

represents the tendency toward oxidation, that is, the reducing activity of ascorbic acid. The concentration of ascorbic acid checks its reducing activity, which determines the constant of the potential of solutions ( $pH$  2.7 at  $20^\circ$ ) near 0.4  $N$ . In complex media, the vitamin activity can obviously be limited by factors other than its own concentration. In biological media with  $rH$  clearly above 16.2, the stability of ascorbic acid is due principally to this cause. In fact, it is known that the normal oxidation-reduction potential of ascorbic acid<sup>4</sup> corresponds to a  $pH$  of 7 with an  $rH$  of approximately 16.2, while the average value of the blood  $rH$  is<sup>5</sup> 20.8.

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<sup>1</sup> Bezssonoff, N., and Woloszyn, M., *Bull. Soc. Chim. Biol.*, 21, 203 (1939).

<sup>2</sup> Bezssonoff and Woloszyn, *loc. cit.* The  $pH$  stability of the ascorbic acid solutions was ascertained by means of an antimony electrode system.

<sup>3</sup> Heintz, *C.R. Acad. Sci.*, 208, 1893 (1939).

<sup>4</sup> Borsook, H., and Keighley, G., *Proc. Nat. Acad. Sci.*, 19, 1875 (1933). Wurnser, R., *Actual. Sci. et Indust.*, 244, 56 (1935). Ball, E., *J. Biol. Chem.*, 118, 219 (1937).

<sup>5</sup> According to the potentiometric measurements of Vles, Reiss and Deloyers, *C.R. Soc. Biol.*, 108, 37 (1931). Outevskaya, *Bull. Biol. et Med. Exper. U.R.S.S.*, 5, 226 (1933). Rafalkes, Tzoutzoukowskaya et Chmerson, *Bull. Soc. et Med. Exper. U.R.S.S.*, 5, 191 (1933).

#### Ozonization of *O*-Xylene and the Structure of the Benzene Ring

A. A. Levine and A. G. Cole<sup>1</sup> ozonized *o*-xylene and were able to isolate diacetyl from the reaction product, while methylglyoxal and glyoxal were isolated in the form of the osazones by means of *p*-nitrophenylhydrazine. This result is of great importance for the theory of the structure of the benzene nucleus. In their paper, Levine and Cole give no evidence about the yield of the substances isolated. Therefore we have reinvestigated this reaction using another analytical method. We have transformed the decomposition products of the ozonides into the corresponding oximes and we have worked out a method for separating the oximes quantitatively.

*O*-xylene (solidification point  $-27^\circ$ ) was ozonized in chloroform solution at  $-25^\circ$ , using oxygen with 2.5 per cent ozone until about the calculated quantity of ozone had been taken up. From the reaction product we isolated:

(1) The oxime of dimethylglyoxal melting at  $233-234^\circ$ , mixed melting point with an authentic sample (m.p.  $234-235^\circ$ )  $233.5-234^\circ$ . (2) The oxime of methylglyoxal melting at  $150.5-151.5^\circ$ , mixed melting point with an authentic sample (m.p.  $152.8-153.1^\circ$ )  $152-152.5^\circ$ . (3) The oxime of glyoxal melting at  $170.5-171.5^\circ$ , mixed melting point with an authentic sample (m.p.  $172.5-173^\circ$ )  $171.2-172^\circ$ .

The yield of oximes amounted to about 20 per cent of the theoretical yield calculated on *o*-xylene. The composition of the mixture was roughly determined as follows: 0.88 mol. of dimethylglyoxime, 2 mol. of methylglyoxime and 3.2 mol. of glyoxime,

while the calculated ratio should be 1 : 2 : 3, if the two Kékulé forms each contribute 50 per cent to the structure of *o*-xylene. It will be necessary to improve our analytical method before we can give the exact value for the molecular ratio of the oximes.

The work of two independent groups of workers now affords chemical evidence for the occurrence in *o*-xylene of two resonating Kékulé structures. The investigation is being continued from the quantitative point of view; full details will be published in the *Recueil des Travaux Chimiques des Pays-Bas*.

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<sup>1</sup> Levine, A. A., and Cole, A. G., *J. Amer. Chem. Soc.*, 54, 333 (1932).

#### Photo-oxidation of Pyrrole

FRESHLY redistilled pyrrole dissolved in water, alcohol or acetone and mixed with  $0.5 \times 10^{-4}$   $M$  methylene blue rapidly takes up oxygen in the light but not in the dark. Pyrrole alone is not oxidized. The rate of oxygen uptake is a linear function of light intensity. Eosin, but not fluorescein, can be used instead of methylene blue. Tests with standard optical filters showed the effective wave-lengths to be within the range of 5200-5800 Å. On the basis of 5500 Å., the energy necessary to institute the reaction is  $3.4 \times 10^{-12}$  ergs/molecule.

Two atoms of oxygen are taken up per molecule pyrrole. There was no decarboxylation or deamination. The methylene blue was removed by a minimal amount of charcoal and the solution evaporated to dryness. The residue was extracted with acetone which on evaporation left crystals. These were recrystallized several times from boiling benzene. The analysis was C, 49.1 per cent, H, 5.3 per cent, N, 14.1 per cent; m.p.,  $102.5^\circ$ ; yield, 58 per cent. Mixed melting point determinations showed that the substance was not succinimide. On alkaline hydrolysis, it yielded 14 per cent ammonia nitrogen and 72 per cent succinic acid.

*n*-methyl and *n*-ethyl pyrrole are also oxidized under the same conditions, but only one atom of oxygen is taken up per molecule.

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#### Crotoxin

IN May of last year<sup>1</sup> we announced that we had been able to purify and crystallize the chief toxic principle of rattlesnake venom, which we called crotoxin. Crotoxin contains the whole neurotoxic and hæmolytic activity of the crude venom; but both have a 25 per cent greater activity than the crude.

The following are the main points of evidence that crotoxin is a pure and homogeneous substance:

(1) The analytical data<sup>2</sup> obtained from different samples of crotoxin at different times are the same (C: 50.77 per cent and 50.56 per cent; H: 6.41 per