to the preparation of some simple organic compounds not previously produced by the methods employed.

3. Comparative data are supplied in several cases concerning the relative efficiency of various methods of preparation used for the same compound.

4. The compounds studied were the alkyl iodides; trimethylene bromide, iodide, bromohydrin and iodohydrin; trimethylene glycol monoacetate, β-chloro-ethyl acetate, γ-chloropropyl acetate, γ-bromopropyl acetate, β-iodo-ethyl acetate, γ-iodopropyl acetate and the methylene ether of trimethylene iodohydrin.

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THE ISOMERIC MODIFICATIONS OF METHYLENE AMINO-ACETONITRILE AND THEIR BIOCHEMICAL SIGNIFICANCE

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In the various biochemical transformations which take place or function in the normal processes of plant growth, the three reagents ammonia, formaldehyde and hydrocyanic acid undoubtedly play a very fundamental part. In fact, we have good reasons for believing that these very reactive and unsaturated substances are the primary constructive chemical units which lead through fundamental and perfectly definite chemical changes to the final synthesis of the naturally occurring compounds containing the elements carbon, nitrogen, oxygen and hydrogen. Support for the assumption that these reagents actually operate in the normal processes of growth is given by the fact that all three compounds have been shown to be present in the free state in the sap or fluids of plants.[3] Hydrocyanic acid occurs widely in nature especially in many of the important glucosides. Formaldehyde may be considered as a primary assimilation product leading by polymerization to the formation of sugar, while ammonia in the form of the base or its salts is known to serve as a nitrogenous food. While the unsaturated nature of these three reagents is favorable

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[1] Constructed from a Dissertation presented by H. W. Rinehart to the Faculty of the Graduate School of Yale University in June, 1922, in Candidacy for the degree of Doctor of Philosophy.

[2] Communicated to the Organic Section of the American Chemical Society at New Haven, Conn., April, 1923.

[3] Hebert, *Bull. soc. chim.*, [3] **19,** 310 (1898). Ravenna and Tonegutti, *Jahresber.*, **1910,** 1672. Stoklasa and Zdobnicky, *Monatsh.*, **32,** 53 (1911). Müntz, *Jahresber.*, **1890,** 2739. Mayer, *ibid.*, **1874,** 892. Curtius and Franzen, *Ber.*, **45,** 1715 (1912). Gentil, *Bull. soc. chim. sucr. dist.*, **27,** 169 (1909); *C. A.*, **3,** 2882 (1909).

for chemical reactivity, their molecular activity in nature's processes is also undoubtedly influenced and stimulated by the forces of light radiation[4] and the catalytic action of specific enzymes.

In the light of these fundamental facts it is very important to develop, therefore, the chemistry of the system, ammonia-formaldehyde-hydrocyanic acid, and to investigate every organic combination which is found to be formed as a result of the interaction of these three reagents. While this system has been productive of several combinations whose chemical behavior and constitution are well understood, there is, however, one product of synthesis which has been known for a long time that has not received the attention it deserves. We refer to methylene amino-acetonitrile, $C_3H_4N_2$, which was first described by Jay and Curtius[5] in 1894.

It is very probable that a searching investigation of this nitrile will not only disclose it as a substance of immediate biochemical interest, but at the same time will also reveal to us a clearer picture of the manner in which nature actually appropriates ammonia, formaldehyde and hydrocyanic acid. At present we have to deal with a synthetic reaction involving these three reagents which we do not know how to control for maximum production of the nitrile in question, and furthermore, the constitution of the nitrile itself has not been definitely established. In other words, the chemistry of methylene amino-acetonitrile is in a state of disorder. The study of this nitrile, which is easily synthesized in quantity for research work, presents therefore a problem of immediate biochemical interest, and this paper may be considered as the first of a series dealing with the synthesis and chemistry of this interesting compound.

The pronounced reactivity of the aldehyde group, in its simplest aliphatic combination, towards ammonium cyanide was revealed in an early paper by Ljubavin,[6] in which he showed that glyoxal interacts with this salt giving a product which is transformed into glycocoll by hydrolysis with dil. sulfuric acid. The mechanism of this interesting transformation is explained best by an interpretation of the reaction recently proposed by Dakin[7] and may be expressed by the following equations:

$$CHO.CHO + NH_4CN = HCONH_2 + HOCH_2CN;\ HOCH_2CN + NH_3 = H_2O + NH_2CH_2CN;\ NH_2CH_2CN + 2H_2O = NH_2CH_2COOH + NH_3.$$

Twelve years after Ljubavin's work Jay and Curtius[8] investigated the behavior of ammonium cyanide towards formaldehyde under specific conditions and succeeded in isolating a characteristic crystalline compound melting at 129° to which they assigned the constitution of *methylene amino-*

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[4] Ciamician and Silber, *Ber.*, **39**, 3942 (1906).

[5] Jay and Curtius, *Ber.*, **27**, 59 (1894).

[6] Ljubavin, *Ber.*, **15**, 1448 (1882).

[7] Dakin, *J. Biol. Chem.*, **55**, 489 (1923).

[8] Jay and Curtius, Ref. 5; *Ber.*, **31**, 2490 (1898).

acetonitrile, $CH_2{:}NCH_2CN$. These investigators assumed this to be a monomolecular compound and accepted this simple formula as an expression of constitution on the basis of the following reactions.

$$CH_2{:}NCH_2CN + HCl + H_2O = HCl.NH_2CH_2CN + HCHO \quad (1)$$

$$NH_2CH_2CN + 2H_2O + C_2H_5OH + 2HCl = HCl.NH_2CH_2COOC_2H_5 + NH_4Cl + H_2O \quad (2)$$

That the formation of this nitrile is accompanied by deep-seated changes was known to Curtius and in a paper by this investigator and Welde[9] it is stated that under certain conditions the nitrile is obtained as a viscous substance which is difficult to purify. This behavior has been observed by other workers who have applied this reaction and it is now known that the cause of this is the formation of secondary products of reaction of unknown constitution. Cambier and Brochet[10] repeated the work of Jay and Curtius and furthermore attempted to determine the molecular weight of the nitrile, but nothing was contributed as a result of their research which altered in any way the conclusion of Jay and Curtius.[8]

For the final conclusions to date regarding the conditions controlling the formation of methylene amino-acetonitrile we are indebted to the investigations of Klages.[11] He has shown that the system, ammonia-formaldehyde-hydrocyanic acid, is productive of the nitrile melting at 129° when the condensation is applied in either alkaline or acid media, and he has reported in his papers analytical values for the molecular weight indicating that this is a dimolecular compound and is to be expressed by the formula $(C_3H_4N_2)_2$. Outside of this analytical result and the characteristic behavior of methylene amino-acetonitrile on hydrolysis under different conditions, the outstanding feature of Klages' investigations is the isolation of a second product of condensation melting at 86°, which was shown by analysis to be isomeric with the nitrile melting at 129°. Klages apparently was not interested in the chemistry of this substance, and it has received, so far as the writers are aware, no attention since the publication of his papers. In conclusion, it can be said that Klages contributed practically no new chemical data which can be used to elucidate the molecular constitution of methylene amino-acetonitrile. That this nitrile is not a monomolecular substance was confirmed later by Delepine,[12] who showed that it does not interact normally as a Schiff base with hydrocyanic acid to give the nitrile of iminodiacetic acid. $CH_2{:}NCH_2CN + HCN = NH(CH_2CN)_2$. This observation has since been confirmed by Bailey and Lochte.[13]

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[9] Curtius and Welde, *Ber.*, **43**, 868 (1910).

[10] Cambier and Brochet, *Bull. soc. chim.*, [3] **13**, 416 (1895).

[11] Klages, *J. prakt. Chem.*, **65**, 192 (1902); *Ber.*, **36**, 1508 (1903).

[12] Delepine, *Bull. soc. chim.*, [3] **29**, 1201 (1903).

[13] Bailey and Lochte, THIS JOURNAL, **39**, 2443 (1917). See also Bailey and Snyder, *ibid.*, **37**, 935 (1915).

In order to provide ourselves with material for research we have applied Klages' directions for the preparation of methylene amino-acetonitrile and have made the interesting observation that the lower-melting isomer (melting at 86°) is always formed along with the nitrile melting at 129°. This product remains dissolved in the filtrate after separation of the higher-melting nitrile and on allowing this filtrate to stand the lower-melting isomer is finally deposited in a crystalline condition. Klages reports that he obtained this isomer when he allowed ammonia, formaldehyde and hydrocyanic acid to interact in aqueous solution acidified with sulfur dioxide. The fact that this isomer is always formed along with the nitrile melting at 129° accounts partly for the low yield of the latter after purification, and consequently for the low yield of glycocoll which is formed by hydrolysis of the crude nitrile. It has not yet been shown that the isomer melting at 86° is converted into glycocoll by hydrolysis. Both of these nitriles are characterized by their crystalline habit, and in the experimental part of this paper are recorded crystallographic data which sharply differentiate the two compounds.

In his determinations of the molecular weight of methylene amino-acetonitrile melting at 129° Klages employed both the cryoscopic and ebullioscopic methods using naphthalene and chloroform, respectively, as solvents. As stated above, the analytical values obtained by him were in agreement with that calculated for a dimolecular substance, ($C_6H_8N_4$). We have repeated this work and also determined the molecular weight of the isomer melting at 86°. We have obtained in our measurements analytical values which are not in agreement with those reported by Klages. We have made molecular-weight determinations of both isomers by the cryoscopic method with naphthalene as solvent, and also by the ebullioscopic method using acetone as solvent, and have obtained results in both cases which agree consistently with the calculated values for a trimolecular compound.

In other words, we are led to conclude that the dimolecular formula of Klages is incorrect and that the two compounds melting at 129° and 86° are structural isomers to which is to be assigned the empirical formula $C_9H_{12}N_6$. The facts which lead us to conclude that we are dealing here with two compounds possessing entirely different molecular structure are (1) inability to rearrange one isomer into the other, (2) difference in behavior on hydrolysis, and (3) difference in crystalline form of the 129° and 86° isomers, which crystallize in the orthorhombic and monoclinic systems, respectively.

In the light of these facts, and the new experimental data which we will report in our next publication, it is very evident that the term methylene amino-acetonitrile is a misnomer. It is an extremely unstable combination and apparently has not been isolated thus far in a free monomolecular condition. We propose, therefore, to designate hereafter this higher-melting isomer (129° product) by the name -*α-hydroformamine cyanide* and the lower-melting compound (86° product) by the name -*β-hydroformamine cyanide*. The name methylene amino-acetonitrile applies strictly to the monomolecular, unsaturated compound represented by the formula $CH_2:NCH_2CN$.

Experimental Part

Preparation of the Isomeric Modifications of Methylene Amino-acetonitrile, $(CH_2:NCH_2CN)_3$.—The method employed was essentially described by Klages,[11] and recently developed and modified by Adams and Marvel.[14] The crude nitrile is washed with cold water several times and then dried at 50–60°. After crystallization from 95% alcohol or acetone, the nitrile is obtained in colorless crystals melting at 129°. In eight runs the yields varied between 270 and 310 g. and averaged exactly 295 g. This corresponds to a yield of 52%.

When the temperature of the reaction mixture is allowed to rise above 13° during the addition of the cyanide solution it is our experience that the reaction product tends to separate in a plastic form which is extremely difficult to purify. When ammonium sulfate (350 g.) and ammonium oxalate (240 g.) were substituted for ammonium chloride in the above process we obtained 305 g. and 270 g., respectively, of the crude nitrile.

The Isomer Melting at 129°.—Molecular-weight determinations were made by the cryoscopic and ebullioscopic methods using naphthalene and acetone, respectively, as the solvents.

Calc. for $C_9H_{12}N_6$: mol. wt. 204. Found: (naphthalene) 201.7, 189.8; (acetone) 220, 212, 202.

The Isomer Melting at 86°.—The aqueous filtrate left behind after separation of the higher-melting isomer (129° product) was allowed to stand at ordinary temperature in an open vessel. The lower-melting isomer deposited slowly in a colorless crystalline form which generally melted at 80–84° without further purification. This modification was always obtained as a secondary product of reaction in every one of our runs, and the yield varied between 20 and 30 g. It is easily purified by crystallization from 95% alcohol or acetone, and melts at 86° to an oil. When crystallized from acetone it separates in the form of characteristic transparent, prismatic crystals. It is insoluble in ether, benzene, chloroform and carbon disulfide.

Molecular-weight determinations were made by the cryoscopic and ebullioscopic methods using naphthalene and acetone, respectively, as solvents.

Calc. for $C_9H_{12}N_6$: mol. wt., 204. Found: (naphthalene), 202, 217; (acetone), 213, 215, 203.

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[14] Adams and Marvel, Univ. Illinois, *Bull.*, **49**, [6] 19 (1921), "Organic Chemical Reagents," III.

Crystallographic Study of the Two Modifications of Methylene Amino-acetonitrile Melting at 129° and 86°, Respectively.—Crystals of both were prepared by crystallization from acetone. Gradual cooling of the solutions produced supersaturation which was followed by slow crystallization. By this method large, well-developed crystals were grown easily without resorting to a constant temperature bath. The weight of a single, perfectly developed crystal from acetone was very frequently as much as 25 g. These were very frangible, however, and had a high coefficient of expansion. Consequently sudden changes of temperature were sufficient to cause the large crystals to crack easily.

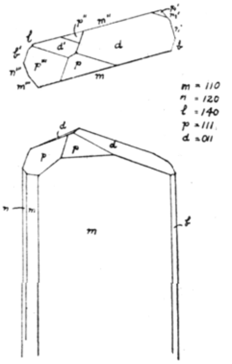

Fig. 1.—**Isomer melting at 129°.**

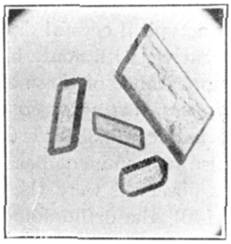

Fig. 2.—Microphotograph of the isomer melting at 129°.

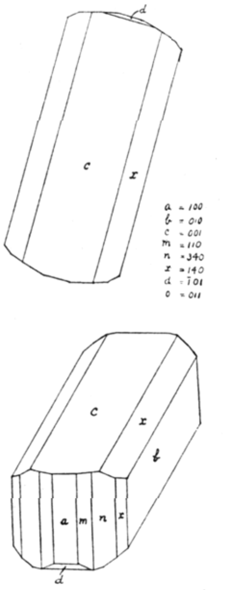

Fig. 3.—Isomer melting at 86°.

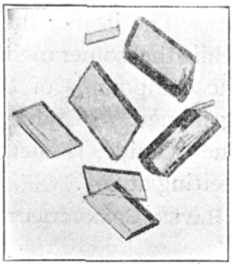

Fig. 4.—Microphotograph of the isomer melting at 86°.

Both forms crystallize from acetone without solvent of crystallization. We are indebted to Professor William E. Ford of the Department of Mineralogy for his assistance in obtaining the following crystallographic measurements of the two isomers.

The isomer melting at 129°.—Orthorhombic crystals. Axial ratio, a:b:c = 0.6690:1:0.4575. Crystals are prismatic in habit showing the following forms: the prisms, m(110), n(120), f(140), the pyramid p(111) and the brachy-dome d(011). The crystals are usually distorted through the larger development of a pair of prism faces. Fig. 1 represents a typical crystal drawn to show this distortion. Optically the crystals are biaxial, negative, with acute bisectrix normal to the basal pinacoid.

The appearance of these crystals is shown in the microphotograph, Fig. 2.

The isomer melting at 86°.—Monoclinic crystals. Crystal constants are =71°; axial ratio, a:b:c = 0.3379:1:0.4114. Crystals are tabular parallel to the clinopinacoid showing also a prominent basal place with narrow truncations of prisms and domes. The forms observed were the pinacoids, a(100), b(010), c(001), the prisms m(110), n(340), x(140), the ortho-dome d(101), and clino-dome x(011). Fig. 3 represents an ideal development of these crystals. Optically the crystals are biaxial, positive, with the acute bisectrix normal to the clino-pinacoid, b(010). Crossed dispersion with $\nu > \rho$, 2E = 96°.

The appearance of these crystals is shown in the microphotograph, Fig. 4.

Summary

1. Methylene amino-acetonitrile occurs in two isomeric modifications melting at 129° and 86°.
2. Molecular-weight determinations have been obtained which are not in agreement with values previously reported by Klages.[11] Both forms gave values conforming to the expression $C_9H_{12}N_6$ proving that neither isomer is correctly expressed by the formula $CH_2:NCH_2CN$.
3. The two isomers are sharply differentiated by their crystallographic properties. The isomer melting at 129° crystallizes in the orthorhombic system while the isomer melting at 86° crystallizes in the monoclinic system.
4. The compounds of this series will hereafter be designated by the following names: methylene amino-acetonitrile, $CH_2:NCH_2CN$; α-hydroformamine cyanide, isomer melting at 129°; β-hydroformamine cyanide, isomer melting at 86°.

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