ketone, ϵ 50. In consequence the presence of even minute quantities of acid in the ketone causes a radical change in spectrum, which may serve as a basis for analysis; complete spectra synthesized on the basis of this single-point estimation faithfully reflect the experimental curves of deteriorated mixtures. The rapidity of the oxidation follows from our finding that a sample of ketone, on standing in air at room temperature for one month, contained *ca.* 40% of γ-benzoylbutyric acid; on the other hand, a sample of 99.9% spectrally pure ketone remained more than 99% pure after standing under nitrogen in the refrigerator for one month. The sensitivity of the method may be gauged by examination of the slight shoulder at 241.5 mμ in curve B: it can be estimated that this shoulder, if due to admixture of γ-benzoylbutyric acid, represents 0.03–0.04% contamination.

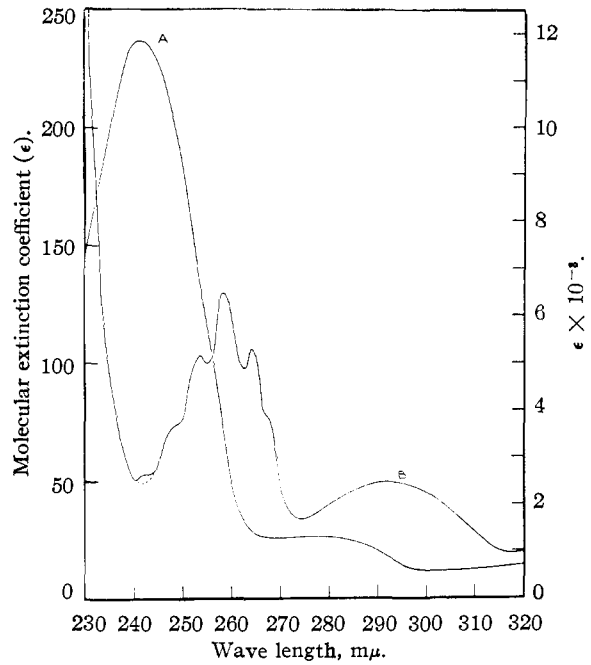


Fig. 1.—Ultraviolet spectra of γ-benzoylbutyric acid (curve A), and of 2-phenylcyclopentanone (curve B). The values of the extinction coefficients are recorded on the right-hand ordinate for A, and on the left-hand ordinate for B; solvent 95% ethanol.

Obviously earlier preparations[3] of 2-phenylcyclopentanone had yielded, in fact, γ-benzoylbutyric acid; of more immediate interest is the observation that the particular sample used for the earlier determination of the ultraviolet spectrum[1] was contaminated by γ-benzoylbutyric acid to the extent of about 14%. Any slight oxidation of the optically active ketone[1] which might have gone unrecognized would have had the effect of lowering its specific rotation, the product of oxidation being itself optically inactive. The conclusions advanced[1] therefore retain their validity. However, it is unlikely that the relative magnitude of the rotation of the cyclic ketone can be attributed, as originally felt, to a large difference in the ultraviolet absorption spectra of 3-phenyl-2-butanone and of 2-phenylcyclopentanone; the spectra of these two compounds are in fact closely similar. We shall reserve further comment pending an examination of the rotatory dispersions of these compounds.

(3) V. M. Mitchovitch, *Compt. rend.*, **200**, 1601 (1935); J. Pascual, J. Sanchez and R. Crespo, *Anales real soc. espan. fis. y quim.*, **45B**, 583 (1949).

Experimental

2-Phenylcyclopentanone.—This compound was prepared[1] by treatment of 2-chlorocyclopentanone with phenylmagnesium bromide. In the workup of the chloroketone care was taken to isolate the fraction boiling at 75° (11 mm.), it being known[4] that the chlorination of cyclopentanone results in a variety of products. Freshly distilled 2-phenylcyclopentanone, b.p. 96° (0.4 mm.), was recrystallized from ligroin five times, m.p. 36.5–37.5°. Its ultraviolet spectrum (curve B) was determined three hours subsequent to the final crystallization. A sample was sealed under nitrogen and kept in the refrigerator for one month; its spectrum after storage was substantially unchanged.

Air Oxidation of 2-Phenylcyclopentanone.—A sample of 2-phenylcyclopentanone was allowed access to air for a period of six weeks. During this time spectra were determined at intervals of 11, 21 and 33 days, revealing successively increasing amounts (4.8, 7.4, 39%) of γ-benzoylbutyric acid. Over this period the material changed from large, flat, colorless, transparent plates to a liquid, which gradually turned light yellow, and eventually to an oily, high-melting solid. The last product was triturated with a mixture of carbon tetrachloride and 5% sodium carbonate; the aqueous layer was acidified and the resulting solid, recrystallized from benzene–ligroin and water, melted at 127–127.5°, mixed m.p. with authentic γ-benzoylbutyric acid 126.5–127°.

The semicarbazone melted at 210–212° dec. The ultraviolet spectrum is reproduced in curve A.[5]

A sample of ketone was allowed to stand, with intermittent access to air, in the refrigerator for one month. In appearance the material consisted of large, lustrous plates characteristic of the ketone. Recrystallized three times from ligroin, the material melted at 36–37°. Its spectrum, recorded three hours subsequent to its last crystallization, revealed that it was 9.8% contaminated by γ-benzoylbutyric acid. Appearance and melting point cannot, therefore, be employed as sole criteria of purity in the case of this ketone.

Acknowledgment.—The authors gratefully acknowledge assistance afforded them by Dr. Alvin I. Kosak in the determination of the spectra. A grant from Research Corporation supported a part of this work.

(4) H. W. Wanzlick and G. Gollmer, *Chem. Ber.*, **88**, 281 (1955).

(5) C. D. Gutsche and K. L. Seligman, THIS JOURNAL, **75**, 2579 (1953), report λ_{max}^{EtOH} (ε) 242 mμ (12,200), 280 (1180).

WM. H. NICHOLS CHEMICAL LABORATORY
NEW YORK UNIVERSITY
NEW YORK 53, N. Y.

The Structure of 2-Methyl-1,4-naphthoquinone Addition Compounds with Bisulfites

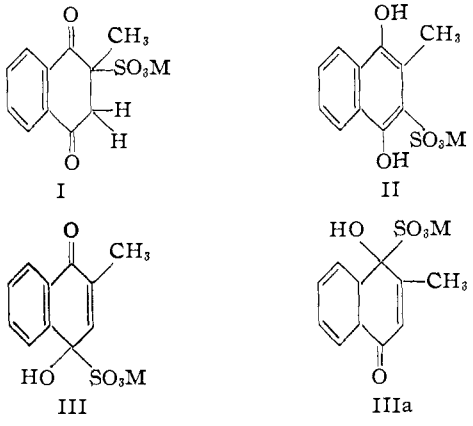
BY M. B. MOORE AND W. H. WASHBURN

RECEIVED AUGUST 15, 1955

In a previous paper,[1] the structure of the antihemorrhagically active addition compound of menadione with bisulfites was formulated as I rather than II, III or IIIa (M represents a metallic or ammonium cation).

Additional confirmation of this structure now has been obtained from infrared spectrosopic data on a purified specimen of the potassium salt, synthe-

(1) M. Carmack, M. B. Moore and M. E. Balis, THIS JOURNAL, **72**, 844 (1950).



sized by the method of Baker, *et al.*[2] The recrystallized active salt was dried for five days in a vacuum desiccator (P_2O_5).

The infrared spectrum of this material, while exhibiting a very weak absorption in the 3 μ region probably due to a trace of moisture in the potassium bromide used in the pellet, very clearly indicates lack of hydroxyl in the sample.

(2) B. R. Baker, T. H. Davies, L. McElroy and G. H. Carlson, THIS JOURNAL, **64**, 1096 (1942).

ABBOTT LABORATORIES
NORTH CHICAGO, ILLINOIS

Hydrolysis of Isomaltotriose by Oligo-1,6-glucosidase

BY JOSEPH LARNER

RECEIVED JULY 18, 1955

The enzymatic hydrolysis of isomaltose, "panose" and branched α-amylase dextrins by oligo-1,6-glucosidase from hog intestinal mucosa has been reported.[2,3] When coupled to the hexokinase, Zwischenferment, TPN system, the enzyme has no activity on dextran.[3] The present report demonstrates the enzymatic hydrolysis of isomaltotriose (6-α-isomaltopyranosyl-D-glucose),[4] the next higher homolog of isomaltose.

Hydrolysis of isomaltotriose has been followed by increase in reducing power, paper chromatography and by coupling with the hexokinase, Zwischenferment, TPN system. Incubation of 4.0 mg. of isomaltotriose with 120 units[2] of oligo-1,6-glucosidase at 30° in the absence of buffer resulted in a 60.2% hydrolysis in 120 minutes. Aliquots of the reaction mixture were deproteinized by the $Ba(OH)_2$-$ZnSO_4$ method of Somogyi[5] and reducing power determined by the method of Nelson.[6] The more alkaline reagent 60 of Shaffer and Somogyi[7] and a boiling time of 30 minutes were used. Paper chromatographic analysis at 22.0, 29.3, 39.8, 52.9 and 60.2% hydrolysis revealed only isomaltose and glucose as products, when about 100 γ of total sugar was applied to each spot.

An experiment in which the rate of hydrolysis of equimolar amounts of isomaltose and isomaltotriose are compared is presented in Table I. The results are expressed both as amount of reducing sugar (as glucose) appearing per time period as well as percentage of hydrolysis. In terms of appearance of reducing sugar, isomaltotriose is hydrolyzed more rapidly than isomaltose. Since, on a molar basis, isomaltose has one susceptible linkage for each two of isomaltotriose, isomaltose is more rapidly hydrolyzed when compared in terms of percentage of hydrolysis. When calculated in terms of linkages hydrolyzed, 1.5 linkages of isomaltotriose[8] were hydrolyzed for each linkage of isomaltose during the 25 minute time period, and 1.9 during the 90-minute period.

TABLE I

HYDROLYSIS OF ISOMALTOSE AND ISOMALTOTRIOSE BY OLIGO-1,6-GLUCOSIDASE

Reaction mixture contained 100 units oligo-1,6-glucosidase, 1.98 μmoles isomaltotriose, or 2.22 μmoles isomaltose; total volume, 2.2 ml.

Substrate	Time, min.	Δ Reducing sugar, as glucose, μg./2.2 ml.	Hydrolysis, %
Isomaltose	25	172	44.8
	90	358	92.2
Isomaltotriose	25	224	33.8
	60	448	67.5
	90	566	85.4

In the presence of hexokinase, Zwischenferment, and TPN formation of glucose could be conveniently followed by the increase in optical density at 340 mμ (Fig. 1). With equimolar amounts of substrates, the rate of TPN reduction with isomaltotriose was 80% that of isomaltose (slope of curve). When calculated in terms of linkages hydrolyzed, 1.6 linkages of isomaltotriose were hydrolyzed for each linkage of isomaltose in good agreement with the results of Table I.

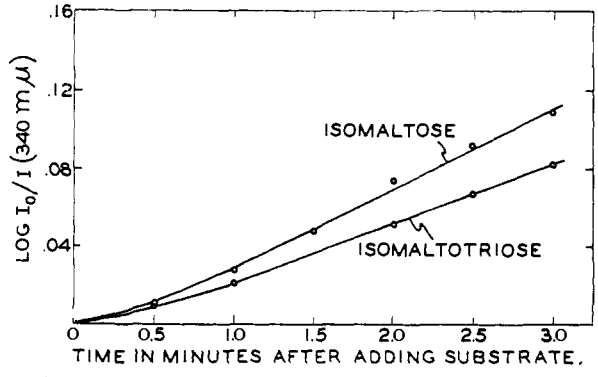


Fig. 1.—Microenzymatic determination of isomaltose and isomaltotriose hydrolysis by oligo-1,6-glucosidase. The reaction mixture (3.0 ml. volume) contained 41 units oligo-1,6-glucosidase and 1 μmole of either isomaltose or isomaltotriose. All other components as previously described.[3]

K_m values have been determined for the two substrates using the hexokinase Zwischenferment sys-

(1) Supported in part by a grant from the Graduate College, University of Illinois, Urbana, Illinois.

(2) J. Larner and C. M. McNickle, THIS JOURNAL, **76**, 4747 (1954).

(3) J. Larner and C. M. McNickle, *J. Biol. Chem.*, **215**, 723 (1955).

(4) We gratefully acknowledge the gifts of isomaltose and isomaltotriose from Dr. A. Jeanes, Northern Utilization Research Branch, Peoria, Illinois.

(5) M. Somogyi, *J. Biol. Chem.*, **160**, 69 (1945).

(6) N. Nelson, *ibid.*, **153**, 375 (1944).

(7) P. A. Shaffer and M. Somogyi, *ibid.*, **100**, 695 (1933).

(8) It is not known which of the two linkages of isomaltotriose the enzyme hydrolyzes most readily.