2OH$

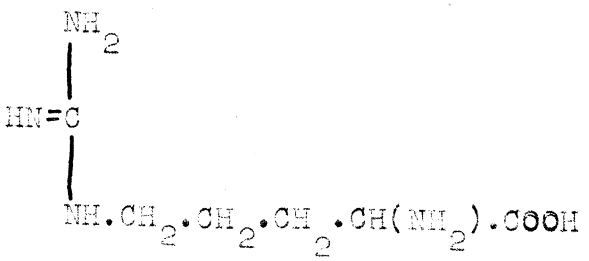
Choline



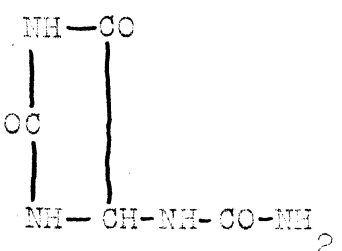
Guanine



Histidine



Arginine



Allantoin

Relations of Creatine and Creatinine to other Substances

Of Biochemical Importance

paration. Biological sources must be used, and two have been most convenient: (1) meat, from which creatine may be obtained directly and creatinine indirectly, and (2) urine, primarily a source of creatinine but will yield creatine on appropriate manipulation.. More recently commercial creatine, a by-product in the manufacture of meat extracts, has become so cheap[1] that it is the most convenient source of starting material. This commercial creatine contains one molecule of water of crystallization and no organic impurities except a small proportion of creatinine, traces of other meat extractives, and a little colloidal material. These can easily be removed[2].

In considering the biological distribution of creatine and creatinine, actual proof for creatinine can be supplied only through its isolation and identification as such, which is seldom convenient, or through the production and isolation of some characteristic derivative; for creatine proof must depend upon either its isolation and identification by analysis, or the production of creatine from it by treatment with acid, and the creatinine then identified as above. With either the ease of transformation of one to the other must be kept in mind. By the above criteria, Hunter[3] sums up the biological distribution as follows: complete and un-

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1. Edgar, G. (1922) J. Ind. Eng. Chem. 14, 984

2. Hunter's monograph, page 54

3. Hunter's monograph, page 112

equivocal evidence exists (1) for the presence of creatine in the voluntary muscles of every class of vertebrate, in mammalian heart muscle, in the electric organ of Torpedo, in the brain, in the testis in the blood of dogs with ligated ureters, in the urine of birds, and under certain conditions in the urine of mammals; (2) the presence of creatinine in the urine of mammals; (3) the presence of one or the other or both in normal blood and its serum, in the pituitary, in the ovary, in milk, in soil, and in certain of the higher plants. Direct evidence, but less complete, indicates that the gastric muscle, the gravid uterus, the kidney and the amniotic fluid contain some creatine, certain cultures of bacteria some creatinine, and lung tissue one or the other of these substances. Considering the evidence from colorimetric methods also, there would seem to be hardly any organ or fluid among vertebrate animals from which creatine or creatinine is entirely absent. For their presence in invertebrates there is no convincing evidence.

The creatine found in various non-muscular tissues may be of considerable physiological importance, but it is important to realize that in quantity it is relatively insignificant beside that of the muscles. The pre-eminence of the latter is not only by their greater content of creatine, but by their much greater mass. Burger[1] has calculated that the muscles of the human body contain ninety-eight per cent of its total creatine. In absolute terms the muscle creatine of a man of seventy kilograms is estimated by Burger at one hundred and twelve grams; by

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1. Burger (1919) Z. ges. exp. Med. 9, 262 - 284

Hahn and Meyer[1] at one hundred and forty grams.

Work on the creatine content of blood is somewhat contradictory, but the data critically considered indicates (1) that normal blood and serum contain at least one of the two substances, creatine or creatinine; and (2) that creatine accumulates in the blood when excretion is prevented[2]. Using a method which practically precludes the transformation of creatine into creatinine, Gaebler and Keltch[3] have isolated from 0.3 to 0.4 mg. of creatinine from 100cc normal human blood. Considering all the evidence, one may take 4 mg. per 100 cc as the best approximation at present available[4] for the average creatine content of normal human blood.

The chief seat of creatine and creatinine in the body is the muscle and the urine respectively, and the function of creatinine as an end product and derivative of creatine is accepted. This being the case the simplest application of the physico-chemical data would lead to the view that the mechanism in the life process is a simple dehydration of creatine, governed solely by the pH, the temperature and the creatine content of muscle. A necessary consequence of this view is that the creatine of the muscle be free, that is, present as such, otherwise it may exist in such

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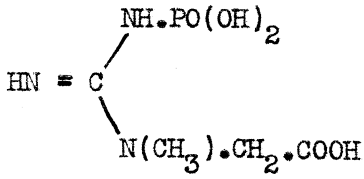
1. Hahn and Meyer Z. Biol. 80, 195 - 210 (1922)
2. Hunter. Monograph, p. 92
3. Gaebler and Keltch. J. Biol. Chem. 74, Proc. Am. Soc. Biol Chem (1927)
4. Hunter. Monograph, p. 97

a state as to be incapable of direct conversion to creatinine.

The evidence has been accumulating for some time indicating that the greater portion of the creatine in muscle is not present as such. Numerous investigations have postulated the existence of a creatine-containing complex, but until recently no such substance has been identified. In any case, if such a complex exists, the data require that it be a combination of the loosest possible kind. Fiske and Subbaron[1] reported final success in isolating such a creatine complex in muscle.

According to this work and that reported later in confirmation by Eggleston and Eggleston[2] the compound consists of one molecule of creatine and one molecule of phosphoric acid, phosphocreatine or phosphagen being the result. It is readily hydrolyzed in acid medium and has heretofore evaded discovery in that the usual conditions for the determination of phosphorous in muscle caused its destruction with the phosphorous appearing as inorganic phosphorous and the creatine as such. Fiske and Subbaron find its calcium salt to have the composition $C_4H_8O_5N_3Ca.4H_2O$ and suggest as its most probable structure:



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1. Fiske and Subbaron. Science 65, 401 (1927); 67, 169 (1928)

2. Eggleston and Eggleston. Nature 119, 194 (1927)

They point out that its most characteristic chemical property, marked instability in acid solution, is characteristic of the few other compounds containing the group $-NH.PO(OH)_2$. It is the first substance in which phosphorous is attached to nitrogen to be isolated from natural sources, and the instability of the phosphamic group makes it one of considerable importance biologically[1].

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1. Shiver. Chem. Reviews 6, 441 (1929)

THEORETICAL DISCUSSION

The first really quantitative methods for determining creatine and creatinine were supplied by Folin[1]. His method is based upon the red color reaction of creatinine with alkaline sodium picrate[2]. In its original form, which was adapted only to the determination of relatively considerable quantities (7 to 15 mg.) of creatinine in a volume of limited range (5 to 15 cc) the color produced in the solution under analysis was compared with a fixed standard consisting of an 8 mm. layer of N/2 potassium bichromate. The later introduction as a standard of creatinine itself[3] or one of its compounds rendered the method much more flexible, so that it became possible to use it under a variety of conditions and for the measurement of creatinine concentrations as low as 0.2 mg. or less in 100 cc. Folin's method for creatine consisted in converting the creatine by appropriate treatment with acid into creatinine, in determining then by the new colorimetric method the "total creatinine" ("preformed" creatinine + creatinine from creatine), and in deducting from this the

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1. Folin (1904) Z. physiol. chem. 41, 223 - 242
   (1905) Am. J. physiol. 13, 45 - 65
   (1906) Festsckr. f. Olof Hammarsten iii, Upsala

2. Hunter. Monograph. p. 25

3. Folin (1914) J. Biol. Chem. 17, 493, 502

creatinine originally present as such. The creatine (in terms of its creatinine equivalent) was thus determined by difference. This method in one form or another is the one at present in general use. The alternatives which have been proposed[1] are applications of the colour reaction of creatine with alpha-Diketones, thus giving a direct determination of the creatine is present.

In the determination of creatinine by the Folin method, a measured quantity (5 to 15 cc) of oxalated blood, containing not more than 20 mg. of oxalate in 10 cc is diluted with seven volumes of water, and mixed with one volume of a 10 per cent solution of sodium tungstate ($Na_2WO_4.2H_2O$). To the mixture is added from a burette, slowly and with frequent shaking, one volume of 0.66 N-H_2SO_4. After five minutes the mixture is filtered to remove the coagulum. A suitable volume (15 to 25 cc.) of the perfectly clear filtrate is treated with an excess of dry powdered picric acid, amd shaken for from five to ten minutes on a shaking machine until saturated. The excess of picric acid is then removed by filtration, and to a measured portion (10, 15 or 20 cc.) of the filtrate there is added from a burette exactly one-twentieth of its volume of 10 per cent sodium hydroxide. After ten minutes the color resulting is compared with that obtained by adding one-twentieth volume of the alkali to a standard solution of creatinine in saturated picric acid. For normal human blood this standard will contain 0.1 mg. creatinine in saturated picric acid. In analysing pathological bloods which may contain up to 10 mg. or more, it is therefore necessary to have a series of additional standards. The number of such

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1. Walpole, G. S. (1911) J. Physiol. 42, 301 - 308
   Lang, K. Z. Physiol. Chem. 208, 273 - 80 (1932)

standards may be reduced by referring the colorimeter readings to empirical curves[1].

In the determination of blood creatine, ten cc. of the protein-free blood filtrate, prepared as for the determination of creatinine, are placed in a 25 cc. volumetric flask, treated with 2cc. of normal HCl, and heated in an autoclave to 130 C. for twenty minutes. To the cooled product there are added one drop of methyl red indicator (0.02 per cent) and, drop by drop, sufficient 10 per cent NaOH to just discharge the red color. The mixture thus neutralized is diluted to 25 cc., saturated on a shaking machine with solid picric acid, and filtered. Ten cc. (or more) of the filtrate is treated with 0.5 cc. (or a proportionately greater amount) of 10 per cent NaOH, and the resulting color is compared with a standard simultaneously prepared. As an alternative to heating in the autoclave the acidified blood filtrate may be diluted with about 75 cc. of water, and boiled upon the hot plate for three hours so that the final volume is approximately 10 cc. This procedure is according to Hunter[2].

It can be seen very easily that the method described above is tedious and indirect, and that a method for the direct determination of creatine in the presence of creatinine would be of great value, particularly if sensitive enough to be used on blood. Two methods have been suggested for the direct determination of creatine, by Walpole[3] using diacetyl in

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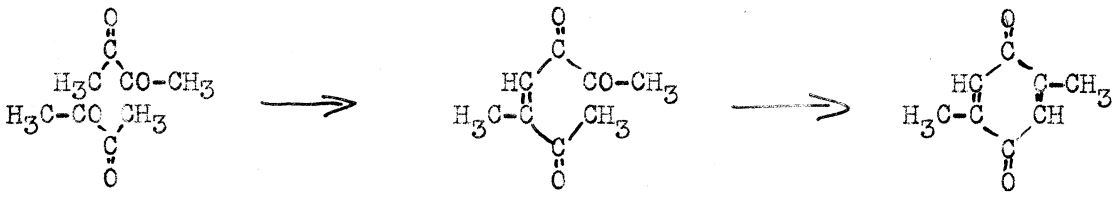
1. Hunter. Monograph, page 65

2. Hunter. Monograph, page 68

3. Walpole. J. Physiol. 42, 301 - 308 (1911)

an alkaline solution, to determine the creatine in urine, and by Lang[1] using acetylbenzoyl in alkaline solution for the determination of creatine in muscle and of arginine in protein.

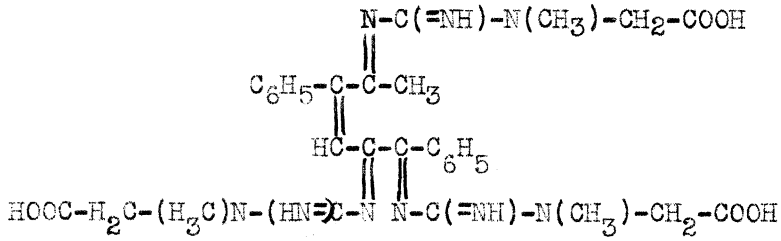
Both of these methods are based on what is known as the diacetyl reaction with guanidines. Harden and Norris[2] have shown that all substances with the atomic grouping $NH{=}C(NH_2){-}NH{-}R$ when warmed with diacetyl in water solution made strongly alkaline give a color, variously described as pink or violet. Among the substances Harden and Norris found gave this color were arginine agmatine, creatine, dicyanamide, and guanidine acetic acid. The mechanism of this reaction was explained by Lang[3]. He says that an alpha diketone is required, having the two C=O groups adjacent and a CH_2 group adjacent to one C=O group; in other words, the grouping $RCOCOCH_2R$. Pechmann[4] has shown that when diacetyl is warmed with alkali in water, two molecules of diacetyl condense with the elimination of a molecule of water to form dimethylquinogen. With the elimination of another molecule of water, a molecule of dimethylquinone is formed.



1. Konrad Lang. Z. Physiol. Chemie 208, 273 - 80 (1932)
2. Harden and Norris. J. Physiol. 42, 332 - 336 (1911)
3. K. Lang. Z. Physiol. Chem. 208, 273 - 80 (1932)
4. Pechmann. Ber. 21, 1411 (1908)

Lang obtained similar condensations with alpha-diketones of the type $RCOCOCH_2R$, but could obtain no condensation with phenantheroquinone or camphorquinone. In this work we have tested benzil $C_6H_5COCOC_6H_5$ and obtained no condensation. The CH_2 group adjacent to one of the CO groups seems to be necessary for the reaction to take place,

Lang varied the proportions of diketone and creatine in the reaction mixture, and could obtain no satisfactory results with an excess of diacetyl in alkaline solutions of creatine. But using acetylbenzoyl as the diketone, he obtained a definite compound. Analysis showed that 2 mols of diketone had reacted with 3 mols of creatine. For this to take place, the two mols of the diketone condense to form the quinogen compound, the three CO groups of which then condense with the NH_2 group of three molecules of creatine to form the colored compound. According to this hypothesis Lang writes the formula for the colored compound thus:



The color is probably due to the accumulated double bonds in the compound. A further conclusion from this formula is that only guanidine derivatives containing at least one free NH_2 group give this reaction; therefore it is given by creatine but not creatinine.

In general, the procedure followed, both by the workers mentioned above and in the present work, is as follows: A certain volume of a solution of creatine is taken, a small amount of diketone added (usually

in the form of a 2% alcohol solution) and the solution made alkaline (using Na_2CO_3, KOH, or NaOH). It is then warmed on the water bath for thirty minutes, the color appears, and may be compared with a standard creatine solution which has been subjected to the same treatment at the same time.

Because of its faults, Walpole's[1] method has never come into general use. When it is used in comparison with that of Folin, results are obtained which are sometimes higher and sometimes lower than Folin's.

Lang[2] prefers to use acetylbenzoyl instead of diacetyl as did Walpole, because it is less volatile and thus easier to handle, and because it gives a purer color. The brown coloring matter observed at greater dilutions of creatine when diacteyl is used, does not appear when acetylbenzoyl is the diketone employed. Lang's method in detail is as follows: Reagents Required: (a) saturated solution of $UO_2(OCH_3)_2$ uranyl acetate; (b) 1% acetylbenzoyl in ethyl alcohol (the solution should not be more than two days old); (c) 60% KOH; (d) 5% NH_2OHCl hydroxylaminechlorhydrate; (e) creatine standard solution containing 0.4 mg. per cc. Procedure: Grind 1 gram of muscle with quartz sand and 7 cc. H_2O. Add 1 cc. of (a) and filter. Treat 1 cc. of filtrate with 1 cc. of (c) and 0.2 cc. of (b) Treat the standard creatine solution (e) in the same way. Heat both tubes

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1. Walpole. J. Physiol 42, 301 - 308 (1911)

2. K. Lang. Z. Physiol. Chem. 208, 273 - 80 (1932)

30 minutes in boiling H_2O, cool and add 1cc. of (d). Dilute to 10 cc. and compare in colorimeter or photometer within 30 minutes. Calculations follow from the principle of the colorimeter. The creatine in 0.1 gram of muscle has been determined. When applied to the determination of creatine in pure solutions, the accuracy of Lang's method is claimed to be within 5% by the author. But the method is applied to the determination of creatine in muscle, where the creatine concentration does not fall below 0.2 mg in 10 cc. in the method used. According to Lang, the method fails when the creatine content is 0.05 mg. or less in 10 cc., and is therefore not applicable to the determination of creatine in blood and other substances where the content is very low. This may be true, but in the course of the present work, a distinct color was obtained with acetyl-benzoyl in a creatinesolution containing 4 parts creatine to 5,000,000 parts of water. This indicates a much greater sensitivity of this reaction than reported by Lang. More about this later.

The object of this work was to find a direct method of determining creatine in the presence of creatinine in the blood, and the most promising possiblility seemed to be this colorimetric reaction of creatine with alpha-diketones in alkaline solution. The creatine content of normal human blood is about 4 mg. per 100 cc.[1], and in an effort to find an alpha-

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1. Hunter. Monograph, page 97

diketone sensitive enough to give a color reaction with creatine at this concentration and thus furnish a basis for a method for the direct quantitative determination of creatine in the blood, a number of alpha-diketones were prepared.

The three most important series of alpha-diketones may be represented as follows:

(1) A-CO-CO-A; where A and A' are aliphatic groupings; for example $CH_3COCOCH_3$ diacetyl.

(2) R-CO-COA, where A is an aliphatic grouping and R is an aromatic grouping; for example $C_6H_5COCOCH_3$ acetyl benzoyl.

(3) R-CO-COR', where R and R' are aromatic groupings; for example $C_6H_5COCOC_6H_5$ benzil.

Group (3) can be disregarded, because this type does not have the CH_2 adjacent to one of the CO groups, which is necessary for the condensation to the quinogen compound that gives the colored compound by condensation with the creatine. Just to make sure, however, an attempt was made to secure this color reaction with benzil, $C_6H_5COCOC_6H_5$; no color could be obtained, although the conditions were varied in a number of ways.

The members of series (1) prepared:

(a) Diacetyl; 2,3 Butanedione; $CH_3COCOCH_3$

(b) Acetyl propionyl; 2,3 Pentanedione; $CH_3COCOCH_2CH_3$

(c) Acetyl butyryl; 2,3 Hexanedione; $CH_3COCOCH_2CH_2CH_3$

(d) Acetyl n-valeryl; 2,3 Heptanedione; $CH_3COCOCH_2CH_2CH_2CH_3$

(e) Propionyl butyryl; 3,4 Heptanedione; $CH_3CH_2COCOCH_2CH_2CH_3$

The members of series(2) prepared:

(a) Acetylbenzoyl; 1,2 propanedione 1, phenyl; $CH_3COCOC_6H_5$

(b) Propionylbenzoyl; 1,2 butandione-1, phenyl; $CH_3CH_2COCOC_6H_5$

(c) n-Butyrylbenzoyl; 1,2 pentanedione-1, phenyl $CH_3CH_2CH_2COCOC_6H_5$

(d) Acetylpara-brombenzoyl; 1,2 propanedione-1, para-bromphenyl

Also pyruvic acid[1], $CH_3COCOOH$, was tested for the colorimetric reaction with creatine.

Various methods of preparing these compounds have been used by workers in the past:

Diacetyl. b.p. 89° C, sp. gr. 0.9734 (22°).

(1) From isonitrosoethylmethyl ketone by hydrolyzing with H_2SO_4 (Ber. 40, 4337; 20, 3213; 21, 1411; 22, 527).

(2) From methylethyl ketone or methylethylcarbinol by oxidation with HNO_3 (accompanied by dinitro ethane).

(3) From oxalicdiacetic or ketiptic acid, by elimination of CO_2 (Ber. 20, 3183).

(4) By oxidation of tetrinic acid with $KMnO_4$ (Ber. 26, 2220).

(5) By electrolysis of pyroracemic acid $CH_3COCOOH$ (Ber. 33, 650).

(6) From vinylidine oxanilide and methyl magnesium iodide (Ber. 40, 186).

(7) Distillation of dimethylglyoxime with dilute H_2SO_4.

(8) For additional methods, see Beilstein vol. 1, page 1015; and supplement vol 1, page 530.

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1. Organic Synthesis XV 90 (WILEY 1935)

Acetyl propionyl. b.p. 108°C.

(1) From isonitrosodiethylketone by hydrolyzing with dilute H_2SO_4 (Ber. 24, 3956).

(2) By the hydrolysis of alpha-bromethylidine acetone $CH_3CH{=}CBrCOCH_3$ (Ber. 34, 2092).

(3) From ethylacetoacetic ester (Ludvig Vanino Prep. Chemie, page 85).

(4) By warming isonitrosodiethyl ketone with isoamyl nitrite (Manasse. Ber. 21, 2377).

(5) By warming methylpropyl ketone with nitric acid (Dl:38) (Filete, Pongio, G. 25I 239).

(6) From amyl keto pseudonitrol by heating (Pechmann J. Pr. [2] 59, 495).

Acetyl Butyryl. b.p. 128°sp. gr. 0.9343 19/4.

(1) From ethyl n-propyl ketone by the isonitroso reaction.

(2) From hydrolysis of acetoxymesityloxide (Ber. 33, 500)

(3) see Ber. 22, 2115.

Acetyln-valeryl. b.p. 148 - 150°C.

(1) From ethyl n-butyl ketone by the isonitroso reaction.

Propionyl n-butyryl.

(1) From dipropyl ketone by the isonitroso reaction.

(2) By the oxidation of dipropyl ketone with nitric acid (also forms dinitropropane) (Fileti, Ponzio, J. pr. [2] 55, 194).

Pechmann[1] was one of the first workers to prepare compounds of this series in the pure state and determine their physical constants. See reference given.

Acetyl Benzoyl. b.p. 222°; 164°/116 mm. 123°/23 mm. sp. gr. 1.1006 20/4

(1) By hydrolyzing isonitroso propiophenone (1 part) with 5% H_2SO_4 (30-35) (Ber. 21, 2119; 22, 2128).

(2) By warming isonitroso propiophenone with 1 1/2 mol isoamyl nitrite (Ber. 21, 2376).

(3) By hydrolizing 1-isonitrosophenylacetone, $C_6H_5C(:NOH)COCH_3$ with dilute H_2SO_4 (Ann. 291, 286).

(4) From 300 cc. absolute ether, 88 grams ethylacetate, 48 grams of acetophenone, and 10 grams sodium (Claisen, Ann. 291, 51).

Propionyl Benzoyl. b.p. 238 - 240°C.

(1) By distillation of isonitroso butyrophenone with dilute H_2SO_4 (Ber. 22, 2131).

n-Butyryl Benzoyl. No reference to this compound in Beilstein, International Critical Tables, or Heilbron. b.p. 256-258°C (estimated).

(1) Prepared by isonitroso reaction from phenylbutyl ketone.

Acetyl para-brombenzoyl. No reference to this compound could be found. Prepared by the isonitroso reaction from para-brom propio-phenone.

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1. Otte and Pechmann. Ber. 22b 2115 (1888)

The most generally applicable and convenient method of preparation of the alpha-Diketones of series (1) and (2) makes use of the monoketone as a starting material. This is converted to the isonitroso ketone, which is then hydrolyzed by means of dilute H_2SO_4 to the diketone.

(1) $R.CH_2.CO.R' + C_5H_{11}.ONO \rightarrow R.C(:NOH).CO.R'$

(2) $R.C(:NOH).CO.R' \xrightarrow[5\%]{H_2SO_4} R.CO.CO.R' + H_2NOH$

An organic nitrite is used to furnish the (:NOH) group. Illustrated is the isoamyl, because that was the most convenient material at hand; however ethyl nitrite has been used[1], and in some cases it was used with good results in this work; also butyl nitrite has been used[2] and reference is also made to methyl nitrite[3].

The introduction of the (:NOH) group is not an easy matter. A catalyst must be used, and in many cases the yields are extremely poor. The earliest catalyst used was concentrated hydrochloric acid[4]. This reaction was carried out in water solution; and although it was used a great deal in this work for want of a better method, the yields of the isonitroso ketone are small (20-25%). A much better method of catalyzing

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1. Aston, Menard, and Mayberry. J. Am. Chem. Soc. 54, 1530 (1932) Aston and Mayberry J. Am. Chem. Soc. 57, 1888 (1935)

2. Hartung and Munch. J. Am. Chem. Soc. 51, 2262 - 6 (1929)

3. Slater. J. Chem. Soc. 117, 587 (1920)

4. Otte and Pechmann. Ber. 22b, 2115 (1888)

this reaction makes use of dry HCl gas in anhydrous ether solution[1]. Yields as high as 97% have been reported by this method, and a yield of 50% or higher was obtained with no trouble. Soduim ethylate in absolute alcohol solution[2] has been used with excellent results.

This assumption seems to have been made by workers in the past: that the (:NOH) group enters in place of two hydrogen atoms on the carbon atom adjacent to the CO grouping; and that when there is a possibility of entering on both sides of the CO grouping, that it will go in on the side having the smallest grouping:

$$\underset{1}{CH_3}\cdot\underset{2}{CH_2}\cdot\underset{3}{CO}\cdot\underset{4}{CH_2}\cdot\underset{5}{CH_2}\cdot\underset{6}{CH_2}\cdot\underset{7}{CH_2}\cdot\underset{8}{CH_3}$$

In the above example, the (:NOH) group will go in on carbon atom number [2], or at least the greater part will go in on that carbon atom. No definite information was found on this subject, and since the formulas of the diketones prepared here were not checked, (except in the cases where benzoic acid was identified as will be discussed later) it must be borne in mind that this assumption has been made in many instances.

The conversion of the isonitroso compound to the diketone by hydrolysis with dilute H_2SO_4 (see reaction 2 above) is practically quantitative[3] in the case of acetylbenzoyl, and is very good in other in-

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1. Slater. J. Chem. Soc. 117, 587 (1920)
   Hartung and Munch. J. Am. Chem. Soc. 51, 2262 - 6 (1929)
   Tiffeneau, Levy, and Ditz. Bull. Soc. Chim.(5) 2, 1848-55(1935)

2. Lang. Z. Physiol. Chem. 208, 273 - 80 (1932)

3. Borsche. Ber. 40, 740 (1907)

stances.

Yields as high as 86.66% were obtained for the hydrolysis of isonitrosopropiophenone to acetyl benzoyl; a yield of 40 - 45% from isonitroso butyrophenone to propionyl buteryl; other yields were not calculated, since the isonitroso ketone was not isolated and purified. But on the whole, the yields seemed to be 50% or better.

According to evidence observed in the hydrolysis of mixed alkyl-aryl isonitroso ketones, there is a split of the molecule at some stage in the reaction, to give two molecules of acid. This may be a split of the isonitroso ketone, or a split of the diketone, under the influence of the H_2SO_4 used in hydrolyzing. In the reaction mixture on standing for 24 hours or more, white flakes were seen to separate out. This occured in a number of instances. These white crystals were filtered off; they were soluble in dilute alkali, and recrystallized on acidifying the solution. After two crystallizations, the product was weighed, and identified as benzoic acid by its melting point (121° C) and formation of the anilide[1]. For benzoic acid to be formed under these conditions, a split of the carbon chain must occur in one or both of two ways:

(1) $C_6H_5.CO.C(:NOH)R \rightarrow C_6H_5COOH+?$

(2) $C_6H_5.CO.COR \rightarrow C_6H_5COOH+RCOOH(?)$

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1. Mulliken. vol.1, page 82 (IDENT. PURE ORG. CMPDS - WILEY-1914)

Results observed are tabulated below:

Ketone used		Benzoic acid recovered		% of Theoretical Yield(on ketone)	
$C_6H_5COCH_2CH_3$	40 gr.	0.51	grams	1.40	%
	30	0.5017	grams	1.80	%
	10	0.3466	grams	4.28	%
$C_6H_5COCH_2CH_2CH_3$	1.59	0.0708	grams	0.73	%
	9.0	0.3653	grams	4.83	%
$C_6H_5COCH_2CH_2CH_2CH_3$	9.0	0.1850	grams	2.64	%

Increased concentration of H_2SO_4 used in hydrolysis increases the split, and more benzoic acid is formed. If the concentration is kept about 5%, very little splitting takes place.

The yields given above are based on the amount of monoketone taken as the starting material. A much more accurate statement of the facts could be given if the isonitroso ketone had been isolated in every case, because it is from that point on that the fission takes place. In the two instances in which the intermediate compound was isolated and weighed, the yields were: isonitrosopropiophenone, $C_6H_5COC(:NOH)CH_3$, 4.01% yield of benzoic acid; isonitrosobutyrophenone $C_6H_5COC(:NOH)CH_2CH_3$, 3.37% yield of benzoic acid. From this evidence it seems probable that about 4% of the theoretical amount of benzoic acid is formed by a split of the carbon chain somewhere in the course of the hydrolysis of the isonitrosoketone to the diketone. This may possibly be advanced as evidence for the structure of the alkyl-aryl diketones.

The method used most for the preparation of the diketones described in this paper follows in a general way the method of Diels and Stephan[1],

1. Diels and Stephan. Ber. 40, 4337 (1907)

which does not involve the isolation and purification of the isonitroso compound. The general procedure is given below:

A weighed amount of the ketone was placed in a three necked 1000 cc. flask equipped with reflex condenser, stirrer, and dropping funnel, and 10 cc. concentrated HCl was added. The calculated equivalent amount of nitrite was then added dropwise with vigorous stirring over a period of 30 minutes to 1 hour. In the preparation of the lower members of the series, the reaction flask was cooled in an ice bath: in the higher members, the reaction was carried out at room temperature, or at an elevated temperature. The temperature conditions must be determined for the individual reaction. After the addition of the nitrite was complete, stirring was continued for 15 minutes, and then the mixture allowed to stand over night. A green color was observed after the addition of the nitrite; this changed to dark brown on standing. The next day the mixture was poured slowly into a 20 - 30% solution of NaOH containing pieces of ice, and shaken for 10 - 15 minutes. The oily layer was extracted several times with cold alkali to insure the complete removal of the product, the alkaline portions being added to that from the first extraction; the combined alkaline layer was then extracted twice with ether to remove alcohols, and then poured cautiously into a beaker containing concentrated HCl (or 1:1 H_2SO_4) and pieces of ice. When the solution was slightly acidified, the isonitroso ketone separated out. With no further purification of the product, enough concentrated H_2SO_4 was added to make the concentration 5 - 10%, and the mixture was slowly brought to

boiling. Then a rapid current of steam was passed through, and the diketone separated as a yellow oil in the distillate. It was separated by means of a separatory funnel and purified by distillation when the yield was great enough to do so, otherwise the diketone was used without further purification.

Vanino's method[1] is very similar, except that it involves the separation and purification of the isonitroso ketone. Pechmann used a similar method[2].

Yields by this method are very low, in most cases 10% of theory based on the monoketone was the maximum obtained. Yields up to 25% have been reported by various workers using this method.

The best means of introducing the (:NOH) group is based on the work of Slater[3] and later enlarged and improved by Hartung and Munech[4]. Tiffeneau, Levy, and Ditz[5] also used this method. The method employs dry hydrogen chloride gas as the catalyst in an anhydrous ether solution of the ketone, the (:NOH) group being furnished by an organic nitrite. The method in detail follows.

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1. Ludwig Vanino. Prep. Chemie, page 85

2. Pechmann. Ber 22, 2115 (1888)

3. Slater. J. Chem. Soc. 117. 587 (1920)

4. W. H. Hartung and J. C. Munch. J. Am. Chem. Soc. 51, 2262 - 6 (1929)

5. Tiffeneau, Levy, and Ditz. Bull. soc. chim.[5]2, 1848 - 55 (1935)

In a 1 liter three-necked round bottomed flask, fitted with stirrer, reflex condenser, and delivery tube for hydrogen chloride gas, was placed a solution of the monoketone in anhydrous ether (100 cc. for each 20 grams ketone taken). The solution was saturated with HCl gas, and HCl at the rate of 2 - 3 bubbles per second was kept bubbling through the solution throughout the course of the reaction. Then isoamyl nitrite was added through the reflux condenser in 2 - 3 cc. portions until the calculated equivalent amount of nitrite had been added. After the addition of the first portion the reaction mixture slowly became a yellow brown and after several more minutes a light yellow color, after which a second portion was added; now the color change took place more rapidly, and a third portion was added, etc. The mixture gradually warmed up and the ether began to reflux gently. The total time required for addition of the nitrite was about 60 minutes. Stirring and bubbling of the HCl were continued for another 15 minutes and the mixture was then allowed to stand over night, during which time it became dark. The next day the ethereal solution was slowly stirred into 20% sodium hydroxide containing pieces of ice, and the ethereal layer was repeatedly extracted with cold alkali until no more product was obtained. The alkaline extracts were slowly stirred into concentrated HCl containing sufficient ice to keep the reacting mixture cold. In this way white crystals of the isonitroso ketone were obtained, and purified by recrystallizing from toluene. Yields have been reported as high as 97% of the theoretical

by this method. A yield about 50% was obtained. The purified isonitroso ketone was placed in a distilling flask with 30 - 35 parts of 5% H_2SO_4 and the mixture slowly brought to boiling. 100 grams (approximately) of salt was added and a rapid current of steam passed through. The diketone separated as a yellow oil in the distillate, and was separated by means of a funnel or pipette. Yield from the isonitroso ketone, 80 - 90%.

A third method of catalysis has been used by Lang[1], and he reports good results. This procedure was not used, but is given here because of its excellent yields and possible use in future work.

4.6 grams of sodium were dissolved in 92 grams of absolute alcohol, then 23 - 4 grams of amylnitrite added; then slowly with cooling 26.8 grams of commercial ethylphenyl ketone were dropped in. The reaction mixture was allowed to stand 2 days, then 500 cc. of water was added and the reaction mixture extracted with ether several times. On the introduction of CO_2 the isonitrosopropiophenone separated out of the water layer. The isonitroso compound was filtered with suction and without any further purification transferred to a distilling flask and covered with 30 times its weight of 5% H_2SO_4 and distilled. During the distillation the water evaporated was frequently replaced to keep the volume constant, and so on until no more yellow oil came over. Then the distillate

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1. Lang. Z. Physiol. Chem. 208, 273 - 80 (1932)

was extracted with ether, the ether layer dried over K_2CO_3 and then warmed to drive off the ether. The acetyl benzoyl thus obtained was subjected to vacuum distillation. B.p. 123/23 mm. Yield of purified substance 40 - 50% of theoretical based on the propiophenone employed.

The diketones prepared were tested for the "diacetyl" reaction with creatine[1] in water solution. Ten cc. of the creatine solution was placed in a small test tube, made alkaline with Na_2CO_3, NaOH, or KOH, and 1cc. of an alcoholic solution (1%) of the diketone was added. The mixture was shaken vigorously and heated in boiling water for 30 minutes, then cooled and examined for color. With the exception of pyruvic acid and benzil, all the substances tested gave a color with alkaline creatine solutions containing 1 gram of creatine in 100 cc. water. The bases used were Na_2CO_3, NaOH, and KOH; KOH gave the best results, using a 60% solution. The substances most sensitive to the reaction were diacetyl and acetyl benzoyl, which gave distinct colors at a dilution of 4 parts creatine to 100,000 parts water for diacetyl and 4 parts creatine to 5,000,000 parts water for acetyl benzoyl.

In general, the intensity of the color fades out with increase in molecular weight of the diketone used. This is probably due to the effect of the larger groups on the polymerization of the diketone to the quinogen compound. The lowest members of the series, diacetyl and acetyl benzoyl, seem to polymerize faster and to a greater extent than those having a larger interfering groups present. The color appears

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1. The creatine used in these tests was furnished through the courtesy of Mr. Braxton Valentine, of the Valentine Meat Juice Co., Richmond, Virginia.

sooner, is more intense, is less effected by standing, and is much more sensitive, i.e. color appears at greater dilutions of creatine solution. Of the two, acetylbenzoyl seems to offer greater possibilities, because it is less volatile and therefore easier to handle, gives a purer color, and is more sensitive. Diacetyl will polymerize to give a pigment described as brownish[1], though here observed as greenish-yellow, which obscures the pink color given by the reaction with creatine in very dilute solutions. This difficulty was not experienced with acetylbenzoyl.

Acetylbenzoyl is sensitive to this color reaction with creatine, more so than is theoretically required by the concentration of creatine in normal human blood (4 to 100,00), and seems to offer the best possibilities for a direct quantitative determination of creatine.

Dr. W. A. Peabody of the Valentine Meat Juice Company, Richmond, Virginia was kind enough to make an attempt to apply the acetylbenzoyl reaction to the determination of creatine in the blood. His results were not very satisfactory with the method used, as the following excerpt from his letter will show:

"We did not have opportunity for a very extensive trial of the acetylbenzoyl. We made the usual tungstic acid filtrate from 10 cc. of blood and used 50 cc. of this, equivalent to 5 cc. whole blood, for the reaction according to the directions which you sent us. At the end of one half hour's heating the corresponding standard showed some of the

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1. G. S. Walpole. J. Physiol. 42, 301 - 308 (1911)

color due to the reaction with creatine. In the blood filtrate solution, something, probably tungstic acid, inhibited the creatine color and at the one half hour mark had somewhat inhibited the brownish black color which the reagent alone gave on heating with alkali. With more prolonged heating, the color in the unknown became darker than that in the standard, but the creatine color in the standard seemed to have bleached out, and the resulting colors in both tubes apparently were simply the blanks.

"We then tried to make a zinc hydroxide filtrate but apparently too much zinc remained in the filtrate; upon heating this, $Zu(OH)_2$ precipitated and no color whatever developed.

"We may point out that the amount of blood filtrate used in the first trial is more than is usually available for the various routine clinical analyaes. Of course the dilution of the blood ten times as in preparation of the usual filtrate, puts a burden upon the none too sensitive acetyl benzoyl reaction. Probably with special means of deproteinizing a non-diluted filtrate could be obtained which would contain enough creatine to permit determinations by this method. The creatine color, however, would be rather faint and it would be necessary to work out a suitable color filter to take out the black color before very accurate results could be obtained."

EXPERIMENTAL DATA

Diacetyl; 2.3 Butandion; $CH_3COCOCH_3$

Diacetyl was prepared from dimethylglyoxime $CH_3C(:NOH)C(:NOH)CH_3$ by distillation with 30 times its weight of 15% H_2SO_4. 10 gr. dimethylglyoxime was covered with 300 cc. 15% H_2SO_4 and the mixture slowly brought to boiling. The solution turned green;100 gr. NaCl was added and a rapid current of steam passed through until no more yellow oil distilled over. The diacetyl was separated by means of a separatory funnel, and the product from several runs distilled under atmospheric pressure. Fraction boiling at 86 - 90° C was taken, b.p. 89 C. Yield 10% or less. A yellow oil with a peculiar pungent odor. Vapors yellow like chlorine gas. Water solution green.

Acetyl propionyl; 2,3 Pentandion; $CH_3.CO.CO.CH_2.CH_3$

Acetyl propionyl was prepared from diethyl ketone by forming the isonitroso ketone and then hydrolyzing this to the diketone.

(a) Diethyl ketone

Diethyl ketone was prepared by distilling the barium salt of propionic acid. 25 gr. propionic acid was added slowly to 30 gr. barium

hydroxide, and the mixture boiled to drive off the water formed in the reaction. When most of the water had been distilled off, the salt was dried, then subjected to dry distillation. The ketone distilled over as a brown liquor, which was purified by distillation at atmospheric pressure. Fraction boiling at 98 - 102° C was taken. Yield on two runs about 65% of theoretical, based on the propionic acid.

(b) Isonitroso reaction to form acetyl propionyl.

To 20 gr. diethyl ketone in a three necked flask fitted with reflux condenser, stirrer, and dropping funnel, was added 10 cc. conc. HCl. Then with rapid stirring 29 gr. isoamyl nitrite was added dropwise. The reaction flask was cooled in running water. Addition of the nitrite required 30 min. Then allowed the flask to stand over night. The green color first observed turned to a dark brown, with a small amount of a white crystalline product. The next morning the liquor was shaken with 50 cc. 30% NaOH and small pieces of ice to keep it cool. The dark oil was separated off and repeatedly extracted with cold alkali to remove all the product. The combined alkaline extracts were then acidified with conc. H_2SO_4 , ice being added as necessary to keep the solution cool. The isonitroso ketone separated, and with no further purification enough H_2SO_4 was added to make the solution 5% H_2SO_4 , and then distilled. The yellow oil in the distillate was separated and the diketone purified by distillation at atmospheric pressure. The fraction boiling at 106 - 110° was taken, b.p. 108° C. Yellow oil similar to diacetyl.

Acetyl butyryl; 2,3 Hexanedione; $CH_3.CO.CO.CH_2.CH_2.CH_3$

Acetyl butyryl was prepared from ethyl propyl ketone by forming the isonitroso ketone and hydrolyzing this to the diketone.

(a) Propyl bromide.

This was prepared from n-propyl alcohol and bromine using red phosphorous as a catalyst. Phosphorous and alcohol placed in a three necked flask equipped with reflux condenser, stirrer, and dropping funnel. The bromine was added over a period of 1 hr. with stirring and cooling. Refluxed 1 hr. and distilled, taking fraction 60 - 75° C, b.p. propyl bromide 70° C, and the product was purified by repeated distillation. Very good yield was obtained.

(b) Propionyl chloride

Prepared by the reaction of phosphorous pentachloride on propionic acid. The propionic acid was dropped on the PCl_5 in a three necked flask, allowed to stand 3 hrs., refluxed 1 hr. on boiling water bath, and distilled. The product was purified by distillation; the fraction boiling at 77 - 81° was taken, b.p. propionyl chloride 80° C.

(c) Grignard synthesis of ethyl propyl ketone.

20 gr. clean magnesium shavings were covered with 200 cc. anhydrous ether, and 100 gr. anhydrous propyl bromide allowed to drop into the reaction mixture, which was contained in a three necked flask. The reaction was started by the addition of a few crystals of iodine, and when refluxing became too vigorous, the flask was cooled with cold running water. When practically all of the magnesium had been dissolved, 80 gr.

propionyl chloride was slowly added, with cooling to control the reaction. After all had been added, the mixture was allowed to stand 1 hr. and then refluxed gently on the water bath for 1 hr. Enough water was added to decompose the excess Grignard and propionyl chloride, and to dissolve the $MgBr_2$ and $MgCl_2$ formed. The ether layer was separated and washed with water, then dried over $CaCl_2$ and distilled to purify the ketone. Fraction taken 122 - 125°, b.p. ethyl propyl ketone 124° C. Yield 40 grams; 50% of theoretical.

(d) Isonitroso reaction and preparation of acetyl n-butyryl. Same procedure as in preparation of acetyl propionyl.

Acetyl n-valeryl; 2.3 Heptanedione; $CH_3.CO.CO.CH_2.CH_2.CH_2.CH_3$

This compound was prepared from ethyl n-butyl ketone prepared by S. B. Row. The ketone was purified by fractional distillation at atmospheric pressure; the residue was steam distilled and fractionated also, and fractions added. Fraction 145 - 150° C taken, b.p. 148.5° C. Ethyl nitrite was used in place of isoamyl nitrite.

(a) Ethyl nitrite. Prepared according to the procedure given by Gatterman and Wieland, page 137.

70 cc. alcohol, 48 grams $NaNO_2$, and 45 cc. concentrated HCl were used.

(b) Isonitroso reaction to form the diketone. Procedure same as in preparation of acetyl propionyl and acetyl buteryl. Comparative tests on alkaline creatine solutions at this point indicated that diacetyl

gives the deepest color, and the color appeared in a shorter time. As we go up the series the color decreases with increasing molecular weight until it is barely visible with acetyl n-valeryl in 1:100 creatine solution. The color faded out on exposure to the air.

Propionyl butyryl; 3,4 Heptanedione; $CH_3.CH_2.CO.CO.CH_2.CH_2.CH_3$

Prepared from dipropyl ketone (Eastman Kodak) by the isonitroso reaction using ethyl nitrite. Same procedure as for acetyl propionyl, etc. The yield was very good.

When tested for the color reaction, color appeared in 1:100 and 1:1000 creatine solutions within 10 minutes. After standing 2 - 3 weeks the original pink color changed to a deep blue, almost purple shade.

Acetyl benzoyl; 1,2 Propanedione 1-phenyl $CH_3.CO.CO.C_6H_5$

Three slightly different methods of synthesis were used:

1. Propiophenone was prepared by the Gignard synthesis and converted to the diketone by forming the isonitroso ketone and then hydrolyzing it to the diketone.

(a) Ethyl iodide was prepared according to the method suggested in Organic Synthesis[1].

(b) 32 gr. dry magnesium turnings were covered with 100 cc. anhydrous ether in a 1 liter three necked round bottom flask equipped with stirrer, reflux condenser and dropping funnel. 206 gr. ethyl iodide was diluted with 300 cc. more anhydrous ether and added dropwise with stirring over a period of 2 hrs. Refluxed 1 hr. When the magnesium was almost all dissolved, 100 gr. benzoyl chloride was added dropwise with cooling to pre-

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1. Organic Synthesis Volume xlll, page 60 (WILEY 1933)

vent too vigorous refluxing. The mixture was refluxed 1 hr. on a water bath (60° C), then poured slowly into water containing enough H_2SO_4 to make the solution acid. The ether layer was separated off and washed with dilute Na_2CO_3 to remove benzoic acid, then dried over $CaCl_2$ and fractionally distilled at atmospheric pressure. Only a small fraction came over above 200° C, and a large amount of tar was formed. Free iodine seemed to be present in large proportions, possibly accounting for the decomposition of the product.

Repeated, using 90 grams ethyl iodide 15 grams magnesium turnings, and 50 grams benzoyl chloride. The fractional distillation was carried out under reduced pressure however, with less decomposition of the product. Fraction boiling at 105 - 115° C under 35 mm. (approx.) pressure taken (b.p. 218° C under 760 mm. pressure).

(c) Isonitroso reaction and preparation of acetyl benzoyl. 40 gr. impure propiophenone from two runs and 8 gr. prepared by W. W. Sweeney were treated with 25 cc. conc. HCl and 50 gr. isoamyl nitrite in the usual manner (see acetyl propionyl, etc.) The oxime was distilled with dilute H_2SO_4; the distillate was colored green, and a few drops of yellow oil separated out on bottom of flask. This was drawn off by means of a pipette.

2. Propiophenone (Eastman Kodak) was converted to the isonitroso ketone and then hydrolyzed to the diketone. 9 grams propiophenone, 12 cc. isoamyl nitrite and 3 cc. concentrated HCl used. Reaction carried out in three necked flask as usual (see acetyl propionyl, etc.). The reaction mixture was heated to 95° C on the water bath, allowed to stand over night heated again for one hour. The oxime was extracted with dilute NaOH and

hydrolyzed to the diketone. Yield about 2 grams acetyl benzoyl.

3. In this procedure the propiophenone was prepared by the Friedel Crafts synthesis[1] , converted to the isonitroso ketone (or oxime) and this hydrolyzed to the diketone with dilute H_2SO_4.

(a) Propionyl chloride (see Gatherman and Wieland, page 110) 72 gr. PCl_3 allowed to run drop by drop into a distilling flask containing 110 grams (1.5 mol.) anhydrous propionic acid. The flask was fitted with a reflux condenser and warmed in a pan of water at 50 - 60° until the vigorous evolution of HCl slackened and the originally homogeneous liquid had separated into two layers. The propionyl chloride was then distilled away from the lower layer of H_3PO_3 by means of a vigorously boiling water bath. A small filter flask attached to the lower end of the condenser served as the receiver and its contents were protected from moisture by a $CaCl_2$ tube. The product was purified by distillation at atmospheric pressure. The fraction boiling at 76 - 82° C was taken (b.p. 80° C). Yield 80 grams (50 - 60% theory)

(b) Friedel Crafts synthesis of propiophenone: 200 cc. anhydrous benzene was placed in a three necked flask fitted with reflux condenser, stirrer, and dropping funnel. 113 gr. $AlCl_3$ (anhydrous) was added with stirring; then 32 gr. propionyl chloride was added drop by drop. The reaction mixture was refluxed gently for 2 - 3 hours until the evolution

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1. N. O. Calloway. Chem. Reviews 17, 3, 327 - 392 (1935)

of HCl had almost stopped; then the liquid was poured cautiously into 200 cc. 20% H_2SO_4 containing cracked ice. The oily layer was separated by means of a funnel and washed twice with water to remove mineral acid and aluminum salts. The water layer was extracted twice with benzene to prevent loss of product, and the extractions added to the oily layer, which was then warmed on the water bath to expel excess benzene. The residual oil was steam distilled to extract the propiophenone from non-volatile condensation products. The oily layer in the distillate was dried over $CaCl_2$ and warmed on the water bath to expel any remaining benzene, and the oil was subjected to vacuum distillation. 35 gr. of propiophenone, b.p. 124° C at 45 mm. pressure, obtained; yield 70% of the theoretical.

(c) Isonitroso propiophenone was prepared according to the procedure of Hartung and Munch[1]. In a 1 liter 3 necked flask fitted with mechanical stirrer, reflux condenser, and delivery tube for hydrogen chloride gas, was placed a solution of 30 grams propiophenone in 200 cc. anhydrous ether; hydrogen chloride was passed through the stirred solution until it was saturated, and at the rate of 2 - 3 bubbles per second throughout the reaction. 26 gr. isoamyl nitrite was added in 2-3 cc. portions through the reflux condenser. After the addition of the first portion the reaction mixture slowly became a yellow-brown and after several minutes

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1. Hartung and Munch. J. Am. Chem. Soc. 51, 2264. (1929)

a light yellow collor, after which a second portion was added; now the color change took place more rapidly, and a third portion was added, etc. The mixture gradually warmed up and the ether began to reflux gently. The total time required for addition of the nitrite was about 60 minutes. Stirring and bubbling of the HCl were continued for another 15 minutes and the mixture was then allowed to stand over night, during which time it became quite dark. The next day the ethereal solution was slowly stirred into dilute NaOH containing pieces of ice; the ethereal layer was repeatedly extracted with cold alkali until no more product was obtained. The alkaline extracts were slowly stirred into concentrated HCl containing sufficient ice to keep the reacting mixture cold. In this manner white crystals of isonitroso propiophenone were obtained; these were recrystallized from toluene and melted at 114° C. Yield 17.23 grams, 48.5% of theoretical.

(d) Hydrolysis of isonitrosopropiophenone to acetyl benzoyl. The isonitroso compound was covered with 30 parts 5% H_2SO_4 and brought slowly boiling. NaCl was added and a rapid current of steam passed through. The yellow oil was separated from the distillate by means of a pipette. Yield 13.74 grams acetyl benzoyl, 86.66% of theoretical from isonitrosopropiophenone.

Tests on acetyl benzoyl for the color reaction with creatine solutions gave some interesting color changes. 10 cc. of 1:100 creatine, 5 cc. 20% NaOH and one drop of acetyl benzoyl, when shaken vigorously, gave first a pale green, then a dirty light brown, then pale lavendar

deepening to violet after about three minutes. With 1:1000 creatine solutions the color change was the same, but took place over a space of 10 minutes time.

Propionyl benzoyl; 1.2 Butanedione 1-phenyl $CH_3.CH_2.CO.CO.C_6H_5$

Prepared from phenyl propyl ketone (Eastman Kodak) by forming the isonitroso ketone and hydrolyzing this to the diketone; see (1c) and (2) under acetyl benzoyl. Yield on two runs very small (10% or less).

n-Butyryl benzoyl; 1,2 Pentanedione 1-phenyl; $CH_3.CH_2.CH_2.CO.CO.C_6H_5$

Prepared from phenyl n-butyl ketone (Eastman Kodak) by forming the isonitroso ketone and hydrolyzing this to the diketone. See (1c) and (2) under acetyl benzoyl. Yield very small (10% or less).

Acetyl p-brom benzoyl; 1,2 Propanedione 1-(p-brom phenyl); $CH_3.CO.CO.$ p-Br C_6H_5

Parabrom propiophenone was prepared by the Friedel Crafts synthesis from brombenzene and propionyl chloride. This was then converted to the isonitroso derivative and hydrolyzed with dilute H_2SO_4 to the diketone.

(a) para-brom propiophenone by the Friedel Crafts synthesis. 60 gr. brombenzene and 200 cc. more to act as solvent were placed in a three neck 1 liter flask fitted with stirrer, reflux, and funnel, and 113 gr. $AlCl_3$ added. Then with stirring 45 gr. propionyl chloride was added drop by drop. The reaction mixture was heated by a water bath kept at 80 - 85° C for 4 - 5 hrs. and then allowed to stand overnight at room temperature. The next morning the mixture was poured slowly into 20% H_2SO_4 and enough ice to keep the reactants cool. The oily layer containing the ketone dissolved

in excess brom benzene was separated from the aqueous portion and then washed several times to remove mineral acid and aluminum salts. To avoid loss of product, the aqueous layer was extracted with several small portions of brom benzene before discarding. These portions were distilled, first using a bath of vigorously boiling water and ordinary steam, then with an oil bath at 150 - 160° C and super-heated steam (using a copper coil heated with a bunsen burner for the super-heating). Nonvolatile condensation products remained behind. The oily layer in the distillate was separated and distilled at atmospheric pressure and 165° C to remove excess brom benzene, then at 35 - 40 mm. and 100° to remove last of solvent. The oil remaining solidified on cooling to white crystals. These were dissolved in hot alcohol and recrystallized. About 10 gr. pure substance was obtained m.p. 45° C (Beilstein); yield, 11% of theoretical based on propionyl chloride.

(b) The ketone was converted to the isonitroso derivative and this was hydrolyzed to the diketone in the same way as acetyl benzoyl (1c) and (2), and propionyl benzoyl, etc., were prepared, Yield of product was very small; a yellow oil which partially crystallized on standing for 48 hours.

In the hydrolysis of the mixed alkyl-aryl oximes to the corresponding diketones, a white crystalline mass was noticed after standing a few days. This was filtered, purified by dissolving in dilute alkali and recrystallizing by acidifying the solution, and identified as benzoic acid by its melting point (121° C) and formation of the anilide[1].

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1. Mulliken 1, 82

Quantities recovered given below (see also Theoretical Discussion, this thesis)

From:	Benzoic acid recovered:
$C_6H_5COC(:NOH)CH_3$	(1) 0.51 gr. (2) 0.5017 gr. (3) 0.3466 gr.
$C_6H_5COC(:NOH)CH_2CH_3$	(1) 0.0708 (2) 0.3653
$C_6H_5CO(:NOH)CH_2CH_2CH_3$	(1) 0.1850

The following diketones were tested for the color reaction with creatine:

A 1. Diacetyl, $CH_3.CO.CO.CH_3$

2. Acetyl propionyl, $CH_3.CO.CO.CH_2.CH_3$

3. Acetyl butyryl, $CH_3.CO.CO.CH_2.CH_2.CH_3$

4. Acetyl valeryl, $CH_3.CO.CO.CH_2.CH_2.CH_2.CH_3$

5. Propionyl butyryl, $CH_3.CH_2.CO.CO.CH_2.CH_2.CH_3$

B 6. Pyruvic acid, $CH_3.CO.CO.OH$

C 7. Benzil, $C_6H_5.CO.CO.C_6H_5$

D 8. Acetyl benzoyl, $CH_3.CO.CO.C_6H_5$

9. Propionyl benzoyl, $CH_3.CH_2.CO.CO.C_6H_5$

10. Butyryl benzoyl, $CH_3.CH_2.CH_2.CO.CO.C_6H_5$

11. Acetyl p-brom benzoyl, $CH_3.CO.CO.C_6H_4Br(p)$

Series are designated by A, B, etc. The diketones are referred to by number in the following tests.

In carrying out the tests, 10 cc. creatine solution was placed in a clean test tube, 1 cc. of 1% alcoholic solution of the diketone added, and then the solution was made alkaline with Na_2CO_3, NaOH, or KOH and the tube heated in boiling water for 30 minutes. Observations as to color were made before and after heating.

Test no. 1: on 1:100 solutions of creatine using 5 cc. 20% NaOH as base.

No heating: Deep orange-pink in 1, faint pink in 2, deep violet in 8, pale lavendar in 11, very faint blue in 5, no color in the remaining tubes.

After standing over night: Color had deepened in 1, 5, 8, and 11; color faded out of 2, but a pale violet had appeared in 9. A precipitate had appeared in all tubes (colored in 1, 5, 8 and 11).

Test no. 2: on 1:100 creatine solutions. 5cc. 10% Na_2CO_3 used as a base.

Before heating: A deep true pink color in 1, faint pink in 2, pale violet in 8 and 11, no color in remaining tubes.

After heating: Orange color in 1, pink in 2, 3, 4, 5, deep violet in 8, fainter in 9 and 10, pale violet precipitate in 11.

Test no. 3: on 1:1000 creatine solution, 5 cc. 20% NaOH used as base. Heated in boiling water. Selected 1, 2, 5, 8, 9 and 11 for test. All gave color to slight extent, but that with diacetyl and acetyl benzoyl was by far the deepest in shade.

Repeated using 5 cc. 10% Na_2CO_3. Color appeared in 1, 2, 5, 8, but not so deep in shade as that using NaOH. No color in 9 and 11.

Test no. 4: on 1:2500 creatine solutions. With 20% NaOH, color appeared in 1, 8, 5, 2 and 9; none in 11; with Na_2CO_3, color in 1, 2, and 8. These shades lighter than those with NaOH.

Test no. 5: on 1:5000 creatine solution. With NaOH, color in 1 and 8; with Na_2CO_3, color in 1, very faint in 8.

Test no. 6: 1:10,000 creatine solution. With NaOH, color in 1; barely visible in 8.

Test no. 7: 1:100.000 creatine solution. Using NaOH, a faint color was obtained with 1(diacetyl) but it was largely due to the brownish pigment from polymerized diacetyl. No color appeared in 8 (acetyl benzoyl) at this dilution.

In another series of tests 5 cc. 60% KOH was substituted for NaOH and Na_2CO_3. In all cases the color was more intense and of a deeper shade, particularly in the mixed alkyl-aryl series. A pale violet color was distinctly visible using acetyl benzoyl on 4:5,000,000 creatine solution. This was duplicated.

Note that no color was obtained with benzil, $C_6H_5.CO.CO.C_6H_5$ in any case. The test with pyruvic acid, $CH_3.CO.COOH$ reported by Miss Stahl[1] could not be duplicated although the same lot of pyruvic acid was tested.

In an attempt to apply this color reaction to the quantitative determination of creatine, the following procedure was used:

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1. Julia Stahl. B.S. Thesis (V.P.I.) 1933, page 14

30 cc. of a solution containing 1 mg. of creatine per cc. was pipetted out into a Nessler tube. 5 cc. 60% KOH, and 1 cc. 1% alcoholic acetyl benzoyl solution added and the tube heated in boiling water for 30 minutes. At the end of this time the tube was cooled repidly and made up to the 50 cc. mark. Same for tubes containing 0.5 and 0.1 mg. creatine per cc. These were then compared in the colorimeter (DuBoss) but the tints were entirely different, and no true comparison could be made. The concentration of creatine was varied in succeeding attempts, but no satisfactory results were obtained. A method of creatine determination using this reaction with acetyl benzoyl to be successful,must be carried out under rigorously maintained standard conditions, since slight variations of technique and various interfering factors will greatly effect the color produced. A series of standards after the manner of Walpole will probably be necessary.