isomers (II, XV, and XVI) were characterized. In conformity with their structure they smoothly gave the ketone (IX) when treated with dil. hydrochloric acid, whereas the natural dihydro-rotundine was proved to be quite stable to hydrochloric acid even at an elevated temperature.

Similar experiments were also conducted with synthetic *rac*-dihydro-isorotundine (I).

(Received March 2, 1961)

UDC 614.31 : 612.015.3 : 547.563.13

33. Masuo Akagi and Isamu Aoki : Studies on Food Additives. VIII.[1] Metabolism of α-Hydroxy-2,6-di-*tert*-butyl-*p*-cresol. Isolation of Metabolites.

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In the previous work[1] of this series, a glucuronide as its methyl acetate derivative was isolated from the urine of a rabbit receiving 2,6-di-*tert*-butyl-*p*-cresol (BHT) and Aoki assumed its structure as methyl [(3,5-di-*tert*-butyl-4-hydroxybenzyl)-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate.

In the present series of work, as a means for certifying the structure of its glucuronide, the isolation of metabolites from the urine of a rabbit receiving α-hydroxy-2,6-di-*tert*-butyl-*p*-cresol (BHT-alc) was carried out, and unchanged BHT-alc (M_1), 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-ald, M_2) as its 2,4-dinitrophenylhydrazone, 3,5-di-*tert*-butyl-4-hydroxy-benzoic acid (BHT-acid, M_3), 4,4′-ethylenebis(2,6-di-*tert*-butylphenol) (BHT-diphenylethane, M_4), were isolated, similarly as in the case[1] of BHT-dosed rabbits, and also a glucuronide (M_5) as its methyl acetate derivative.

This glucuronide was clearly different from the glucuronide[1] isolated from BHT-dosed rabbits or synthesized methyl [(3,5-di-*tert*-butyl-4-hydroxy-benzoyl)-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate.

Experimental

Materials——BHT-ald was prepared by oxidation of BHT in a mixture of AcOH-H_2O (5:1) with Br_2 according to the method of Fujisaki.[2]

BHT-alc was prepared as follows : BHT-ald (3 g.) was suspended in 30 cc. of MeOH with stirring and $NaBH_4$ (0.5 g. in 5 cc. of 0.1*N* NaOH) was added to this suspension in small portions at room temperature. After standing overnight at room temperature, the white crystals that separated were collected and washed with a small amount of H_2O. Recrystallization from 100 cc. of ligroine (b.p. 80~120°) gave colorless crystals (2.8 g.), m.p. 137~138° (reported[*2] m.p. 137°). *Anal.* Calcd. for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24. Found : C, 76.34; H, 10.17.

The preparation of BHT-acid and BHT-diphenylethane was described in the previous paper.[3]

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*2 This compound was prepared through reduction of BHT-ald with $LiAlH_4$ by Coppinger. *et al.* [J. Am. Chem. Soc., **75**, 734 (1953)] and through hydrolysis of α-bromo-2,6-di-*tert*-butyl-*p*-cresol in H_2O-Me_2CO by Cook, *et al.* (*Ibid.*, **77**, 1783 (1955)).

1) Part VII. I. Aoki : This Bulletin, **10**, 105 (1962).
2) T. Fujisaki : Nippon Kagaku Zasshi, **77**, 731 (1956).
3) M. Akagi, I. Aoki : This Bulletin, **10**, 101 (1962).

The preparation of the derivative of ester-type BHT-acid-glucuronide will be described in a later section.

Paper Chromatography of the BHT-alc Metabolites——The methods were the same as in the previous work.[3] Toyo Roshi No. 51 filter paper was used by the ascending method, at 20°. Moving phase : MeOH-H_2O (4:1), iso-PrOH-H_2O-NH_4OH (20:2:1). Coloring reagent : Gibbs, diazo, and phosphomolybdic acid reagents. Unchanged BHT-alc, BHT-acid, and BHT-diphenylethane were detected also in the urine of a rabbit receiving BHT-alc.

Isolation and Characterization of Glucuronide (M_5)——The animals used in this work were rabbits weighing 2.54~2.74 kg. They were housed in metabolism cages and were fed with a mixture of 50 g. of oats, 100 g. of carrots, and 200 g. of cabbage daily. BHT-alc filled in a capsule was administered orally and the decomposition of metabolites in the urine was prevented by addition of toluene.

A total dose of 4.0 g. of BHT-alc was administered orally to three rabbits. The collected 24-hr. urine (770 cc.) was filtered through cotton, the filtrate was adjusted to pH 2 with 5*N* H_2SO_4, and was continuously extracted with Et_2O for 60 hr. The extract was carefully warmed to remove Et_2O. The residue (9 g.) dissolved in 40 cc. of Et_2O was poured into 250 cc. of petr. ether (b.p. 40~60°) to precipitate the glucuronide. The clear yellowish brown petr. ether supernatant was used for separation of other metabolites. The brown-colored syrup which deposited was dissolved in 30 cc. of Et_2O and insoluble material was removed by filtration.

To the filtrate under cooling in an ice-bath, Et_2O solution of CH_2N_2 (obtained from 15 g. of nitrosomethylurea) was added cautiously and the reaction mixture was allowed to stand overnight in a refrigerator. The solvent was removed by evaporation in a reduced pressure and the syrupy residue was dissolved in 20 cc. of pyridine and 25 cc. of Ac_2O. The mixture was allowed to stand overnight at room temperature, poured into ice-water (500 cc.) with stirring, the crude powder that separated was collected, and recrystallized three times from EtOH to 0.4 g. of white prisms, m.p. 170~171°; $[\alpha]_D^{10}$ −37.7° (c=0.53, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{38}O_{11}$: C, 60.22; H, 7.11. Found : C, 60.10; H, 7.10.

This compound showed intensive naphthoresorcinol reaction and carbazole reaction as glucuronic acid, and this glucuronide showed absorption in infrared region at 3590 cm^{-1}, consistent with the presence of a hydroxyl group (Fig. 2).

This glucuronide was easily hydrolysed with 10% KOH on a water bath. This behavior to hydrolysis reminded of an ester glucuronide, but this compound showed depression of melting point with synthetic methyl [(3,5-di-*tert*-butyl-4-hydroxy-benzoyl)-2,3,4-tri-O-acetyl-β-D-glucopyranosid]-uronate and also with BHT-glucuronide[1] which was isolated from the urine of rabbits receiving BHT.

After hydrolysis with 10% KOH, the Et_2O extract was used for paper chromatography. In the case of solvent system of iso-PrOH-H_2O-NH_4OH, one spot was found at Rf 0.73, which gave a yellowish green color with phosphomolybdic acid reagent and a violet color with Gibbs reagent. The Et_2O extract showed ultraviolet absorption at 249 mμ (in EtOH).

From the result obtained by analysis, it was assumed tentatively that this compound might be methyl [(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate, but further study would be required to establish its structure.

Isolation of Other Metabolites——After removal of the glucuronide, the clear yellowish brown petr. ether supernatant was extracted with saturated $NaHCO_3$ solution and $NaHCO_3$ extract was acidified with dil. HCl. The yellowish solid that separated was collected and sublimed in a reduced pressure (water pump, 10~15 mm. Hg, bath temperature, 130°) in order to remove BzOH. The residue was redissolved in $NaHCO_3$ solution, filtered, and reprecipitated with acid. Recrystallization from 50% *tert*-BuOH gave 0.1 g. of 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (M_3), m.p. and mixed m.p. 211°.

The remaining petr. ether solution was further extracted with Na_2CO_3 solution and successively with 10% KOH solution.

The KOH extract was acidified and immediately separated yellowish brown crystals. The crystals collected by filtration were sublimed in a reduced pressure (water pump, 10~15 mm. Hg, bath temperature 150°) and resublimed compound was recrystallized from ligroine (b.p. 80~120°) to 0.03 g. of unchanged BHT-alc (M_1), m.p. and mixed m.p. 136~137°.

After separation of the sublimate, the residue was dissolved in EtOH and reacted with 2,4-dinitrophenylhydrazine to give 0.05 g. of 2,4-dinitrophenylhydrazone, m.p. and mixed m.p. 234~235°, of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (M_2).

The residual petr. ether solution, after washing with H_2O, was evaporated to dryness in a reduced pressure at 30°. From the residual gummy material, yellowish crystals were separated by sublimation in a reduced pressure and recrystallized from EtOH to 0.05 g. of BHT-diphenylethane (M_4), m.p. and mixed m.p. 170°.

The four compounds isolated as above showed the same behavior as the authentic compounds both in ultraviolet and infrared absorption spectra.

Preparation of the Derivatives of Ester-type BHT-acid Glucuronide——3,5-Di-*tert*-butyl-4-hydroxy-benzoic acid (2.5 g., 0.01 mole) was dissolved in 50 cc. of EtOH containing 0.68 g. (0.01 mole) of EtONa, and the solvent was evaporated in a reduced pressure to dryness. The residue (needles) was dissolved in 30 cc. of Me_2CO and 4.0 g. of methyl (2,3,4-tri-O-acetyl-α-D-glucopyranosyl bromide)-uronate[4] in 10 cc. of Me_2CO was added dropwise into the solution with cooling in an ice-bath. The reaction mixture was allowed to stand for 24 hr. at room temperature. After removal of the separated NaBr by filtration, the filtrate was evaporated to dryness in a reduced pressure. The residue was dissolved in 100 cc. of $CHCl_3$ and the $CHCl_3$ solution was washed with H_2O to remove NaBr. The $CHCl_3$ solution was dried over Na_2SO_4 and evaporated to dryness in a reduced pressure. The residue was dissolved in 10 cc. of EtOH and allowed to stand overnight in a refrigerator. The square crystals which separated from EtOH were collected and washed successively with H_2O and EtOH. After recrystallization from EtOH, 0.5 g. of methyl [(3,5-di-*tert*-butyl-4-hydroxy-benzoyl)-2,3,4-tri-O-acetyl-β-D-glucopyranosid]uronate, m.p. 144~145°, was obtained. *Anal.* Calcd. for $C_{28}H_{38}O_{12}$: C, 59.22; H, 7.09. Found: C, 59.07; H, 6.83.

This compound gave 3,5-di-*tert*-butyl-4-hydroxy-benzoic acid by hydorlysis with 10% KOH and showed intensive naphthoresorcinol and carbazole reactions for a glucuronide.

The infrared and ultraviolet absorption spectra of this compound are shown in Figs. 1 and 2.

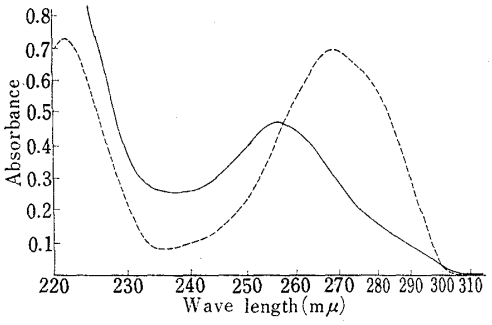

Fig. 1. Ultraviolet Absorption Spectra of Glucuronides

—— isolated glucuronide derivative, λ_{max}^{EtOH} mμ (log ε): 256 (4.39).

····· synthesized BHT-acid glucuronide derivative, λ_{max}^{EtOH} mμ (log ε): 222 (4.45), 267 (4.43).

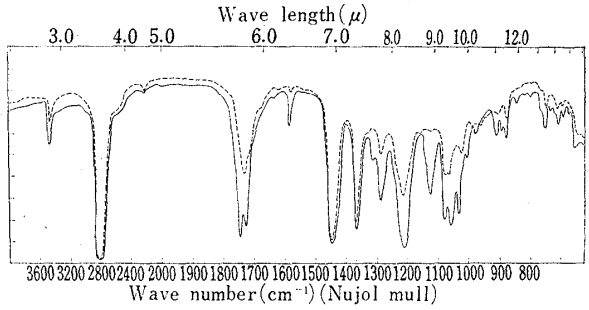

Fig. 2. Infrared Absorption Spectra of Glucuronides

····· isolated glucuronide derivative

—— synthesized BHT-acid glucuronide derivative

Chemical Reaction of BHT-alc——Reaction with CH_2N_2: To 1.0 g. of BHT-alc in 10 cc. of EtOH, Et_2O solution of CH_2N_2 (obtained from 5.0 g. of nitrosomethylurea) was added and the mixture was allowed to stand overnight in a refrigerator. The solvent was removed by evaporation in a reduced pressure and the light yellowish residue was recrystallized from ligroine to the starting BHT-alc.

Reaction with Methanolic Hydrochloric Acid: To the solution of 1.0 g. of BHT-alc dissolved in 30 cc. of MeOH, HCl gas was introduced to 3% saturation. The mixture was refluxed on a water bath for 15 min., the cooled solution was added to 50 cc. of distilled water, and extracted with three 50-cc. portions of Et_2O. The Et_2O extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The white residue was recrystallized from MeOH-H_2O to short blunt needles, m.p. 101°. This compound sublimed at 120° and showed no depression of melting point with the authentic α-methoxy-2,6-di-*tert*-butyl-*p*-cresol. *Anal.* Calcd. for $C_{16}H_{26}O_2$: C, 76.75; H, 10.47. Found: C, 76.70; H, 10.55.

Antioxidative Effect of BHT Derivatives on Vitamin A in Fish Liver Oil——This test was made by the method described previously.[5] The substrate used was shark liver oil containing vitamin A in 44.000 USP U/g. Stored at 37°. A comparative test is shown in Table I.

4) G.N. Bollenback, *et al.*: J. Am. Chem. Soc., **77**, 3310 (1955).
5) M. Akagi, I. Aoki: Yakugaku Zasshi, **81**, 492 (1961).

Table I. Antioxidative Effect of BHT Derivatives on Vitamin A in Fish Liver Oil (Vitamin A, 44.000 USP U/g., stored at 37°, added to 0.1% of sample)

Sample	Induction period (days)						
	5	6	7	8	9	10	12
Control	25.300	16.700	4.900				
BHT	40.200	39.800	36.900	36.300	33.600	28.600	8.500
BHT-alc	39.700	38.800	35.600	35.300	28.100	12.800	4.300
BHT-ald	35.100	22.300	11.600	5.300			
(I)	30.100	21.400	13.100	6.100			
(II)	39.300	38.900	34.400	31.300	21.100	13.000	3.700
(III)	40.100	39.500	34.500	32.400	27.100	21.700	8.100
BHA	38.200	35.400	25.000	23.900	18.440	15.600	7.700

(I) 2-*tert*-Butyl-*p*-cresol
(II) 3,5-Di-*tert*-butyl-4-hydroxy-benzaldehyde mercaptal-β,β-di-propionic acid
(III) α-Methoxy-2,6-di-*tert*-butyl-*p*-cresol

Discussion

During the course of this experiment, the BHT-alc-dosed rabbits maintained their normal growth as the control rabbits. The fact that BHT-ald (M_2) and BHT-acid (M_3) were isolated as metabolites of BHT-alc can be explained sufficiently from the metabolic fate of BHT.

From the biochemical view-point, it is interesting that BHT-diphenylethane (M_4) was also isolated as one of the metabolites of BHT-alc. As demonstrated in Chart 1, BHT-diphenylethane, synthesized by many investigators in various ways from BHT and its derivatives, was not obtained from BHT-alc by chemical procedure.

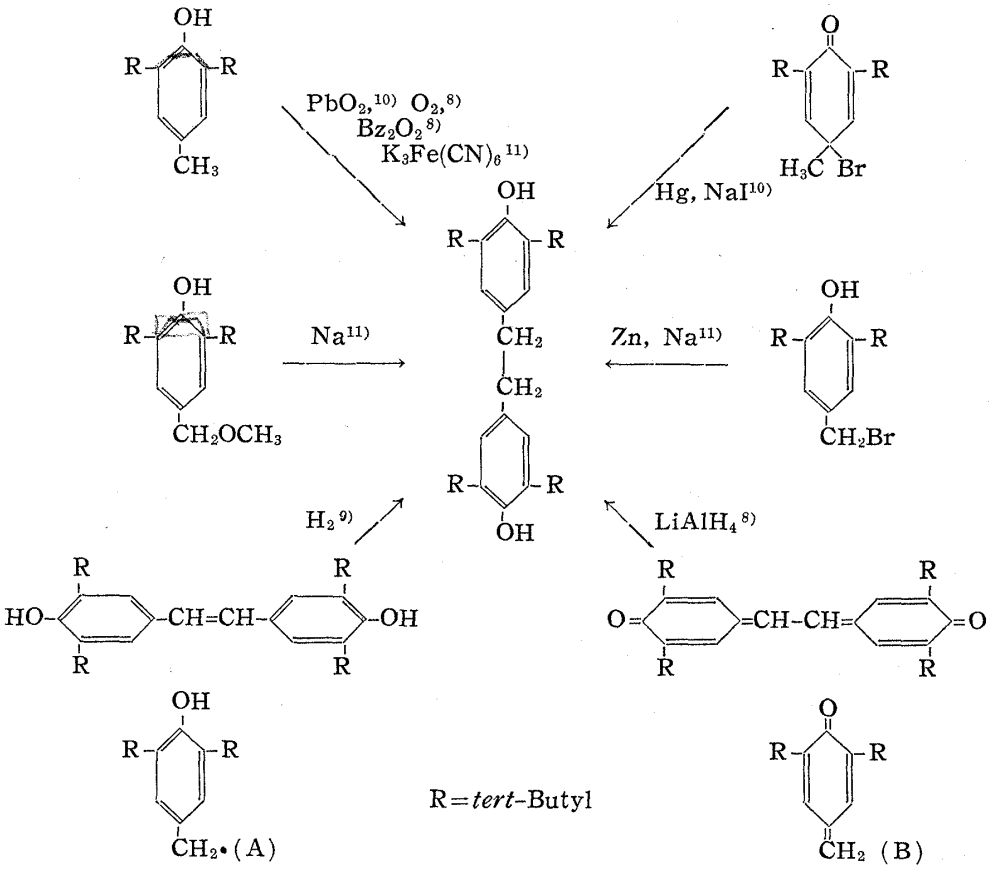

Chart 1.

In addition, Cosgrove and Waters,[6] Yohe and Hill,[7] and Moore and Waters[8] proposed (A) as the intermediate of (M_4) from BHT in the reaction with various oxidizing agents, while Cook[9] and Fujisaki[10] estimated (B) as this intermediate. With consideration of these data, it is interesting that BHT-alc changed to a dimer *in vivo*.

On the determination of antioxidative effect on concentrated vitamin A oil, BHT-alc and BHT-alc-Me (oxidation products of BHT) showed an excellent effect like BHT. Previously, Cook[9] reported that BHT-diphenylethane also exhibited an excellent effect.

The authors are indebted to Prof. T. Ukita of the University of Tokyo for his kind advice and suggestion, and also to Mr. Narita of the Analysis Room of this Faculty for elementary analyses.

Summary

3,5-Di-*tert*-butyl-4-hydroxy-benzoic acid, 4,4′-ethylenebis(2,6-di-*tert*-butylphenol), and unchanged α-hydroxy-2,6-di-*tert*-butyl-*p*-cresol were isolated and characterized from the urine of a rabbit dosed with α-hydroxy-2,6-di-*tert*-butyl-*p*-cresol. The isolated 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde was identified as its 2,4-dinitrophenylhydrazone. A glucuronide was isolated as its triacetyl-methyl ester. Antioxidative effect of 2,6-di-*tert*-butyl-*p*-cresol and its derivatives on vitamin A was examined.

(Received March 3, 1961)

6) S. L. Cosgrove, W. A. Waters: J. Chem. Soc., **1951**, 388.
7) G. R. Yohe, D. R. Hill, J. E. Dunbar, F. N. Scheidt: J. Am. Chem. Soc., **75**, 2688 (1953).
8) R. F. Moore, W. A. Waters: J. Chem. Soc., **1954**, 243.
9) C. D. Cook, N. G. Nash, H. R. Flanagan: J. Am. Chem. Soc., **77**, 1738 (1955).
10) T. Fujisaki: Nippon Kagaku Zasshi, **77**, 869 (1956).

UDC 547.834.3.07

34. Masao Shimizu, Fumihiko Uchimaru, and Bumpei Kurihara: Studies on N-Substituted Nortropane Derivatives. V.[1] High-pressure Catalytic Hydrogenation of N-Substituted 3-Nortropanones and their Methiodides.

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As described in Part II[2] of this series, one of the authors obtained the epimeric mixture of alcohols, 3α(axial)- and 3β(equatorial)-ols, by sodium borohydride reduction of several kinds of N-substituted 3-nortropanone derivatives. The present paper describes further studies on these 3-ketones and their methiodides.

Keagle and Hartung[3] obtained 3α-tropanol by the reduction of 3-tropanone over platinum oxide, and Stoll, *et al.*[4] reported the formation of corresponding 3α-ols by the reduction of various N-substituted 6-hydroxy(or alkoxy)-3-nortropanone derivatives. In

*1 2810, Minamifunabori-cho, Edogawa-ku, Tokyo (清水正夫, 内丸文彦, 栗原文平).
1) Part IV: This Bulletin, **9**, 313 (1961).
2) Part II: *Ibid.*, **9**, 304 (1961).
3) L. C. Keagle, W. H. Hartung: J. Am. Chem. Soc., **68**, 1608 (1946).
4) A. Stoll, E. Jucker: Angew. Chem., **66**, 376 (1954).