Hirst and Teece[2] when methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranoside was methylated with the Purdie reagent. In that instance the acetyl migrated to give the 3,4,6-tri-*O*-acetyl derivative and methylation took place at carbon atom C-2. Thus the present reaction is another illustration that acetyl groups cannot be used to block particular hydroxyl groups in a sugar derivative, if the derivative is at any time subject to mild alkaline treatment.

Proof that the methyl ether group is at carbon atom C-2 was established through the 2-*O*-methyl-D-glucophenylhydrazone derivative, the D-glucophenylosazone derivative, and the strong dextrorotary shift of the methyl mono-*O*-methyl-α-D-glucopyranoside in cuprammonium solution.[3] The latter property is characteristic and readily distinguishes the methyl 2-*O*-methyl derivative from the methyl 4-*O*-methyl-α-D-glucopyranoside which exhibits a strong levorotatory shift in cuprammonium.[4]

Experimental

2-*O*-Methyl-D-glucophenylhydrazone.—Methyl mono-*O*-methyl-α-D-glucopyranoside (1 g.), prepared as previously described,[1] was hydrolyzed with 7% aqueous hydrochloric acid (100 ml.) to constant rotation. The solution was then neutralized with Amberlite ion exchange resin IR 4B. Evaporation under vacuum of the filtrate yielded a crystalline product which on recrystallization from methanol gave pure 2-*O*-methyl-D-glucose, m.p. 157°. Treatment of this 2-*O*-methyl-D-glucose (0.5 g.) in methanol (1 ml.) with phenylhydrazine (1 ml.) and a few drops of glacial acetic acid at room temperature for 24 hours yielded the phenylhydrazone,[5] m.p. 175–176°.

Anal. Calcd. for $C_{13}H_{20}O_5N_2$: OMe, 10.9; N, 9.86. Found: OMe, 11.0; N, 10.08.

D-Glucophenylosazone.—A second sample (0.2 g.) of 2-*O*-methyl-D-glucose was heated on the steam-bath for 30 minutes with a solution containing phenylhydrazine hydrochloride (0.2 g.), sodium acetate (0.3 g.) and water (2 ml.). Recrystallization of the crude product from ethanol–water gave D-glucophenylosazone, m.p. 204°. Mixed melting point with authentic D-glucophenylosazone gave no depression. The X-ray pattern of this specimen was also identical with authentic D-glucophenylosazone.

Anal. Calcd. for $C_{18}H_{22}O_4N_4$: OMe, nil; N, 15.64. Found: OMe, nil; N, 15.54.

Optical Rotation of the Methyl Mono-*O*-methyl-α-D-glucopyranoside in Cuprammonium Solution.—The rotational shift of the methyl mono-*O*-methyl derivative in cuprammonium[4] was determined by Dr. R. E. Reeves and found to be +2080°; $[\alpha]^{25}_{436}$ +1300 ± 20° (*c* 0.7, in cuprammonium); $[\alpha]^{25}_{436}$ +300 ± 5° (*c* 0.83, in water).

(2) W. N. Haworth, E. L. Hirst and E. G. Teece, *J. Chem. Soc.*, 2858 (1931).
(3) R. E. Reeves, *Advances in Carbohydrate Chem.*, **6**, 107 (1951).
(4) R. E. Reeves, THIS JOURNAL, **71**, 215 (1949).
(5) P. Brigl and R. Schinle, *Ber.*, **62**, 1716 (1929).

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5-Hydroxy-5-hydroperoxybarbituric Acid ("Alloxan Hydroperoxide") and Other Alloxan Adducts[1]

BY BERNHARD WITKOP, SIDNEY GOODWIN AND THEODORE W. BEILER

RECEIVED APRIL 5, 1954

It has been shown in the preceding paper that the yellow solution of dimethyl diketosuccinate in absolute ether is readily decolorized by the addition of one mole of ethereal hydrogen peroxide with the formation of a very labile hydroperoxide, presumably dimethyl α-keto-α′-hydroperoxysuccinate. We have now applied this reaction to anhydrous alloxan (I), a lemon-yellow compound, easily obtained by vacuum sublimation of its hydrate (III).[2] The colorless hydroperoxide obtained in this way had the composition $C_4H_2N_2O_4 \cdot H_2O_2$ (or less likely the dimeric structure) and differed from the ill-defined compound $(C_4H_2N_2O_4)_{10} \cdot H_2O_2$ isolated by Stoltzenberg[3] as the product resulting from the reaction of 30% hydrogen peroxide on an aqueous solution of alloxan. The infrared spectra of alloxan hydrate[4] and of the hydrogen peroxide adduct were similar. The hydrogen peroxide adduct may exist in the chelate from II (OOH at 2.95 instead of 2.85).

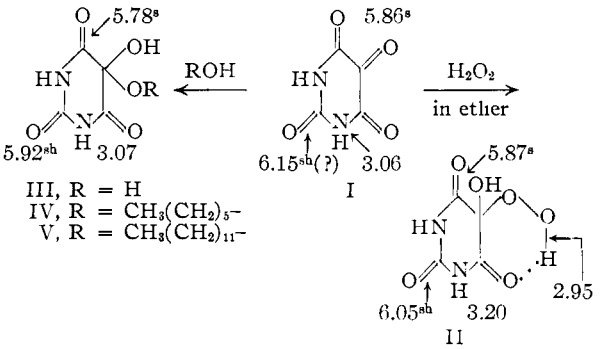
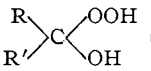


Table I shows that the polarographic reduction waves of the hydrate and hydroperoxide of alloxan at *p*H 6 do not differ appreciably from a mixture of alloxan and hydrogen peroxide or from hydrogen peroxide alone. The exchange of alloxan hydrate with H_2O^{18} (48% after 16 hours at 25°)[5] shows the lability of the water molecule in alloxan "hydrate." By comparison, hydroxyalkylhydroperoxides of the type



(R and R′ = alkyl)

are not known, but the equivalent dimeric structures are more stable than the corresponding hydrates.[6]

TABLE I

POLAROGRAPHIC REDUCTION OF ALLOXAN ADDUCTS

The solvent was Eimer and Amend Standard Buffer Solution *p*H 6.0 at 25°. The concentration of the sample was approximately 1 mmole/l. The figures mean volts *vs.* SCE.

Compound	Wave I	Wave II	Wave III[b]
Alloxan hydrate[a]	−0.115	−0.965	−1.52
Alloxan hydroperoxide	− .120	− .950	−1.51
Equimolar mixt. of alloxan hydrate and H_2O_2	− .110	− .945	−1.55
Hydrogen peroxide	.....	− .950[c]	....

[a] *Cf.* G. Sartori and A. Liberti, *Ricerca sci.*, **16**, 313 (1946); *C.A.*, **43**, 7835 (1949). [b] Wave III is a catalytic hydrogen wave. [c] Reported −0.94 [I. M. Kolthoff and C. S. Miller, THIS JOURNAL, **63**, 1013 (1941)].

(1) Oxidation Mechanisms. XIII. Preceding paper in this series, S. Goodwin and B. Witkop, THIS JOURNAL, **76**, 5599 (1954).
(2) H. Biltz, *Ber.*, **45**, 3659 (1912).
(3) H. Stoltzenberg, *ibid.*, **49**, 1545 (1916).
(4) R. S. Tipson and L. H. Cretcher, *J. Org. Chem.*, **16**, 1091 (1951).
(5) M. Senkus and W. G. Brown, *ibid.*, **2**, 569 (1938); *cf.* B. Stehlík, *Chem. Zvesti*, **3**, 325 (1949); *C. A.*, **45**, 561 (1951).
(6) *Cf.* R. Criegee in Houben-Weyl, "Methoden der Organischen Chemie," Georg Thieme Verlag, Stuttgart, 1952, Vol. VIII/III, p. 43.

Alloxan hydroperoxide is less stable than the hydrate and loses its peroxide character completely on standing for several hours in the presence of traces of moisture and after several weeks in the desiccator.

Dr. Byron Olson[7] has kindly tested alloxan hydrate (III) and alloxan hydroperoxide (II) as well as equimolar mixtures of alloxan hydrate and hydrogen peroxide in the desensitization test against tubercle bacilli (BCG) in guinea pigs.[8] The statistical evaluation of the tests involving several hundred animals is still in progress and will be reported elsewhere. There seem to be distinct differences between II and III.

The adducts IV and V of alloxan with hexyl and dodecyl alcohol,[9] prepared in the hope of obtaining lipophilic compounds suitable for diabetogenic tests, were soluble in ether and hexane but easily hydrolyzed by the addition of water.

Experimental

Anhydrous Alloxan (I).—Alloxan hydrate was sublimed at 210° (0.1 mm.) and formed lemon-yellow crystals, m.p. 253° (reported 250–255°).[2]

Anal. Calcd. for $C_4H_2N_2O_4$: C, 33.81; H, 1.42. Found: C, 33.55; H, 1.48 (the previous analysis[5] was too high in hydrogen).

Alloxan Hydroperoxide (II).—A suspension of 1 g. (7.04 mmoles) of powdered anhydrous alloxan in 100 ml. of dry ether was treated with 20 ml. (40 mmoles) of hydrogen peroxide in absolute ether.[10] After about two hours the clear colorless solution was decanted from a small amount of solid and concentrated *in vacuo* to 30 ml. The shiny, colorless crystals (1.23 g., 72%) were collected and washed with ether and pentane. On slow heating a sample melted with decomposition at 172–176° (m.p. of alloxan hydrate is 170° dec.). On a hot-stage preheated to 112° the sample evolved gas at 120°.

Anal. Calcd. for $C_4H_2N_2O_4 \cdot H_2O_2$: C, 27.27; H, 2.29; N, 15.91. Found: C, 27.12; H, 2.38; N, 15.95.

Titration for Active Oxygen.—The hydroperoxide (II, 48.8 mg.), dissolved in a mixture of 40 ml. of 0.18 *N* sulfuric acid and 10 ml. of 10% potassium iodide solution, was treated with 2 ml. of 3% molybdic acid solution. After 5 min., titration of the triiodide in solution with 5.05 ml. of 0.1 *N* sodium thiosulfate solution indicated that the sample contained 91.1% hydroperoxide. Samples stored over P_2O_5 contained 89.3% after one day, 87.4% after five days and no active oxygen after two months. Samples kept in screw cap vials outside the desiccator contained 1.76% of active oxygen after two days.

Alloxan–Hexanol Adduct (IV).—The adduct was obtained by adding 1.6 g. of anhydrous alloxan to 15 ml. of hexanol to which some dry hydrogen chloride had been added. The mixture was warmed until the solution became clear. On cooling, the mixture solidified to a waxy white mass. The excess hexanol was dissolved in pentane and the colorless, waxy flakes of the adduct were collected (2.3 g., 85% yield), m.p. 251–265° dec. The compound was soluble in ether but it could not be recrystallized satisfactorily from ether–pentane mixtures. The reaction product, therefore, was analyzed directly.

Anal. Calcd. for $C_4H_2O_2N_2 \cdot C_6H_{13}OH$: C, 49.17; H, 6.60; N, 11.47. Found: C, 48.51; H, 6.42; N, 10.32.

Alloxan–Dodecanol Adduct (V).—The adduct, prepared in a manner similar to IV, yielded shiny colorless leaflets, melting at 104–120°, readily soluble in ether and slightly soluble in hexane; the addition of water caused the separation of dodecyl alcohol in oily drops.

Anal. Calcd. for $C_4H_2O_4N_2 \cdot C_{12}H_{25}OH$: C, 58.51; H, 8.60; N, 8.53. Found: C, 60.49; H, 9.09; N, 7.04.

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(7) We are greatly indebted to Dr. Byron Olson, Laboratory of Tropical Diseases, National Microbiological Institute, for his great interest in this investigation and for kindly making arrangements for the various tests.

(8) D. A. Long, A. A. Miles and W. L. M. Perry, *Lancet*, **902**, 1085 (1951).

(9) *Cf.* H. Biltz and E. Topp, *Ber.*, **45**, 3667 (1912).

(10) See ref. 6, p. 33.

Solubilities of Some 2,3,5-Triphenyltetrazolium Salts

BY SAMUEL WEINER

RECEIVED JANUARY 28, 1954

In spite of the interest in the biochemical properties of the triaryltetrazolium salts, few data are available on their physical properties. Some of the slightly soluble salts of 2,3,5-triphenyltetrazolium were studied in this Laboratory and in the course of the work solubilities were measured at 25.00°.

SOLUBILITIES OF 2,3,5-TRIPHENYLTETRAZOLIUM

Abbrev.: g, gravimetric by evaporation of equilibrated solution; i, iodometric by oxidation to iodate and titration of latter[4]; t, titration with picrate[1]; c, colorimetric; n.c., new compound.

2,3,5-Triphenyltetrazolium	Solubility at 25.00° G./liter	Moles/liter	Method	Lit. Ref.
Iodide	1.308 ± 0.004	$(3.07 \pm 0.01) \times 10^{-3}$	g,i,t	2
Picrate	1.037 ± .002	$(7.0 \pm .4) \times 10^{-5}$	g	3
Triiodide	1.0273 ± .0007	$(40 \pm 1) \times 10^{-6}$	g	2
Dichromate	1.626 ± .004	$(7.69 \pm .05) \times 10^{-4}$	g,c	n.c.
Perchlorate	1.307 ± .001	$(7.71 \pm .02) \times 10^{-4}$	g	n.c.
Thiocyanate	1.51 ± .01	$(4.22 \pm .02) \times 10^{-3}$	g	3

From measurements of the solubility of the iodide in NaCl solutions it was calculated that the ideal solubility at zero ionic strength was 2.95×10^{-3} *M*. Its mean activity coefficients are 0.93 at 0.01 molar ionic strength, 0.89 at $\mu = 0.04$, and 0.85 at $\mu = 0.09$.

Melting points not reported previously in the literature are (all corrected): 2,3,5-triphenyltetrazolium iodide 228–229° dec., perchlorate 269–270°, dichromate 218–219° (dec., with darkening a few degrees below). The picrate, reported melting at 186–188°,[3] melted in this range when first prepared, but after long standing or when well dried or recrystallized from toluene it melted at 193–194°. The thiocyanate, reported melting at 134–136°,[3] melted at 174–174.5°.

The permanganate[2] was found to explode at 135°; it is insoluble in hot water or hot benzene. The ferricyanide, not previously reported, is yellow, insoluble in organic liquids, very slightly soluble in hot water, and explodes at 228°.

The perchlorate is colorless but turns yellow if exposed to light; it is soluble in ethanol or acetone, insoluble in chloroform or CCl_4, recrystallizable from acetic acid as needles. *Anal.* (by Univ. of Wis. microchemical laboratory). calcd.: C, 57.05; H, 4.02; N, 14.00. Found: C, 57.15; H, 4.00; N, 14.19.

The dichromate was 12.8 ± 0.1% Cr (calcd. 12.78); it is lemon-yellow when freshly precipitated but orange-yellow after drying, and explodes if heated much above the melting point.

(1) S. Weiner, *Chem.-Anal.*, **42**, 9 (1953).

(2) H. von Pechmann and P. Runge, *Ber.*, **27**, 2920 (1894).

(3) D. Jerchel and H. Fischer, *Ann.*, **563**, 200 (1949).

(4) I. M. Kolthoff and E. B. Sandell, "Textbook of Quantitative Inorganic Analysis," Rev. Ed., The Macmillan, Co., New York, N. Y., pp. 628–629, modified.