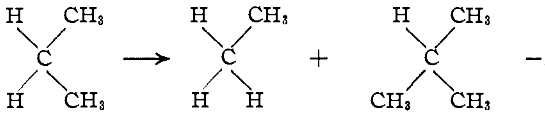
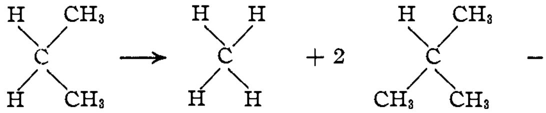
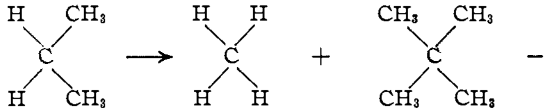
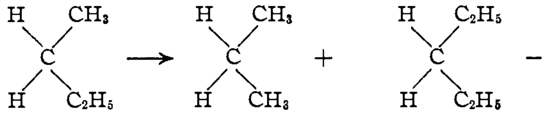
about a central carbon atom. Accordingly the heats of the following reactions were calculated from the data of Rossini.[8] In these reactions the number of carbon–carbon and carbon–hydrogen bonds remains unchanged. There has taken place merely a redistribution of hydrogen atoms, methyl, and ethyl groups about a central carbon atom. The heats of the first three reactions are in the ratio of one, three and four. This may be interpreted by assigning a repulsion to the hydrogen atoms and methyl groups arranged about the central carbon atom and by assuming that the repulsive forces are directed along the six edges of a tetrahedron. The net effect in reaction I is the conversion of two hydrogen–methyl interactions into a hydrogen–hydrogen and a methyl–methyl interaction. In reactions II and III the number of hydrogen–methyl conversions is simply three and four times as many as for I. This result may be summarized as

No.	Reaction'	H, cal.
I	2 	−2041
II	3 	−6315
III	2 	−7775
IV	2 	−59

$$2H{\cdot}CH_3 \longrightarrow H{\cdot}H + CH_3{\cdot}CH_3;\ \Delta H = -2030 \pm 100 \text{ cal.}$$

In reaction IV, the net effect is the conversion of two methyl–ethyl repulsions into one methyl–methyl and one ethyl–ethyl. Since the heat effect is zero, we may write

$$2CH_3{\cdot}C_2H_5 \longrightarrow CH_3{\cdot}CH_3 + C_2H_5{\cdot}C_2H_5 \qquad \Delta H \sim 0$$

The composition of equilibrium mixtures of tetraethyl and tetramethyl lead is determined, according to Calingaert and Beatty,[9] merely by the external symmetry numbers of the molecules involved. Stearn[10] pointed out that this means that the heat of the redistribution reaction must be zero.

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[8] F. D. Rossini, *Chem. Rev.*, **27**, 1 (1940).

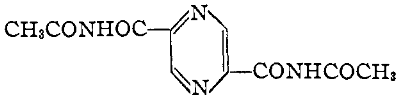
[9] Calingaert and Beatty, THIS JOURNAL, **61**, 2748 (1939).

[10] Stearn, *ibid.*, **62**, 1630 (1940).

Syntheses in the Pyrazine Series. Preparation of 2,5-Dicyanopyrazine and the Diacetyl Diamide of Pyrazine Dicarboxylic Acid-2,5

BY IRVING J. KREMS AND PAUL E. SPOERRI

Since Braun[1] and Tscherniac had reported that phthalic acid diamide undergoes a semi-dehydration to the amido nitrile when treated with acetic anhydride, we subjected the diamide of pyrazine dicarboxylic acid-2,5[2] to the same treatment. No change was detected after four hours; after forty-eight hours of refluxing, crystals of the diacetyl diamide (I) were obtained.



I

When a suspension of the diamide in nitrobenzene was treated with phosphorus pentoxide, the dinitrile was produced in low yield.

Experimental

Pyrazine Dicarboxylic Acid-2,5.[3]—Since the yields were quite poor the conditions of the permanganate oxidation of 2,5-dimethylpyrazine were modified. The results showed that the yield was independent of the permanganate concentration between two and twenty per cent., and the time of addition of the oxidizing solution between one and thirty-six hours, but was favored by an increase in *p*H. An oxidation of the insoluble mercuric chloride double salt instead of the free base indicated no protection to the pyrazine nucleus.

Twenty-seven grams of dimethylpyrazine[4,5] was dissolved together with 5 g. of potassium hydroxide in 150 ml. of water and warmed on the steam-bath in a 2,000 ml. three-necked round-bottom flask fitted with a separatory funnel, thermometer, condenser and stirrer. A hot solution of 158 g. of potassium permanganate in 760 ml. of water was added slowly over a period of one hour with stirring, the temperature being kept at 75°. Stirring was maintained for another half hour at the same temperature, after which the mixture was allowed to cool and stand overnight. The precipitate was filtered off and washed several times with hot water. The filtrate was evaporated to small volume, acidified with dilute nitric acid, and allowed to stand for one hour. The free diacid was filtered off, washed thoroughly with hot water, dissolved with hot dilute ammonium hydroxide and reprecipitated with dilute nitric acid. The precipitate was filtered off and dried *in vacuo* over sulfuric acid; yield 1.68 g., m. p. 253° (seal cap).

The Diacetyl Diamide of Pyrazine Dicarboxylic Acid 2,5 (I).—One-half gram of the diamide of pyrazine dicarboxylic acid-2,5[2] and 15 ml. acetic anhydride were placed in 50 ml. flask fitted with a two-foot length of glass tubing as an air condenser. Care was taken to eliminate moisture, and the air condenser was fitted with a calcium chloride tube. The mixture was refluxed for forty-eight hours at the end of which time it had turned completely black. It was allowed to cool, and then was placed in the ice chest for twenty-four hours. The resultant crystals were filtered

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[1] Braun and Tscherniac, *Ber.*, **40**, 2710 (1907).

[2] Spoerri and Erickson, THIS JOURNAL, **60**, 400 (1938).

[3] Stoehr, *Ber.*, **24**, 4108 (1892).

[4] Étard, *Compt. rend.*, **92**, 460, 795 (1881).

[5] Stoehr, *J. prakt. Chem.*, [2] **43**, 156 (1891); **47**, 439 (1893).

off, washed with acetic anhydride until the filtrate was almost colorless and then recrystallized from glacial acetic acid. After another recrystallization from the same solvent with the aid of a small amount of nuchar small white prisms were obtained; yield 0.15 g., m. p. 270–272°.

Anal.[6] Calcd. for $C_{10}H_{10}O_4N_4$: C, 47.97; H, 4.02. Found: C, 47.56; H, 4.23.

2,5-Dicyanopyrazine.—Attempts to totally dehydrate the diamide of pyrazine dicarboxylic acid-2,5 by a twenty-hour reflux with thionyl chloride, heating with phosphorus pentoxide at 320° for seven hours, and a straightforward distillation with the latter reagent, brought about little but decomposition. The following procedure was successful.

Eight-tenths gram of the diamide of pyrazine dicarboxylic acid-2,5[3] together with 10 g. of phosphorus pentoxide and 35 ml. of freshly distilled nitrobenzene were placed in a 50-ml. flask fitted with an air condenser, precautions against moisture being taken. The mixture was refluxed for four hours. The residue was filtered off, and washed three times with hot nitrobenzene; the combined filtrates were evaporated down to 2 ml. and then chilled. The crystals thus obtained were filtered off and recrystallized from ethyl ether with the aid of a pinch of nuchar. The yield was 0.03 g., m. p. 188–189°; white glistening plates soluble in benzene, nitrobenzene and ethyl ether; insoluble in petroleum ether and water.

Anal.[7] Calcd. for $C_6H_2N_4$: C, 55.38; H, 1.55. Found: C, 55.57; H, 2.03.

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(6) Microanalysis by Wm. Saschek, N. Y.

(7) Microanalysis by Arlington Laboratories, Fairfax, Va.

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Polarographic Analysis of Streptomycin

BY GABOR B. LEVY, PHILIP SCHWED AND J. WARREN SACKETT

In the recovery and purification of streptomycin,[1] as in all similar work (for instance with penicillin), the availability of a rapid and precise method of analysis (assay) is of fundamental importance. Such a method has not been available. Therefore, the development of a chemical method of analysis has been investigated.

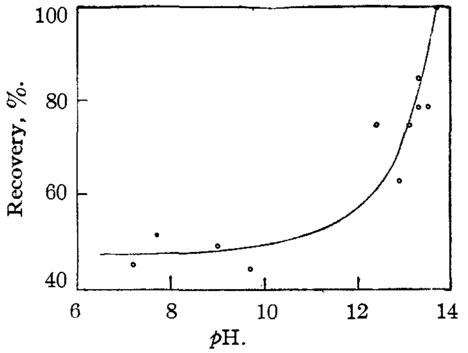

Fig. 1.

It was found that in alkaline medium, well-defined polarographic waves can be obtained with streptomycin solutions under certain conditions. By rigid standardization a method was obtained by which solutions of partially purified[2] streptomycin, which contain 200 μ/ml, or more, can be analyzed.

As a supporting electrolyte we used tetramethylammonium hydroxide (3% in the final solution) the use of which eliminates maxima and which maintains the *p*H at a sufficiently high level. This is important since the lowering of the *p*H causes a rapid diminishing of the diffusion current and concurrently appearance of irregularities. The level of *p*H 13.6 to 13.8 when resulting from tetramethylammonium hydroxide in 3% concentration does not act destructively on streptomycin at 13.6° within an hour. The influence of the *p*H on polarographic analysis is represented in Fig. 1 and it is evident that *p*H conditions should not be varied.

The effect of temperature on the diffusion current is 3%/°C. around room temperature which is comparable to the effect found in other polaro-

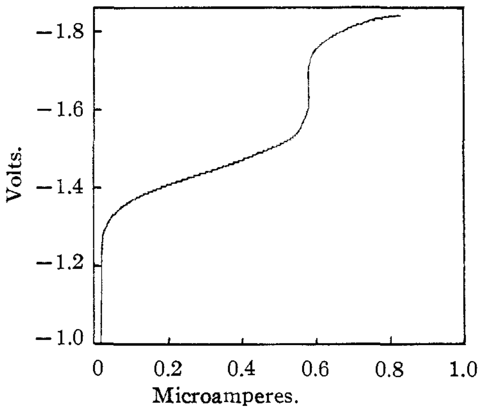

Fig. 2.

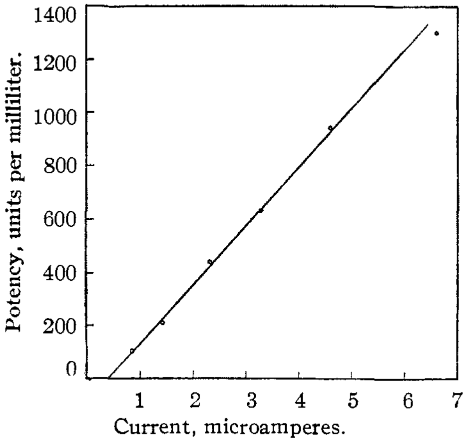

Fig. 3.

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(1) Carter, *et al.*, *J. Biol. Chem.*, **160**, 337 (1945); also Peck, *et al.*, THIS JOURNAL, **67**, 1866 (1945).

(2) Broth containing streptomycin and in some instances salt solutions contain materials which interfere with the analysis by producing additional polarographic waves. Preliminary work indicated that after precipitation of the impurities by lead ions and the removal of the excess lead by sulfuric acid, solutions were obtained which could be analyzed.