acid was added in excess with stirring. The resulting oil was extracted with ether, dried over anhydrous sodium sulfate, filtered and concentrated over a steam-bath. When the temperature of the glutaric acid reached 70°, 280 g. of polyphosphoric acid,[5] previously heated to 70°, was added and the mixture stirred for 20 minutes and then poured onto approximately 1 kg. of crushed ice. On standing in the refrigerator overnight, the slightly yellow material was filtered and dried; yield 27 g. (90%). The product was recrystallized from chloroform–petroleum ether (30–60°); m.p. 178–180°. A mixture melting point with the sample prepared by the sulfuric acid procedure gave no depression. This procedure was used for large-scale (100–200 g.) runs and the same yield realized. The infrared spectrum showed the presence of the carbon–oxygen double bond (1668 cm.$^{-1}$) and of the carboxyl group (1703 cm.$^{-1}$).

*Anal.* Calcd. for $C_{14}H_{16}O_5$: C, 63.63; H, 6.10. Found: C, 63.41; H, 5.93.

The yields of crude product obtained on the polyphosphoric acid ring closure under various conditions were: Mixing reactants previously heated separately to 90° and stirring over steam-bath for 2.5 minutes followed by stirring with no heat for 5 minutes, then adding to crushed ice, 51.3%; same as before except heated for 1 minute only, and standing 5 minutes, 62.8%; heating for 2 minutes and immediately adding to ice, 83%; heating for 1.5 minutes and immediately adding to ice, 90%.

**3,4-Dihydro-6,7-dimethoxy-3-methyl-1-(2)naphthalenone (III).**—A mixture of 25 g. of the tetralonecarboxylic acid, 0.3 g. of cupric oxide and 20 ml. of quinoline was heated in an oil-bath at 200° for 1 hour. After cooling to room temperature it was added with stirring to an excess of cold dilute hydrochloric acid and extracted with chloroform. The chloroform extract was washed successively with dilute sodium bicarbonate, acetic acid and water, then dried over anhydrous sulfate and concentrated to dryness to give 10.3 g. (47% yield) of the desired tetralone. Recrystallization from methanol gave a crystalline material, m.p. 130–132° (reported 132–133°[2]). The infrared spectrum showed the presence of the carbon–oxygen double bond (1662 cm.$^{-1}$).

*Anal.* Calcd. for $C_{13}H_{16}O_3$: C, 70.88; H, 7.32. Found: C, 70.66; H, 7.20.

Acidification of the aqueous sodium bicarbonate extract yielded 4 g. of starting material.

It was found that the yield obtained on decarboxylation decreased materially when impure starting material was used. Attempts to increase the yield by (1) using the copper salt of the carboxylic acid, (2) lower or higher temperatures, (3) heating while under vacuum all gave yields lower than that reported above.

**2-Bromo-3,4-dihydro-6,7-dimethoxy-3-methyl-1(2)naphthalenone.**—To a solution of 5.0 g. (0.023 mole) of the tetralone in 100 ml. of chloroform, there was added dropwise with stirring over a period of 30 minutes a solution of 1.2 ml. (0.023 mole) of bromine in 35 ml. of chloroform. The resulting solution was allowed to stir while in a hood for an additional 12 hours, after which the chloroform was evaporated over a steam-bath. On standing overnight, the oil obtained solidified and was recrystallized from ligroin (90–120°) to give 6.1 g. (90% yield) of colorless crystals m.p. 118–120°. The infrared spectrum showed the presence of the carbon–oxygen double bond (1673 cm.$^{-1}$).

*Anal.* Calcd. for $C_{13}H_{15}BrO_3$: C, 52.19; H, 5.05; Br, 26.71. Found: C, 52.33; H, 4.96; Br, 26.55.

**6,7-Dimethoxy-3-methyl-1-naphthol (IV).**—(a) An intimate mixture of 12.5 g. (0.057 mole) of the tetralone III and 1.85 g. (0.057 mole) of sulfur was heated in an oil-bath at 245° for 1 hour, at the end of which time the evolution of hydrogen sulfide had ceased. The product was then distilled at 160–165° (0.5 mm.). The distillate which solidified in the receiver was removed with ether and after evaporation of the solvent, crystallized first from toluene–petroleum ether (30–60°) and then from ligroin (90–120°); white crystals, m.p. 140–142°, yield 10 g., 80%.

(b) A solution of 5 g. of 2-bromo-3,4-dihydro-6,7-dimethoxy-3-methyl-1(2)naphthalenone in 30 ml. of N,N-dimethylaniline was heated under reflux for 30 minutes. After cooling this solution was poured with stirring onto a mixture of crushed ice and an excess of hydrochloric acid. The red precipitate was filtered, dried and recrystallized from ligroin (90–120°) to give 1 g. (27% yield) of colorless crystals, m.p. 140–142°. A mixture melting point with the sample prepared above gave no depression. When a solution of the bromoketone in N,N-dimethylaniline was heated on the steam-bath for 1 hour and worked up, only starting material was recovered.

The infrared spectra of the two samples were identical and showed the presence of the hydroxyl group (sharp band at 3620 cm.$^{-1}$ and a broad band at 3430 cm.$^{-1}$).

*Anal.* Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.59; H, 6.66.

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(5) Victor Chemical Works, Chicago, Illinois.

## Osazone Formation from Mixtures of Glucose and Fructose[1]

By James Ashmore[2] and Albert E. Renold[3]

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In recent studies on the metabolism of carbon-14 *d*-glucose and *d*-fructose[4] where both sugars were present in the same medium, we have found it necessary to compute the relative contribution of each of these to the osazone formed from the media. Both hexoses form the same osazone, but react at different rates.[5] Maquenne[6] first proposed that the rate of osazone formation might be used as a means of differentiating sugars, and reported that in the course of an hour 1 g. of fructose formed 0.70 g. of osazone compared with 0.32 g. formed from a corresponding quantity of glucose. Garard and Sherman[7] have noted that glucose does not react quantitatively with phenylhydrazine to form glucosazone. Only under the most carefully controlled conditions could yields greater than 70% (max. yield 81%) be obtained. Van Laer and Lombaers[8] have attributed the difference in the reaction of glucose and fructose to the second stage, or oxidation of the adjacent alcohol to a carbonyl.

Neuberg[9] has reported that fructose and methylphenylhydrazine will react to give an 81% yield on heating in a boiling water-bath for only 10 minutes. Glucose forms an osazone with this reagent only after prolonged heating.[10] Methylphenylhydrazine has therefore been suggested as a specific reagent for fructose. We have found this reagent to be of little value for our purpose. Methylphenylosazones are more difficult to form than the corresponding phenylosazones. We have been unable to prepare crystalline methylphenylosazones from

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(2) Schering Fellow of the Endocrine Society, 1953–1954.

(3) Post-Doctoral Fellow of the United States Public Health Service, 1951–1953.

(4) A. E. Renold, A. B. Hastings and F. B. Nesbett, *J. Biol. Chem.*, in press.

(5) E. Fischer, *Ber.*, **17**, 579 (1884).

(6) M. Maquenne, *Compt. rend.*, **112**, 799 (1891).

(7) D. D. Garard and H. C. Sherman, This Journal, **40**, 955 (1918).

(8) M. H. van Laer and R. Lombaers, *Bull. soc. chim. Belg.*, **30**, 296 (1921).

(9) C. Neuberg, *Ber.*, **37**, 4616 (1904).

(10) P. B. Hawk, B. L. Oser and W. H. Summerson, "Practical Physiological Chemistry," 12th ed., Blakiston, Philadelphia, Pa., 1947, p. 65.

pure solutions containing less than 50 mg. per ml. of fructose. On the other hand, phenylosazones are readily formed, even from biological samples, when the concentration of hexose is as low as 2.5 mg. per ml. Using mixtures of carbon-14-labeled glucose and non-isotopic fructose, we have been able to determine that during the 10 minutes heating 4 to 6% of the glucose present reacts with methylphenylhydrazine. The presence of radioactivity in the resultant methylphenylosazone after several recrystallizations and treatment with animal charcoal suggests that glucose has reacted and is not present only as an impurity. Although fructose reacts 3.5 to 4 times as fast as glucose under these conditions, the reaction is by no means specific for fructose.

In our experiments, 10 moles of phenylhydrazine hydrochloride, to 1 mole of hexose and 25 moles of sodium acetate reacted in a boiling water-bath for various lengths of time. The reaction was arrested by cooling in an ice-bath and the yield of glucosazone determined gravimetrically according to the method of Garard and Sherman.[7] The results of such studies are given in Fig. 1. It is apparent that the rates of reaction decline rapidly after 120 minutes to give a maximum yield of 97% for fructose and 48% for glucose. Heating for periods greater than 200 minutes, as previously noted,[7] may actually result in a decreased yield. The relative rates of reaction calculated from the portions of the curves under 60 minutes[11] show $K_F/K_G = 2.6$; thus the mole ratio F/G in the osazone would be 2.6. As the reaction proceeds, this ratio decreases and approaches a limit of 2.0.

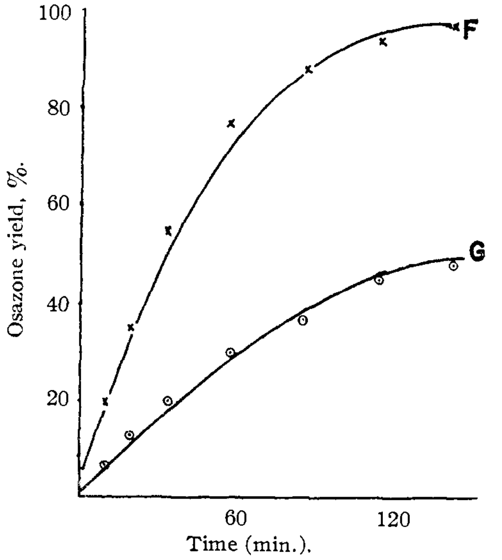

Fig. 1.—The percentage of theoretical yield of phenylosazone obtained from fructose and glucose with time of heating. The sugar was present at a concentration of 3 mg./ml. in the reaction mixture, and the molar ratio of hexose/phenylhydrazine hydrochloride/sodium acetate was 1/10/25. The solutions were heated in a boiling water-bath for the various periods of time.

TABLE I

FORMATION OF PHENYLOSAZONE FROM MIXTURES OF GLUCOSE AND FRUCTOSE[a]

| (A) F/G in the reaction mixture | Sugar labeled | Sp. activity osazone,[b] c.p.m./mM | (B) F/G in the osazone | B/A |
|---|---|---|---|---|
| | | 30 minutes | | |
| 4.00 | G | 9,050 | 10.6 | 2.65 |
| 1.50 | G | 21,800 | 3.8 | 2.54 |
| 0.67 | G | 40,500 | 1.6 | 2.40 |
| .25 | G | 64,000 | .63 | 2.52 |
| | G | 104,000 | | |
| | | 90 minutes | | |
| 0.11 | F | 105,600 | .25 | 2.24 |
| .25 | F | 180,600 | .52 | 2.08 |
| .43 | F | 240,000 | .82 | 1.92 |
| .67 | F | 309,000 | 1.43 | 2.12 |
| 1.00 | F | 351,000 | 2.00 | 2.00 |
| 2.33 | F | 425,000 | 4.00 | 1.72 |
| | F | 528,000 | | |
| 9.00 | G | 38,000 | 15.4 | 1.75 |
| 4.00 | G | 61,400 | 9.0 | 2.26 |
| 2.33 | G | 102,500 | 5.25 | 2.26 |
| 1.50 | G | 143,500 | 3.34 | 2.22 |
| 1.00 | G | 193,000 | 2.22 | 2.22 |
| 0.43 | G | 306,500 | 1.04 | 2.24 |
| | G | 621,000 | | |

[a] Molar ratios of hexose:phenylhydrazine hydrochloride:sodium acetate 1:10:25 were heated in a boiling water-bath for the times indicated. [b] Crystalline osazones were plated directly on planchettes having an area of 1.54 cm.,[2] corrected to a standard of 5 mg. and counted in a gas flow counter.

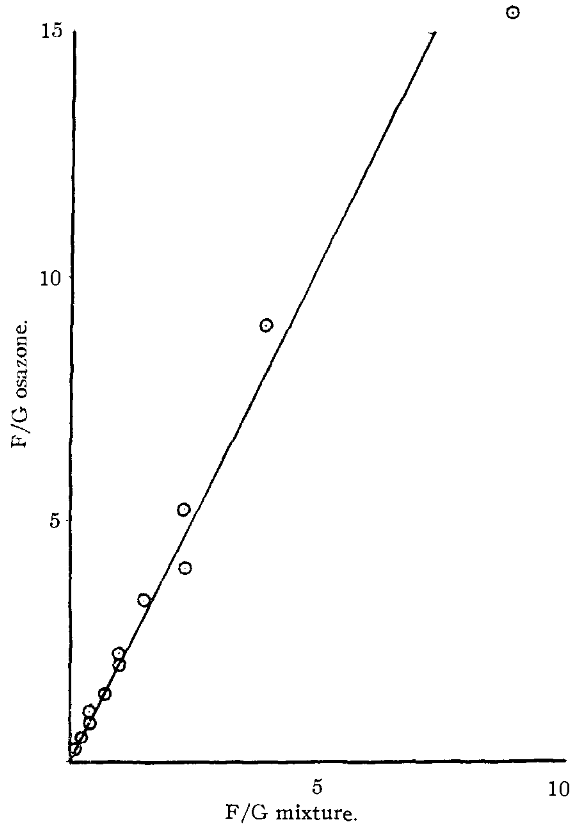

Fig. 2.—The fructose/glucose ratio in phenylosazones formed from mixtures of fructose and glucose.

Because of the inherent errors in the determination of osazones, and because of possible intercon-

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(11) H. B. Bull, "Physical Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1943, p. 42.

version that may occur when the two sugars are heated together during the course of the reaction, we have studied the reaction rates of mixtures of glucose and fructose in which only one of the sugars was labeled with carbon-14. By purification of the resultant osazone and determination of its specific activity, and by knowing the specific activity of the osazone derived from the labeled hexose alone, we can calculate the relative contributions of glucose and fructose to the osazone. Table I gives the calculated ratios of F/G in the osazone formed from the various mixtures. Plotting F/G in the reaction mixture against the calculated F/G in the osazone gave Fig. 2.

We have demonstrated that 2.0 to 2.2 molecules of fructose react per molecule of glucose under the conditions specified. It should be noted, however, that during the early course of the reaction, before the concentrations of the substrates become limiting, fructose reacts 2.5 to 2.6 times as fast as glucose. A maximum yield of phenylglucosazone from glucose of 50% was the highest ever obtained under our conditions, whereas fructose was quantitatively converted to the osazone under the same conditions.

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## Some Physical Properties of *p*-Aminophenol

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During the development of an improved process for the production of *p*-aminophenol, it became necessary either to check or to determine for the first time some of the physical properties of the compound.

**Melting Point.**—The m.p. was determined under nitrogen on two recrystallized samples of *p*-aminophenol. In each case *p*-aminophenol (assaying 99.1% by Kjeldahl, m.p. 189–190° under $N_2$) was recrystallized once from alcohol and once from water, oxygen being carefully excluded. Samples were placed in m.p. tubes (soft glass) and the tubes were four times evacuated to about 1 mm. pressure and flushed with nitrogen. The tubes were then sealed off, bound to a 76 mm. immersion thermometer and placed in a preheated silicone oil-bath (estimated accuracy ±0.3) equipped with mechanical agitation. Both samples melted 191 to 192° (uncorrected) without visible signs of decomposition. Two other samples of *p*-aminophenol were sublimed (110°, 0.3 mm. pressure), and their melting points were determined in the same manner: 189.6 to 190.2° and 189.8 to 190.6°. The latter range could be repeated on the same sample by lowering and raising the temperature. All of these values are appreciably higher than those variously reported in the literature,[1,2] which average around 185° and are in each case accompanied by decomposition. Our higher values are undoubtedly the result of rigorous exclusion of oxygen.

**Vapor Pressure.**—The vapor pressure of a small sample of sublimed *p*-aminophenol (m.p. 190°) was determined in an isoteniscope at temperatures of from 130 to 186°. Table I lists the observed vapor pressures, corrected for that of mercury, as a function of temperature.

TABLE I

VAPOR PRESSURE OF *p*-AMINOPHENOL

| *t*, °C. | *p*, mm. | *t*, °C. | *p*, mm. |
|---|---|---|---|
| 130.2 | 0.3 | 159.6 | 5.1 |
| 143 | 2.2 | 167.0 | 8.0 |
| 145 | 2.2 | 171.1 | 9.9 |
| 150 | 3.0 | 176.5 | 14.9 |
| 151.1 | 3.2 | 185.3 | 26.7 |
| 157.5 | 4.7 | 284 | *ca.* 760 |

A value of 22 kcal./mole for the heat of sublimation can be obtained from these data. The b.p., included above, was determined during distillation under a blanket of nitrogen; there was approximately 7% decomposition during distillation, as determined on the solid distillate by nitrite absorption.[5] The pressure measurement at 185.3° was complicated by the slow evolution of a second gas, correction for which was made by extrapolation to zero time. This gas evolution was probably caused by oxidative decomposition of *p*-aminophenol, since approximately 0.05 mm. partial pressure of air remained from the initial evacuation of the system.

**Solubility.**—The solubility of *p*-aminophenol in water was checked in order to clear up discrepancies between the values reported by Sidgwick and Callow[2,3] and found by Cragwall.[4] At nearly all temperatures, solubilities determined by the former authors are nearly twice as great as those of the

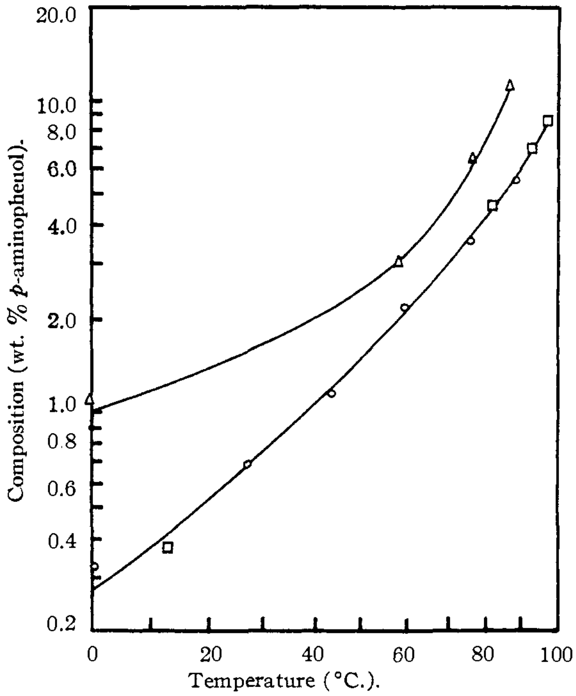

Fig. 1.—Solubility of *p*-aminophenol in water: △, ref. 2; ○, ref. 4; □, present work.

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(1) "Beilstein," Vol. 13, p. 427.

(2) N. V. Sidgwick and R. K. Callow, *J. Chem. Soc.*, **125** 522 (1924).

(3) A. Seidell, "Solubilities of Organic Compounds," 3rd Edition, Vol. 2, D. Van Nostrand Co., Inc., New York, N. Y., 1941, p. 421.

(4) G. O. Cragwall, Jackson Laboratory work.