Summary

The color reaction with benzaldehyde and sulfuric acid known to identify picrctoxin was found to be a sensitive color reaction common to aliphatic compounds in general which have a C=C double bond. This color reaction and the color reaction of double bond by tetranitromethane are similar in some respects, however, the former is specific to aliphatic unsaturated compounds.

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17. **Masuo Akagi, Setsuzo Tejima, and Masanobu Haga :** Reaction Products of Glucuronic Acid with Phenylhydrazine.

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Since, in 1878, D-glucuronic acid was found in nature, it has been studied by many investigators chemically and biochemically.

Hitherto several reports dealing with the glucuronic acid phenylosazone also have been published, however, there are numerous discrepancies among them (Table I). For the cause of these discrepancies, it seemed that, in that time, glucuronic acid was prepared from natural products so the reported osazone might be contaminated, or that glucuronic acid is able to react with phenylhydrazine in various ways so the osazone might be corresponded to one of the isomers.

Previously, two kinds of crystals$^{1)}$ (m.p. 170° and m.p. 124°) were obtained in our laboratory during the identification of glucuronic acid through its osazone. In this paper, the structures of crystals aforesaid are discussed and another crystalline derivative is also described.

Generally, glucuronic acid has two functions to be able to react with phenylhydrazine : formyl and carboxyl group; and on one hand, glucuronic acid exists in two states: glucofuranurono-γ-lactone (I) and glucopyranuronic acid (Ⅱ). It is sufficiently probable that the reaction products vary according to (I) or (Ⅱ), since carboxylic acid does not form phenylhydrazide by only heating on a steam bath, while lactone is able to form it. From this assumption, the authors examined the reaction of (I) and (Ⅱ) with phenylhydrazine individually.

One part of sugar, two parts of phenylhydrazine and two parts of 50% acetic acid were dissolved in twenty parts of water and resulting solution was heated on a steam bath, the reaction was controlled by varying the heating period. As to (I), the mixture changed its color to yellow after *ca.* 5 minutes, to turbid red after *ca.* 15 minutes and yielded dark red syrup ultimately. In the course of reaction, the yellow solution gave pale yellow needles (S_1) m.p. 170~171° by cooling, but the authors failed to obtain constitutionally definite substance from aforesaid dark red syrup. Likewise, (S_1) was obtained either from methyl glucuronate (Ⅲ) under similar conditions as stated above, or by

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1) M. Akagi, S. Tejima, M. Haga : This Bulletin, **8**, 1114 (1960).

2) E. Chargaff, B. Magasanik : J. Am. Chem. Soc., **69**, 1459 (1947).

3) A. Herzfeld : Ber., 28, 442 (1895).

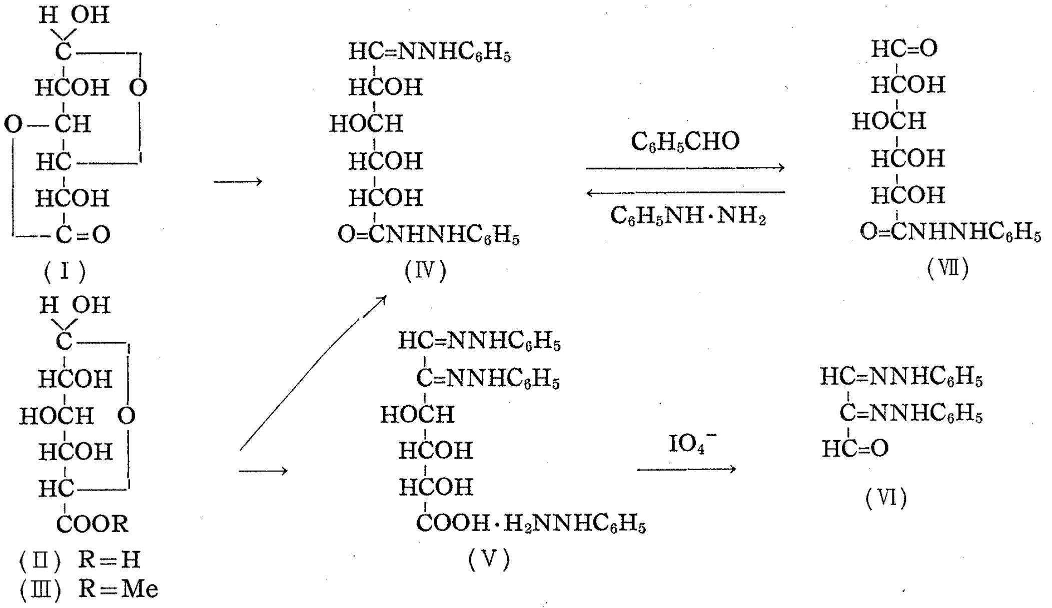
treatment of (I) with excess of phenylhydrazine in hot ethyl alcohol. The chemical composition of (S_1) was shown to be a formula $C_{18}H_{22}O_5N_4$ and its infrared absorption spectrum exhibited no absorption corresponding to lactone (1750 cm^{-1}) and carboxylic acid (1705 cm^{-1}) but acid-hydrazide (1670 cm^{-1}) concerning carboxyl of glucuronic acid.

By oxidation with periodate according to the method devised by Chargaff and Magasanik,[2)] it consumed three moles of periodate and did not give 1,2-bisphenylhydrazone of mesomaloxalaldehyde (VI), which is expected from osazone structure. Besides, (S_1) gave easily glucuronic acid phenylhydrazide (VII) under the condition by which sugar was usually recovered from its phenylhydrazone.[3)] (VII) was easily crystallized from alcohol to colorless needles, and by treatment of (VII) with phenylhydrazine, only (IV) was obtained taking up one mole of phenylhydrazine and no such substance having the structure of osazone was produced. From these facts, it was concluded that (S_1) has the structure as phenylhydrazone of glucuronic acid phenylhydrazide (IV). Of glucopyranuronic acid (II), under the same condition, the mixture gave yellow needles (S_2), m.p. 123~124° irrespective of the heating period. Product (S_2) had the chemical composition of $C_{24}H_{28}O_5N_6 \cdot H_2O$. The infrared absorption spectrum of (S_2) showed characteristic absorption at 1560 cm^{-1} and there was no absorption within the range of 1650~1750 cm^{-1}, accordingly, it is obvious that the carboxyl of glucuronic acid is ionised.

TABLE I.

Author	m.p. (°C)	Formula	Reference
H. Thierfelder	114~115	$C_{42}H_{48}O_{10}N_{10}$	a
J. A. Hirschl	110~114 107~108 150		b
P. Mayer	210~217 159~164		c
C. Neuberg and W. Neimann	200~202 212	$C_{18}H_{20}O_5N_4$ $C_{24}H_{26}O_4N_6$	d
L. Grimbert and R. Bernier	130~132		e
G. Goldschmidt and E. Zerner	192~195 122~123	$Ba(C_{18}H_{19}O_5N_4)_2$ $C_{18}H_{20}O_5N_4$	f
P. A. Levene and J. Lopez-Suàrez	125	$C_{24}H_{26}O_4N_6 \cdot 1.5\,H_2O$	g

a) Z. physiol. Chem., Hoppe-Seyler's **11**, 395 (1887). b) *Ibid.*, **14**, 382 (1890). c) *Ibid.*, **29**, 59 (1900). d) *Ibid.*, **44**, 96 (1905). e) J. Pharm. Chem., **330**, 529 (1909). (Chem. Zentr., 576 (1910)). f) Monatsh. Chem., **31**, 477 (1910). g) J. Biol. Chem., **36**, 118 (1918).



By oxidation of (S_2) with periodate as aforesaid, (S_2) consumed more than three moles of periodate and gave (VI), which was found to be identical with the periodate oxidation product of glucosazone.

These facts showed that (S_2) has the structure of phenylosazone of phenylhydrazine glucuronate (V). These (IV) and (V) can be easily separated by recrystallization from ethyl alcohol, however, it is unsuitable to identify glucuronic acid through osazone.

Experimental

Pheylhydrazone of Glucuronic Acid Phenylhydrazide (IV)——a) From glucofuranurono-γ-lactone (I) or methyl glucuronate (III)[4]: A mixture of 0.5 g. of (I) (m.p. 176~178°) or (III), and 1.0 g. of freshly distilled phenylhydrazine in 1 cc. of 50% AcOH was dissolved in 20 cc. of water and resulting mixture was heated on a steam bath for 5 min. After cooling, the pale yellow precipitates were filtered off and washed with a small amount of water and EtOH. Recrystallization from pyridine-water or EtOH gave almost colorless needles which melted at 170~171°. Yield, 0.6 g. (55%).
b) From (I) and phenylhydrazine in ethyl alcohol: A mixture of 0.5 g. of (I) and 3.0 g. of phenylhydrazine was dissolved in 15 cc. of EtOH and heated on a steam bath under reflux for 30 min. On cooling, (IV) separated in the form of needles, which were filtered off and washed with EtOH. Yield, 0.82 g. (80%).

A substance (IV) was sparingly soluble in water and EtOH, soluble in Me_2CO and pyridine. It had an initial (three min.) rotation of $[\alpha]_D^{16}$ $-1.9°$ (c=0.80, pyridine), which increased to a constant value of $-20.7°$ after 24 hr. *Anal.* Calcd. for $C_{18}H_{22}O_5N_4$: C, 58.06; H, 5.38; N, 15.05. Found: C, 58.40; H, 5.66; N, 15.03.

Glucuronic Acid Phenylhydrazide (VII)——A mixture of 2.0 g. of (IV), 1.6 g. of benzaldehyde, 15 cc. of 95% EtOH and 5 cc. of water was refluxed on a steam bath for 5 hr. To the cooled mixture, 30 cc. of water was added and extracted with three 50 cc.-portions of Et_2O. The aqueous layer was decolorized and evaporated to dryness in a reduced pressure, leaving 0.9 g. (60%) of colorless syrup which was easily crystallized from dehyd. EtOH. After two recrystallizations from dehyd. EtOH, (VII) was obtained as needles. (VII) was easily soluble in H_2O, moderately soluble in MeOH and EtOH, and practically insoluble in Et_2O and benzene. Also (IV) was obtained from (VII) and phenylhydrazine in either conditions stated above. m.p. 165~167°. $[\alpha]_D^{16}$ 58.1° decreased to 24.6° after 24 hr. (c=0.89, H_2O). *Anal.* Calcd. for $C_{12}H_{16}O_6N_2$: C, 50.70; H, 5.67; N, 9.86. Found: C, 50.77; H, 5.75; N, 9.81.

Phenylosazone of Phenylhydrazine Glucuronate (V)——A mixture of 1.0 g. of glucopyranuronic acid (II) (m.p. 150~153°), 2.0 g. of freshly distilled phenylhydrazine and 2 cc. of 50% AcOH was dissolved in 40 cc. of water and heated on a steam bath till yellow precipitates appeared (15 min.). After cooling, the resulting crystals were filtered off and washed with a small amount of cold water. The crude product (0.4 g.) was recrystallized from 95% EtOH to yellow needles. m.p. 123~124°. $[\alpha]_D^{16}$ $-103.1°$ changed to $-86.8°$ after 24 hr. (c=0.92, pyridine). *Anal.* Calcd. for $C_{24}H_{28}O_5N_6 \cdot H_2O$: C, 57.80; H, 6.06; N, 16.85. Found: C, 58.04; H, 6.12; N, 16.69.

Periodate Oxidation of (IV) and (V)——The experiment was carried out in the same way as devised by Chargaff and Magasanik.[2] A product (IV) consumed 3.2 moles of periodate and did not give 1,2-bisphenylhydrazone of mesomaloxalaldehyde (VI). However, (V) gave (VI), m.p. 197~198° and to have the same properties described by Chargaff, *et al.*[2]

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Summary

For the reaction products of glucuronic acid with phenylhydrazine under the condition to form phenylosazone, phenylhydrazone of glucuronic acid phenylhydrazide and phenylosazone of phenylhydrazine glucuronate were obtained. Glucuronic acid phenylhydrazide was also described.

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[4] G.N. Bollenback, J.W. Long, D.G. Benjamin, J.A. Lindquist: J. Am. Chem. Soc., **77**, 3310 (1955).